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April 25, 1962

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Abstract

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The techniques of EPR spectroscopy are being applied in a variety of ways to the study of biological materials. In this paper the theory basic to the EPR experiment is discussed, and the application of EPR spectroscopy to the study of photosynthesis are then reviewed.

The Theory of the experiment is given a simplified treatment. Where possible the measurable parameters of the resonance absorption are illustrated with examples from the literature. Special attention is paid to those aspects of the resonance experiment which become important when the sample consists of amorphous or polycrystalline solid material.

The applications to the study of photosynthesis are divided according to the nature of the materials being studied. The division has been made according to complexity, starting with the simplest (extracts of photosynthetic materials) and proceeding to the most complex systems (complete systems in good physiological condition).

The observations on these various types of materials are correlated. A fairly consistent picture of the basic observation is developed, in which the photo-produced unpaired electrons are related to the photo-physical process of quantum conversion.

I. INTRODUCTION

The material of which living organisms are made is largely organic. Even the inorganic constituents, aside from the sodium chloride, are most often found very closely associated with, if not a component part of, organic substances in living organisms. The structure and change of structure of such materials, that is, organic substances in general, is determined by the electronic configuration of the atoms of which they are made, and by changes in these electronic configurations. Therefore, any method of observation which permits us to look closely into the nature of the electronic configurations and the changes of these electronic configurations is likely to become a major tool in biological studies.

A method of observing both static and, in some cases, changing electronic configurations has recently (that is, in the last decade or so) risen to prominence. This method has been important particularly in the study of the electronic structure of inorganic materials and is now becoming of importance in the examination of both the statics and dynamics of organic and biological materials. This method depends upon the fact that a spinning electron is magnetic as are, in fact, a good many nuclei as well. Therefore, such an electron, when placed in an external magnetic field, will interact with that field in a characteristic way which can be determined by suitable configuration of the magnet and electromagnetic radiation, which we will discuss later.

Now not all the electrons present in chemical substances can be studied by such magnetic resonance methods. Only those substances which contain in them magnetic entities which can respond to an external magnetic field in an observable way are likely to yield information. Certain nuclei of considerable interest to us do have such properties, and we will mention them later.

Most of the electrons in organic materials, however, although they individually do have a spin and a magnetic moment, exist in the organic material in pairs in which the magnetic moments are oppositely directed and are closely coupled so that the individual electrons cannot interact in an as yet observable fashion with an external magnetic field. We are therefore limited in our biological exploitation of this method to such materials that contain electrons not so paired. Since many important chemical transformations which occur in biological materials involve at some stage of their occurrence the uncoupling of these paired electrons in some way or another, it seems likely that this method of observation will find a broad application in the study of biological structures and their changes.

Materials which contain electrons in any of the following conditions might be susceptible to study by electron paramagnetic resonance methods, and many of them have already been observed in biological materials.

(a) Free electrons: Such electrons as are found as conduction electrons in a metal are indeed free, and under certain special conditions their spin lifetimes are sufficiently long so that they may be observed by these methods (e.g. Feher and Kip, 1955, and Wagoner, 1960).

(b) Not-quite-free electrons: Such electrons as are found in the narrow conduction bands in semiconductors are also susceptible to observations. These electrons are produced by being raised from bound, or coupled, states into their conduction, or free, states, usually by thermal energy or light (e.g. Singer and Kommandeur, 1961).

(c) Trapped 'free' electrons: These are electrons which are not paired but which are physically trapped in a solid lattice (either ionic, atomic or molecular) and are definitely readily observable by this method (e.g. Fletcher et al., 1954, and Fortis, 1955).

(d) Unpaired electrons from paired electrons: These unpaired electrons, created by the fission of a paired electron bond which generally involves the separation of atoms as well, are also clearly observable by these methods. Such (ordered) molecules may appear as chemical reaction intermediates (e.g., Yamazaki et al., 1960) or as broken bonds created by high energy radiation (e.g. Shields and Gordy, 1959).

(e) Unpaired electrons in even molecules: These are unpaired electrons from molecules containing an even number of electrons in which two or more have been uncoupled to form what is called a state of higher multiplicity. (When only one electron pair is uncoupled to give two electrons with parallel spin moments, it is called a triplet state.) For example, see Hutchinson and Mangum, 1958.

(f) Unpaired electrons from transition metal ions: These electrons in the transition metal ions such as iron, cobalt, nickel and copper are generally observable by the method of electron spin resonance. It is clear that these unpaired electrons, existing as

they do in the d-orbitals of transition elements, are quite common in biological systems. Unfortunately, they are not always observed, at least under the conditions which are currently known to us. However, much important information is beginning to appear from studies where they have been observed (e.g. Beinert and Lee, 1961).

In what follows we shall discuss the nature of the electron paramagnetic resonance (EPR) experiment by which these various types of unpaired electrons are observed, how this type of experiment has been applied to photosynthetic systems, and the conclusions concerning photosynthesis that can be drawn from the experiments.

In Section II we will describe the parameters associated with the observation of unpaired electrons. Here the emphasis will be placed on those aspects of the observation which become important when the sample material is polycrystalline or amorphous. Some of the resonance parameters are not, as yet, generally useful in making statements about electronic configurations or environment in photosynthetic systems. We will try to indicate these.

In Section III we will list the types of photosynthetic materials on which resonance experiments have been performed. Then in varying degrees of detail we will discuss some of the experiments which have been performed. Our treatment will not be encyclopedic, but, rather, will deal with those experiments that seem to us to be most pertinent.

Section IV will be concerned with the conclusions one can draw from the experiments discussed in Section III. Mention will be made of some experiments which may be profitably carried out in the future.

Finally, a brief appendix is attached in which several terms are defined, and various experimental details are described.

II. THE MAGNETIC RESONANCE EXPERIMENT

The magnetic resonance experiment has been the subject of several text books. These are listed at the head of the list of references. Texts on nuclear as well as electron resonance have been included, since many of the concepts involved in the two types of experiment are the same. A glance at any of the listed texts will show that the subject, when treated in detail, can be quite complicated. The treatment here is considerably simplified; we hope without the sacrifice of accuracy.

A. Zeeman Energy Levels.

If an atomic or molecular system with spin angular momentum of magnitude $\hbar [S(S + 1)]^{1/2}$ is placed in a magnetic field, H , the component of angular momentum along the direction of the field may assume only the values

$$S\hbar, (S - 1)\hbar, \dots, -S\hbar.$$

Each of these $2S + 1$ orientations will have a different energy.

The energy of these states, the Zeeman energy, may be written

$$\mathcal{H} = g\beta H \cdot \underline{S} = g\beta H_z m_s$$

if H is in z direction and $m_s = S, S - 1, \dots, -S$. According to the basic quantum mechanical principle, transitions between

the magnetic states can be induced by providing a quantum of energy, $h\nu$, of the appropriate size.

Except under special circumstances, transitions may be induced only between adjacent levels: i.e. $\Delta m_s = \pm 1$. Thus, the condition for inducing the transitions (the resonance condition) is $h\nu = g\beta H$. In this expression $\beta = \frac{eh}{2mc}$ is the Bohr magneton, and g represents the effective size of the magnetic moment being acted upon by the magnetic field. It is equivalent to the spectroscopic splitting factor

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

of the free atom (White, 1934). When H is 3300 gauss and $g = 2.00$, ν is 9.5 kMc/s ($\lambda \approx 3.2$ cm.).

B. Resonant Energy Absorption.

When the resonance condition is satisfied, transitions from m_s to $m_s + 1$ and from $m_s + 1$ to m_s are induced with equal probability by the high frequency (microwave) magnetic field. This in itself leads to no energy absorption. A net energy absorption arises from two facts: (1) The spin system, in thermal equilibrium, has a few more spins in the state m_s than in the state $m_s + 1$. The slightly greater number of spins in the states of lower energy means that slightly more transitions up than down will be induced by the microwave field. (2) There are alternate routes by which spins excited to the state $m_s + 1$ can be returned to m_s ; the routes through which thermal equilibrium would be re-established should

the microwave field be suddenly removed. These processes are called thermal relaxation processes.

The margin of operation for observation of the net energy absorbed is small. In Table I we have listed values for the Boltzman factor determining the population distribution, and the fractional spin unbalance for an $S = 1/2$ system at three different temperatures. It is seen that even at 4.2°K , of 100 spins there are only 5 more in the ground than in the excited state. The first person to observe these small energy absorptions was Zavoisky (1945) in the USSR. He used solutions containing transition metal ions. In the United States Cumberow and Halliday (1946) made the first observation of this type. The field has been developing rapidly ever since.

We would now like to answer the following two questions: What are the measurable parameters associated with this resonance absorption? And, which of these, if any, might be useful in the solution of problems concerning photosynthesis?

C. Area Under Curve.

The reaction of a system of free electrons to an applied magnetic field is depicted in Fig. 1. In a resonance experiment (holding ν constant) each electron would absorb energy at exactly the same value of H_0 . (H_0 and H_z are used interchangeably to identify the large applied field. If its direction in space is important the z axis is specified.) When unpaired electrons exist inside an actual

Table I
Boltzman Constant and Fractional Spin Unbalance for an $S = 1/2$ System
at Three Temperatures

	300° K	77° K	4.2° K
$\frac{g\beta H}{kT}$	1.48×10^{-3}	5.76×10^{-3}	1.05×10^{-1}
$\frac{n\uparrow - n\downarrow}{n\uparrow + n\downarrow}$	7.4×10^{-4}	2.9×10^{-3}	5.0×10^{-2}

$g = 2.00$; $\beta = 0.927 \times 10^{-20}$ erg/gauss; $H = 3300$ gauss

sample the resonance condition is not satisfied at one unique applied field, but over a range of values. This is because a range of local magnetic fields is contributed by the sample itself. These add vectorially to H_0 to produce the effective resonance field at a particular electron (see below). In the simplest cases the net effect is to produce a resonance curve as shown in Fig. 2.

As the resonance condition is traversed, energy is absorbed from the microwave field, causing an electrical unbalance in the spectrometer. This unbalance is proportional to the amplitude of the absorption curve (Fig. 2) for a particular value of H_0 . Recording the unbalance of the spectrometer as a function of H_0 will thus yield the absorption curve.

The sensitivity of the spectrometer can be considerably enhanced by causing the resonance absorption to unbalance the spectrometer sinusoidally at a frequency ν_m . One then looks specifically at the spectrometer unbalance occurring at frequency ν_m , any other frequency variation in unbalance being ignored. This scheme is accomplished, usually, by modulating H_0 at frequency ν_m as H_0 is also being slowly swept through the resonance condition (Fig. 2). The unbalance that is recorded using this scheme is not proportional to the height of the absorption curve at the mean value of H_0 at a given point, but is proportional to the difference in height of the absorption curve between the extremes of the modulation envelope. The resultant sinusoidal unbalance is designated 'signal' in Fig. 2.

This, then, is the signal leaving the detector (Fig. 3). Its magnitude is proportional to the first derivative of the absorption curve.

In Fig. 3 the lock-in amplifier analyzes the detected and amplified signal for only those components varying at frequency ν_m . The magnitude of these components is recorded, and yields the first derivative of the absorption curve. This is the curve commonly observed in published work.

The integrated area under the absorption curve (the second integral of the derivative curve) is proportional to the number of magnetic species absorbing microwave power. For a voltage-sensitive microwave detector and Lorentzian line

$$\text{Area} \propto H_1 Q_L \chi_0 \left(1 + \frac{1}{4} \gamma^2 H_1^2 T_1 T_2\right)^{1/2}$$

where H_1 is the magnitude of the microwave magnetic field at the site of the sample, Q_L is the quality factor for the microwave cavity in place in the spectrometer, $\gamma = e\hbar/H_0$, T_1 is the spin lattice relaxation time (discussed below) and, for our purposes, T_2 defines the width of the absorption line (see the Appendix for a brief discussion of detector types, line shape functions, Q_L and T_2).

$\chi_0 = N_0 g^2 \beta^2 S(S+1)/3kT$ is the static magnetic susceptibility. Here N_0 is the number of spins per unit volume and T is the absolute temperature.

The constant of proportionality between area and N_0 may be evaluated in terms of the gains, power, time constant, etc., of

the spectrometer. It is easier and more accurate, however, to relate the area under the curve of an unknown to the area under the curve of a sample with a known number of spins.

In view of the form of the above factor, relating area to H_0 , several facts should be observed. (1) The same microwave power level should be applied to both samples, and this power level should be such that both spin populations remain in thermal equilibrium with the lattice ($\frac{1}{T} \gamma^2 H_1^2 T_1 T_2 \ll 1$). (2) The two samples should effect the electrical properties of the microwave cavity in the same way (giving the same Q_L). (3) Most microwave spectrometers obtain the necessary sensitivity by modulating H_0 and employing a phase sensitive detection system (Fig. 3). This modulation scheme broadens the recorded absorption line. If a narrow line is compared with a wide one and the same modulation amplitude is used on both, the narrow line will be relatively more broadened than the wide one.

A double cavity, first used by Kohnlein and Muller (1961), automatically takes points (1) and (2) into consideration. This cavity, illustrated in Fig. 4, allows the simultaneous placement of both samples on equivalent planes of the stationary microwave pattern of the cavity. Overlapping resonance lines are separated by using steel shims or Helmholtz coils to provide an auxiliary field at one sample site. We have used this type of cavity to advantage with samples whose dielectric loss properties vary greatly.

In some situations one wants to know the spin concentration only as a function of time or as a function of some other external variable. Then it is necessary only to follow some resonance para-

meter which is proportional to the area under the curve. Assuming the line shape and width do not change, the amplitude of the resonance is such a parameter.

D. Line Shapes.

1. Resolved Structure. A second measurable parameter is the structure of the resonant absorption. This is capable of providing quite detailed information about the environment of the observed magnetic species. Obviously, this is the sort of thing an experimenter using biological systems would delight in. Unfortunately, it is seldom observed in such samples.

The principal interaction which will produce structure in a resonance absorption is called the hyperfine or 'contact' interaction. The name results from the fact that the electronic wave function must be in 'contact' with the nucleus (i.e. the wave function must have s character) or the interaction is zero. The energy of this interaction may be written

$$H_i = A \cdot \underset{\sim}{I} \cdot \underset{\sim}{S} = A m_I m_S$$

where A is a constant representing the strength of the interaction and I and S are the spin quantum numbers of the nuclear and electronic systems, respectively. The last form of the equation results from the fact that both spin moments are quantized along the same axis, $[m_I = I, I-1, \dots, -(I-1), -I]$. This interaction is isotropic in space. Its effect is to split each electronic energy level into $2I + 1$ levels. Hence, the resonance absorption is split into $2I + 1$ equally spaced, equally intense lines.

Several cases may be distinguished.

(a) The electronic wave function is confined to one atom and is interacting with the nucleus of that atom. An example of this case appears in Fig. 5. The state of the 3d electrons contains some s character so that the Mn^{55} nucleus is seen. $I_{\text{Mn}^{55}} = 5/2$ so that a 6-line spectrum results.

(b) The electronic wave function is delocalized so that the interaction is equally distributed among several nuclei. In this case $I_t = \sum_i I_i$ and $2I_t + 1$ equally spaced lines result. However, now a statistical effect enters. A given value of $(m_I)_t$ may possibly be achieved with several different nuclear configurations. For example, the states with $(m_I)_t = \pm I_t$ can be achieved in only one way (all nuclear spins parallel), while the states with $(m_I)_t \approx 0$ can be made in several ways. The probability of these states (and thus the line intensities) follows the binomial coefficients. This type of behavior is shown for a series of halogenated semiquinones in Fig. 6.

In general, when the electron interacts with several different types of nuclei (the same element but in nonequivalent molecular sites, or different elements in otherwise equivalent molecular sites as in Fig. 6, or a combination of both) each type will contribute its own splitting to the resonance pattern. Presumably in Fig. 6 the splitting produced by the interaction with the chlorine is too small to be observed.

(c) The electron is coupled primarily to one nucleus, but is delocalized enough to interact with neighboring nuclei: a combination of (1) and (2). An example of this situation is found in copper etioporphyrin-II (Roberts and Koski, 1960). The Cu^{++} electrons are coupled most strongly to the Cu^{63} nucleus ($I = 3/2$). This coupling produces a basic four line spectrum. The Cu^{++} electrons interact also, but to a lesser extent, with the four neighboring N^{14} nuclei of the porphyrin ring. This interaction is equally distributed among the nitrogens, and splits each of the four lines due to Cu^{63} into nine (i.e. $2I_t + 1 = 9$). Effects, such as incomplete rotational averaging in solution, prevent the complete resolution of the spectrum.

There are three nuclei of wide biological occurrence that have no nuclear moments and will thus not produce this type of structure. These are listed in Table II with several other nuclei from which splitting might be expected.

As mentioned above, resonance lines with well resolved structures are seldom seen in samples of biological materials. We shall next inquire as to why structures are sometimes unresolved, and what other parameters characteristic of such lines can be measured.

2. No Resolved Structure. There are several mechanisms which might operate singly or in combination to obliterate structures. In extreme cases the entire line might become unobservable.

Table II

Nuclei Capable of (Having Magnetic Moments) - and Incapable of
(Lacking Magnetic Moments) Producing Structure in EPR Absorption

STRUCTURE PRODUCING NUCLEI			NUCLEI WITHOUT MAGNETIC MOMENTS		
Isotope	Spin	% Abundance	Isotope	Spin	% Abundance
H ¹	1/2	99.9	C ¹²	0	98.9
N ¹⁴	1	99.6	O ¹⁶	0	99.8
P ³¹	1/2	100	S ³²	0	95.1
Cl ³⁵	3/2	75.4			
Mn ⁵⁵	5/2	100			
Cu ⁶³	3/2	69.1			

a. Dipole-Dipole Broadening. The effect could be electron-electron (between two unpaired electrons on the same or neighboring molecules) or electron-nuclear (with nuclei on the same molecule). It results from the fact that the electrons and nuclei involved have magnetic moments associated with them. The field of a magnetic moment is anisotropic in space and can be averaged out by the tumbling action of a molecule in solution. In amorphous or multicrystalline solids a broadening results. The order of magnitude of the effect is approximated by the expression $h \approx \mu \gamma^{-3}$, where h is the local field produced at distance γ from a spin with magnetic moment μ . For an electron $h \approx 80$ gauss when $\gamma = 5 \text{ \AA}$. If this type of broadening is a problem, magnetic dilution of the sample (increasing the mean unpaired spin separation) is an obvious solution.

The line shape resulting from this type of broadening is approximately Gaussian. The resonance may be further classified as homogeneous; that is, every magnetic species being observed is equivalent. A spin found resonating in the low field wing of the resonance line may at a slightly later time be found resonating in the high field wing due to a change in its local environment.

b. Inhomogeneous Broadening. In distinction to the definition just given for a homogeneous line, a spin found resonating in the low field wing of an inhomogeneous line will always be found in

that wing because its local environment will never change sufficiently (in the time of the experiment) to move it to any other section of the resonance. This type of broadening can be brought about, of course, by putting the sample in an inhomogeneous external magnetic field where the variation in applied field is large compared to the local fields found in the sample. An entirely analogous situation may arise inside some samples. In these cases the conditions inside the sample are such that the observed resonance is only the envelope of a large number of overlapping narrower lines. This is depicted on the left side of Fig. 11. The classic case of this kind is F centers in alkali-halide crystals (halogen atom vacancies occupied by a single electron). Here, there is a Gaussian distribution of types of magnetic sites. In the other extreme, an unresolved 5 or 7 line structure displays some of the characteristics of an inhomogeneous system.

Fig. 7 shows the idealized behavior of homogeneous and inhomogeneous systems as a function of microwave power. At very low levels, the increase in signal amplitude is approximately linear with power. In this region thermal processes are fast compared with the induced processes, and thermal equilibrium is maintained. As the microwave power is further increased, the induced transitions begin to catch up with the thermal relaxation processes. Saturation begins to set in as the spin system departs from thermal equilibrium; the energy levels are becoming more and

more equally populated. The behavior of the signal amplitude of a homogeneous line is shown in Fig. 7. In addition, the line width of the homogeneous system increases as the microwave power is increased. For example, the width of an homogeneous Lorentzian line (between points of maximum slope) increases as $T_2^{-1}(1 + \frac{1}{4} \gamma^2 H_1^2 T_1 T_2)^{1/2}$. The saturation behavior of an inhomogeneous system is distinctly different. Although each narrow line making up the envelope follows the homogeneous behavior just described, the envelope as a whole does not; the broadening of any one component line is small compared to the width of the envelope; each individual component saturates in approximately the same way so the shape of the envelope does not change. Thus, under saturating conditions this resonance does not broaden, and its amplitude becomes independent of microwave power (Portis, 1953).

c. Exchange Narrowing. This may be thought of as resulting from the actual physical exchange of unpaired electrons among different magnetic environments in the sample. As the exchange becomes rapid, the electron will see an effective magnetic field which is some average of the local fields of the various sites. Because of the averaging effect resolved structure will collapse, with a single narrowed line resulting from sufficiently rapid exchange. The single line tends toward Lorentzian in shape.

Two cases will be mentioned. (1) The unpaired electron is exchanging with other unpaired electrons. After each exchange, a particular electron is still unpaired. The different local environments then result from different nuclear configurations

(the nuclear configuration is 'constant' as compared with the electron relaxation times). (2) A particular unpaired electron becomes paired after an exchange. This case is well illustrated in Fig. 8. In the top picture, 1 in 50 fluoranils has reacted to become a semiquinone radical (at 10^{-4} M concentration), in the middle picture only 1 in 1000 fluoranils has become a radical, while at the bottom only 1 in 9000 has reacted to become a semiquinone radical. The effect of exchange between the fluoranil semiquinone radical and the unreacted fluoranil is clear.

d. g-Value Anisotropy. As noted above, the factor g appearing in the resonance equation represents the effective size of the magnetic moment being acted upon by the magnetic field. In crystal structures of less than cubic symmetry, the value of g can vary as the direction of the magnetic field is varied with respect to the crystal. Why this is so will be mentioned briefly below. The point we wish to make here is that in amorphous or polycrystalline materials an anisotropic g -value can produce quite broad asymmetric absorptions.

As an example of such an effect we can take the resonance of the copper in the protein complex ceruloplasmin (Fig. 9). The extremes in g -value are probably associated with the asymmetry of the molecular field around the copper. In a polycrystalline material there will be contributions from all g -values between extremes. Assuming random orientation in space and tetragonal symmetry (Malmstrom and Vanngard, 1960) the probability of H being parallel to the symmetry plane is twice that of its being perpen-

dicular to this plane. The parallel configurations will therefore contribute more to the resonance absorption and it becomes asymmetric. The spectrum resembles that of a frozen solution in the copper-histidine complex.

This broadening mechanism differs from the others discussed in that its effect is directly proportional to the applied field. Working in two different applied fields will distinguish this type of asymmetry from other possibilities.

e. Lifetime Broadening (Relaxation Broadening). As the lifetime, T_1 , of a spin state becomes very short, the energy of that state should, according to the uncertainty principle, become correspondingly uncertain. In the extreme of very short T_1 's ($\sim 10^{-11}$ seconds) the resonance may go unobserved because of its width. The remedy in this situation is to lengthen T_1 , usually by lowering the temperature. Although this effect can be important for conduction electrons, and for transition metal ions under certain circumstances, it is not generally important for free radicals.

E. g-Values.

Whether or not structure is resolved one can measure the g-value for the resonance. Experimentally this requires measuring, simultaneously, the strength of the magnetic field at which resonance occurs and the frequency of the microwave source.

The value of g differs from the 'free spin' value (2.0023) because the electronic state has some degree of orbital angular momentum associated with it. The electron, as a magnetic moment, interacts with the magnetic field produced by the electron, as an electric charge, moving in an orbit. This interaction is called the spin-orbit interaction, and it is characterized by the relation $\tilde{H}_{so} = \lambda \frac{\mathbf{L} \cdot \mathbf{S}}{\hbar^2}$ where L is the orbital-angular momentum quantum number, and λ is the spin-orbit coupling constant. This perturbation mixes states of higher energy and perhaps different symmetry properties with the ground state. When the Zeeman energy levels are calculated using the new (admixed) wave functions it is found that their separation has been altered by ^{the} perturbation. Also, their separation may be anisotropic with respect to the magnetic field direction because the perturbation has mixed in states of different symmetry. The effect, from the point of view of the resonance experiment, is to shift the g -value. The magnitude of the shift in g -value (from 2.0023) may be roughly approximated as $|\Delta g| \approx \frac{\lambda}{\Delta}$ where Δ is the energy separation between the ground and the first excited state.

$|\Delta g|$ is very small for organic free radicals. Blois et al. (1961), using sophisticated methods, have been able to establish some correlation between g -value and molecular structure or chemical substituents on a given structure. Some of their results are shown in Fig. 10. Most laboratories are not equipped for such refined measurements.

Values of $|\Delta g|$ as high as 2 or 4 have been observed for transition metal ions (Ingram and Bennett, 1955, Bennett et al., 1957, Gibson and Ingram, 1957). For a particular ion $|\Delta g|$ will depend very strongly on the strength of the molecular or crystal fields, and on the symmetry of the site.

g -Values as aids to identification can be useful if they are supported with independent data. This data could be in the form of spectrophotometric observations, chemical analyses, etc.

F. Thermal Relaxation.

The concept of thermal relaxation processes has been mentioned several times. Any process which changes magnetic to thermal lattice energy is such a process. A time characteristic of such a process can be defined in the following way. A spin system with a unique temperature T_s is not in thermal equilibrium with the lattice at temperature T_L ($< T_s$). At a certain time the perturbation keeping the ^{spin} system at T_s is removed. The spin system then returns exponentially to thermal equilibrium (i.e., to the lattice temperature) according to the expression $\exp(-t/T_1)$. T_1 is the spin-lattice relaxation time. This time is characteristic of the environment of the spin system, and will limit its rate of absorption of microwave power.

T_1 may be measured by continuous or transient methods. The amplitude of the resonance signal as a function of applied microwave power can be determined. As noted in Fig. 7, the shape of this curve is dependent upon whether the spin system is homogeneous or

inhomogeneous. But given the type of system, the shape of the curve is determined by the magnitude of the product $H_1^2 T_1 T_2$. T_1 can thus be determined if T_2 and H_1 are known (Portis, 1955). The transient method makes more direct use of the above definition of T_1 . In this method the spectrometer is adjusted so that the amplitude of the resonance line is continuously observed. A short, intense burst of microwave power drives the system from equilibrium. The return to equilibrium is followed. See, for example, Liefson and Jeffries (1961).

G. Miscellaneous Measurements.

Even though a resonance line displays no resolved structure, there is one technique available for deciding which nuclei are coupled to the resonating electrons. The line must be inhomogeneously broadened in the sense that it is made up of unresolved components, and the relaxation times must be long. The technique, called 'electron-nuclear double resonance' (ENDOR), is due to Feher (1959) and is depicted in Fig. 11 for $S = 1/2$, $I = 1/2$.

As in the transient determination of T_1 , the spectrometer is adjusted so that the electron spin resonance amplitude is continuously observed. This resonance is partially saturated. Then, while observing the amplitude of the electron resonance, a second high frequency magnetic field is applied to the sample. This frequency is in the range of nuclear transitions (0 to 100 Mc/s). Assume that while this frequency is being swept, one passes through the resonance of a nucleus coupled to the electron via the contact interaction, $A I \cdot S$. Inducing the nuclear transi-

tions changes the population of the corresponding electronic levels. The latter change is reflected in the resonance amplitude being observed. ENDOR effects will be observed when $h\nu_I = \frac{1}{2} A \pm \gamma_I H_0$ ($A \gg \gamma_I H_0$). The $\pm$ sign comes from the fact that the top and bottom electronic levels are not split quite equally by the nuclei. This, one is able to solve for both A and γ_I . γ_I identifies the nucleus since in the absence of the unpaired electron the nucleus would resonant at frequency $h\nu_I = \gamma_I H_0$.

An example of the ENDOR technique is shown in Fig. 12 for phosphorus impurities in silicon. γ_I for P^{31} at about 3000 gauss is ≈ 6 Mc/s. Approximately twice this frequency is indeed the separation of the resonance peaks in Fig. 12a. Thus, the extra electrons added to the silicon lattice by the phosphorus impurities are most strongly coupled to the phosphorus nuclei themselves (highest value of the coupling constant A), but also interact in a complicated way with near Si^{29} and with many distant Si^{29} nuclei ($A \approx 0$). None of this information can be obtained from the EPR line itself, a broad symmetric line as on the left in Fig. 11.

Only one application of this type of experiment has been made to organic systems (Cole et al., 1961), but it should be generally applicable to inhomogeneously broadened lines.

Long-lived triplet states ($S = 1$) have been observed using EPR techniques. These states were first observed (Hutchinson and Mangum, 1958) using magnetically dilute, single crystals of naphthalene in durene. The transitions observed in the single crystal are depicted by the dashed lines in Fig. 13. Recently van der Waals and

de Groot (1959, 1960) have discovered that the $\Delta m_s = \pm 2$ triplet transitions can be observed in glasses containing the excited molecules. This may make this technique applicable to biological samples in certain instances, although it should be noted that the probability of this transition is an order of magnitude less than that for $\Delta m_s = \pm 1$. The $\Delta m_s = \pm 2$ transitions observed in naphthalene-durene crystals are also shown in Fig. 13 as well as the anisotropy of the energy level system as H_0 is varied with respect to the naphthalene molecule. The $\Delta m_s = \pm 2$, $g = 4$ resonances observed in naphthalene-containing glasses are shown in Fig. 14. The $\Delta m_s = \pm 2$ transitions are allowed for H_1 , both parallel and perpendicular to H_0 .

H. Control of External Factors.

Photosynthetic materials seem to combine most of the features that would seem to obliterate the information carried in an EPR spectrum. Thus, it is not surprising that the measurable parameters of the resonances found in these materials yield, in and of themselves, little information concerning photosynthesis.

The obvious thing to do, of course, is to vary the physico-chemical and 'biological' environments of the sample material while making observations on these resonance parameters. For example, whether the observed radicals are physical or chemical intermediates can be determined from the temperature dependence of the resonance.

At sufficiently low temperatures all chemical reactivity should cease. One might also include as parameters in a temperature study the rise and decay times of the resonance as the light is turned on and off. These can be followed by adjusting the spectrometer to resonance conditions and observing the response to the changing light conditions. If the rise and decay times are faster than the response time of the spectrometer then dispersion techniques, in which the incident light intensity is amplitude modulated at successively higher frequencies (Melville and Burnett, 1953), can be employed to advantage. From such kinetic studies should come information concerning the number of different types of unpaired electrons (biological pools) contributing to the observed resonance. The ambient atmosphere may be important in stabilizing the observed radicals. Pigments important in the production of unpaired electrons can be determined from an action spectrum for unpaired spin production.

Concerning the 'biological' environments: Working with mutant types of photosynthetic species offers many advantages. The correlation of parameters associated with unpaired spin production with quantities long considered a measure of photosynthesis, e.g. oxygen evolution, quantum yield, carbon dioxide fixation, etc. should prove enlightening. Simplified systems, obtained from selective extractions, fragmentations, or by using selective inhibitors, should provide useful information. In the extreme of simplification model systems might be employed.

Most of the approaches just listed have been employed to a greater or lesser extent. In the following section we shall be concerned with several of the experiments in which they are involved.

III. EXPERIMENTAL RESULTS

A. Types of Systems that Have Been Studied.

It might have been noted that most of the functional dependences suggested for determination in the preceding paragraph were oriented more toward identifying the observed radical species than toward making remarks concerning photosynthesis. Indeed, to date this has been the case. Investigators have had to use their knowledge of photosynthesis to try to establish the position of the unpaired electron spins in the photosynthetic cycle rather than the reverse, i.e. making new remarks concerning photosynthesis on the basis of the EPR observations. Still, some statements concerning photosynthesis are emerging.

The first EPR observations on photo-induced unpaired electrons in photosynthetic materials were made on relatively complete systems (Commoner et al., 1956; Sogo et al., 1957). In the earliest work the physiological conditions of the samples was poor; in most cases they were dried. The spectrum observed consisted of a single line approximately 10 to 15 gauss wide, with g-value in the region expected for most organic free radicals. There was no evidence of structure. In short, these resonances yielded a niggardly amount of information.

Since that time the development has been in two directions; one, toward working with complete photosynthetic organisms in relatively good physiological condition, and the other toward simplifying the system. Both approaches are important. It is important to know that the pools of unpaired electrons exist in real functioning photosynthetic systems. On the other hand, it has usually been the case that if one is to understand a complicated situation one must start by first understanding simplified approximations to that situation. In some senses the 'simplified' photosynthetic systems used to date are still much more complicated than one would wish.

The types of photosynthetic materials which have been employed in the various EPR investigations are as follows:

1. Extracts from photosynthetic systems.
2. 'Crystals' composed of pigment molecules. The crystalline property of long range order is probably not too well met by these samples.
3. Very small fragments of photosynthetic systems.
4. Large fragments of photosynthetic systems.
5. Whole or complete photosynthetic systems.

B. Results.

Under the correct conditions photo-induced EPR signals can be observed in each of these classes of materials. Where g-values have been measured they are not (at present) significantly different. The line widths vary from class to class, and there are some

marked differences in the overall shape of the observed resonances. Perhaps the largest variation is found in the kinetics of the rise and decay of the photo-signal. The times involved vary from milliseconds to hours. We shall present the results of the resonance experiments in the order of the class distinctions just made.

1. Extracts. Anderson (1960) has observed photo-induced EPR signals in acetone extracts of spinach chloroplasts which contain essentially all of the pigment molecules in the chloroplast. The extract was evaporated to dryness and tested in vacuo for the presence of a photo-induced EPR signal. No signal, either in the light or in the dark, was observed. However, when small amounts of water vapor were admitted to the system, narrow (≈ 4 gauss wide, $g = 2.00$) photo-induced signals were observed. Their rise and decay times were on the order of seconds and minutes, respectively. The measured rates seem to be dependent on the presence of as yet unknown components in the extract. The equilibrium amplitude of the induced photo signal is a function of the water vapor pressure (Fig. 15).

2. Crystals. A dependence of the photo-induced EPR signal on the presence of water vapor has also been observed by Holmogorov and Terenin (1961) in a recent study on 'crystalline' chlorophyll (a + b). In their experiment the sample space was first evacuated, then water vapor was admitted. They observed two overlapping resonance lines (Fig. 16). One ($g = 2.0035$, half-width = 11 gauss; 'a' in Fig. 16) is always present. The second

($g = 2.0030$, half-width = 7 gauss) is light-induced in the presence of water vapor. The presence of p-benzoquinone (at 2×10^{-3} mm Hg pressure) was also effective in stabilizing^{the} light-induced free radical. The water affects much faster rise and decay kinetics than does the p-benzoquinone.

The role of the water is not entirely clear. By analogy with the effect of the benzoquinone it would appear that it might act as an electron acceptor, a common role for benzoquinone. However, it is difficult to visualize water in this role. Perhaps it is worth noting that the presence of water vapor aids in the initial formation of the chlorophyll crystals (Jacobs and Holt, 1954). Thus, water-induced structural modifications may be responsible for the formation and stabilization of the light-induced unpaired electrons.

3. Very Small Fragments. EPR experiments have been performed on systems composed of very small fragments obtained from both the aerobic and anaerobic growing photosynthetic systems (Androes et al., 1962). These fragments are obtained in both cases by rupturing the cell structure in intense sonic fields, and then using centrifugation techniques to obtain the desired particle size fraction.

The particles obtained from the purple bacterium Rhodospirillum rubrum are called chromatophores (Frenkel and Hickman, 1959). They are spherical, have diameters of approximately $200 \text{ \AA}$, and contain all of the light absorbing pigments of the whole bacterium. There

are two indications that the local pigment environment is left essentially unaltered by the method of preparation. The particles will perform cyclic photophosphorylation reactions, and the optical absorption spectrum is left unaltered by the mechanical disruption of the cell.

The sample of chromatophores is placed in an appropriately buffered aqueous medium (Frenkel and Hickman, 1959), and inserted into the EPR cavity. (See the Appendix for the method of inserting water into the microwave field.) The observed photo-induced resonance absorptions seems to be the same in g-value, width and shape as those observed in the whole bacterium (see Fig. 19). The rise and decay times of the signals are somewhat altered as might be expected when the terminations of the energy transfer system have been removed. In particular, at room temperature the decay scheme consists of a fast and a slow component with time constants of about 2 seconds and 20 seconds, respectively. The observed decay in the whole cell in aqueous suspension is instrument limited, That is, it is completed in less than one second, the time constant of the spectrometer. When the chromatophore sample is prepared as a dried film on a slide, the rise and decay times eventually become instrument limited as the temperature is reduced to -150°C . This is also true of dried films of the whole bacteria (see Fig. 20 and the discussion connected with it).

Where the spin concentration is photo-induced it is of some interest to determine the action spectrum of the equilibrium spin

concentration as a function of the wavelength of the incident light. In previous attempts to obtain such a spectrum (Sogo et al., 1961) the sample has been infinitely thick compared to the distance the active light penetrates into it. Self-absorption effects were pronounced, shifting the maximum in the action spectrum to the long wavelength side of the chlorophyll absorption maximum. The chromatophore samples are nonscattering, and the sample used here was only slightly colored to the eye (the O.D. recorded in Fig. 17 represents a sample of the same thickness, but a factor of 3.3 greater in concentration than those used in the spin determination). As seen in Fig. 17, the action spectrum now peaks at the absorption maximum of the bacteriochlorophyll. This spectrum still suffers from selfabsorption effects, but is nearer the true situation. It is evident that the bacteriochlorophyll is the principal pigment responsible for spin production.

The other type of small fragment can be obtained from spinach leaves (Park and Pon, 1961). The preparative procedure gives one fragments of the lamellar structure of the chloroplast. The smallest fragments are oblate spheres with principal dimensions of 100 and 200 Å. Larger fragments in the preparation appear to be made up of agglomerations, still in sheet form, of the smallest particles. These particles, or the agglomerations of them, will perform the Hill reaction, and when combined with the water-soluble protein leached out in the preparative procedure they will fix carbon dioxide. Again, the optical absorption spectrum

is unaltered by the preparative procedure. These particles have been called quantasomes (Park, 1962). We have made the suspending solution 10% in methanol (Milner et al., 1950) to enhance the chemical stability of the quantasome preparation during the long periods of illumination to which it was subjected.

It is interesting to note that with a modulation amplitude of only one gauss and a signal-to-noise ratio of about 20, only one resonance line has been observed in these particles. This resonance line is approximately 10 gauss wide, has an asymmetry similar to that shown in Fig. 19 for R. rubrum, and has relatively fast kinetics. This contrasts with the observations on whole chloroplasts. In the whole chloroplast two overlapping resonance lines appear (see the following section). The resonance occurring in the quantasomes corresponds to the narrow resonance observed in the whole chloroplasts.

At the time of writing, the action spectrum for the production of unpaired spins in the quantasomes has not been completely determined. When the quantasomes are in aqueous suspension at room temperature the kinetics of the photo-induced signal are similar to those for the signals induced in the chromatophores in equivalent circumstances. However, when the quantasomes are subjected to certain conditions the decay time for the photo-induced signal becomes very long (at least hours). These conditions are (1) when the quantasomes are thoroughly dried, and (2) when they remain in water, but their temperature is reduced to less than 0°C.

Changing the ambient atmosphere from air to nitrogen does not effect the results. Thus, it seems that in these particles diffusing water molecules, or perhaps molecular species carried by the water, must interact with the site of an unpaired electron to bring about its return to the diamagnetic state.

An attempt was made to substitute deuterons for all of the exchangeable protons in both the chromatophores and the quantasomes. This was done by substituting D_2O for H_2O in the preparative procedure. No effect on the resonance line shape was observed. Since a relatively complete exchange of water is probable, the observed radical species either has no protons in its environment or the protons in its environment are nonexchangeable. Replacing H by D in the immediate environment of an unpaired electron should narrow the resulting resonance line. (In this connection see also Sec. III.B.5).

4. Large Fragments. Because of the extremely small dimensions of the particles discussed in the previous section we list the chloroplast as a 'large' fragment of a photosynthetic system. This fragment has been studied widely, and its photosynthetic abilities and characteristics are well known. In the EPR experiment it behaves, when in aqueous suspension, essentially as do the whole green algae, so it will be discussed in connection with them (Sec. III.B.5)

Much larger leaf fragments have been employed in EPR experiments. In particular, eucalyptus leaves give small light-induced signals (Sogo et al., 1957). These signals rise rapidly when the leaf is illuminated (< one second), but remain for hours after the light has been turned off.

Bubnov et al. (1960) report observations on photo-induced EPR in the leaves of the cereals Triticum vulgare, Hordeum vulgare and Avena sativa, and on the variegated leaves of the decorative plant Santheria. In the cereal leaves a doublet (splitting = 1.8 gauss, $g = 2.004$) with fast rise and decay times was observed. In Santheria a broad, complex spectrum was observed. The exact form of this spectrum was different in the chlorophyll and the non-chlorophyll containing parts of the leaves. These observations have not been independently corroborated. Using model photo-chemical reactions as a basis for reasoning, this group has attributed the photo-induced doublet in the cereal leaves to an oxidized form of ascorbic acid.

5. Whole Systems. Systems of this type have been studied while essentially dry, and, more recently, while in aqueous suspension (i.e. in relatively good physiological condition).

The purple bacterium Rhodospseudomonas spheroides also exists in a blue-green mutant form in which the carotenoid pigments are absent (Griffiths et al., 1955). The EPR spectra of the wild and this mutant form of the bacterium were compared in the dry state. Few differences were noted. The mutant is subject to photo-killing when exposed to both light and oxygen (Dworkin, 1958). In the experiments performed the mutant and the wild type both reacted to light-plus-oxygen in a parallel way. The photo-induced resonance amplitude was monitored as a function of time after exposure of the sample, in the form of a thin film, to oxygen. Both resonance amplitudes increased to an asymptotic value of approximately

three times their initial values after about two hours exposure to oxygen in the light. Thus, oxygen appears to stabilize the radicals formed in these bacteria. (In this connection see also Section IV below.)

One difference in behavior which was noted was the way in which the two resonances power saturated. This is shown in Fig. 18. The wild type seems to saturate more like an inhomogeneously broadened system than does the mutant, and to have a somewhat shorter relaxation time. This would suggest that the mutant has fewer varieties of unpaired electrons participating in the production of this signal.

Dried films are a natural choice for the form of the sample if the material is to be studied below zero degrees centigrade. Such studies have been carried out on whole Rhodospirillum rubrum (Fig. 19). The principal points to note are that photo-induced signals can be produced at these low temperatures, that the decay scheme contains multiple decay times, and that the shorter decay times predominate as the temperature is reduced.

This behavior leads one to believe that at a given temperature one is observing free radicals in several biological pools. If this is so, the observed resonance should be of the inhomogeneously broadened type, i.e. it should be the envelope of several narrower overlapping resonance lines. There is additional evidence that this is so. The linewidth does not increase with applied microwave power (one criterion), but the saturation is not ideally in-

homogeneous as in Fig. 7. Rather, its saturation behavior is similar to that observed in R. spheroides (upper curve in Fig. 18).

The derivative of the photo-induced resonance which is observed in R. rubrum is shown in Fig. 20. It is seen that, disregarding the slight asymmetry that is observed, the curve is more nearly Gaussian than Lorentzian. Note that with the modulation amplitude at only 0.6 gauss there is no evidence of resolved structure.

One resonance with easily resolved structure which is observed in chloroplasts and green algae is that of ionic, or cubically bound, Mn^{++} . It is not observed in purple bacteria. This resonance does not seem to be photosensitive. As shown in Fig. 5 the structure consists of 6 lines. The Mn^{++} concentration in the samples is as high as 10^{-6} to 10^{-5} M. ¶ In contrast to the fast low temperature kinetics just cited for R. rubrum, the decay of the photo-induced signal in chloroplasts and algae becomes very long as the temperature is reduced (Sogo et al., 1959). The slowing of the kinetics for these materials is not abrupt at the freezing point of water as it appears to be in the quanta-some particles mentioned above.

In green algae and chloroplasts in aqueous suspension two overlapping photosensitive resonance lines can be resolved with a modulation amplitude on the order of one gauss (Commoner et al., 1957; Commoner, 1961; Allen, et al., 1961; and Weaver, 1961). The resolved structure is made up of a narrower line (~ 10 gauss wide)

with $g = 2.002$, and a broader line (on the order of 20 gauss wide) with $g = 2.005$. The narrow line is fast rising and decaying (a decay time of 50 to 60 milliseconds is reported by Allen et al., 1961) while the broad line takes many minutes to decay. Commoner et al. (1957) have partially resolved this broad resonance into 3 lines each separated by 6 gauss.

Some insight into the origins of these two signals has been obtained through the use of mutants. Allen et al. (1961) have observed the two overlapping resonance lines in several green algae. Some of their results for Chlorella pyrenoidosa are reproduced in Fig. 21. The dependence of the relative signal amplitude on the wavelength of the light incident on the sample made it probable that the two resonance lines were resulting from the absorption of light by two different pigment systems. The pigment system absorbing the light which was producing the broader resonance was implicated with chlorophyll b by using a Chlorella mutant in which this pigment was absent. In this latter case the broad resonance failed to appear.

The magnetic environment partially responsible for the width of both the resonances observed in green algae has been determined. Commoner (1961) reports EPR observations on C. pyrenoidosa cultured in 99.9% D_2O growth medium by Chorney et al. (1960). Both resonances observed in C. pyrenoidosa were significantly narrowed by substitution of D for H. This narrowing results from the much smaller magnetic moment of the deuteron.

It has been shown that few long-lived radicals exist in etiolated as compared with fully greened leaves (Commoner et al., 1954). We are attempting to inquire further into the relationship between the unpaired spin concentration and the chlorophyll content of the sample material. This study is being carried out on a yellow (chlorophyll-less) mutant of Chlamydomonas reinhardtii supplied to us by Dr. Ruth Sager.

In this experiment 6 flasks of cell cultures were grown in the dark until the total cell volume per flask was sufficient for the needs of the experiment. When this condition was fulfilled, all 6 flasks were exposed to uniform illumination. At this time the total chlorophyll content of the cells was quite low. On being exposed to light, though, the cells start to green, regaining their total chlorophyll compliment in 15 hours. One flask of cells was harvested immediately after exposure to light. The others were harvested at intervals of 3 hours thereafter. The uniform illumination in which the cell cultures were greening (~ 700 foot candles) was approximately 5 to 10 times smaller than the illumination to which they were exposed in the later parts of the experiment. The later parts consisted of several independent measurements as follows. On each cell sample, a chlorophyll content, an oxygen evolution rate, a carbon dioxide fixation rate, and an equilibrium EPR signal amplitude (using white light in each case) were determined. The oxygen evolution and carbon dioxide fixation experiments were performed on very dilute suspensions so that variation in observed rates can be attributed to physiological changes in the cell, and not to the

shielding effects of an optically dense suspension. The EPR experiments were performed on very dense aqueous suspensions so that, excepting possibly the first one or two measurements at zero time and at 3 hours, all of the light is absorbed by the sample. All of these measurements were normalized to the same volume of wet packed cells.

Our preliminary results are plotted in Fig. 22. The EPR amplitude recorded is the composite of two overlapping lines. These lines have been resolved in older cultures, but instrument sensitivity has prevented our resolving them during the greening period. The observed line shape and the concentration ratio of chlorophyll a/chlorophyll b remain constant during the greening. The equilibrium EPR amplitude, the rate of $C^{14}O_2$ fixation and the rate of oxygen evolution are all normalized so that the maximum value observed in each variable is equal to one. These are plotted against chlorophyll (a + b) content, also normalized to one at maximum value. The chlorophyll (a + b) concentration as a function of time is given in Table III.

There are several clear results: (1) A photo-induced EPR signal grows in as the chlorophyll content increases. (2) The relationship between chlorophyll content and EPR amplitude is not linear, the larger part of the signal growing with the last 20% of chlorophyll synthesized. (3) The rate of photosynthesis, as measured by rate of oxygen evolution, and the rate of turnover of the carbon cycle, as measured by the rate of $C^{14}O_2$ fixation, are maximal long before the EPR signal starts its steepest rise.

Table III
Chlorophyll Concentration as a Function of Time in a Greening
Chlamydomonas Mutant

Time (hours)	0	3	6	9	12	15
Relative chlorophyll (a + b) concen- tration	0.05	0.08	0.20	0.80	0.84	1.00 [/]

[/] Corresponds to 2.7 mg chlorophyll (a + b)/ml wet
packed cells

IV. CONCLUSIONS

One sequence in the photosynthetic cycle which at present is among the least understood is that series of steps by which the electromagnetic energy of the absorbed light quantum is converted to chemical energy. In recent years a type of spectrophotometry has been developed which allows one to follow spectral changes which take place shortly after the illumination of a photosynthetic system, presumably the steps of quantum conversion. These techniques have been developed especially by Witt et al. (1961) and to a lesser degree by Kok (1959). Witt et al. have been able to make tentative identification of several compounds which undergo electronic transitions in times as short as 10^{-8} seconds after illumination of the system. Some of these changes are temperature independent.

When photo-induced EPR was first observed in photosynthetic systems it was hoped that a method for following the quantum conversion process was at hand. Response times in the EPR experiment are not nearly as rapid as can be obtained in the spectrophotometric experiment, but upon onset of illumination the resonances appeared as rapidly as the EPR spectrometer could follow them, and a portion of the observed resonance is temperature independent. The EPR approach to quantum conversion will probably prove to be valuable, but several years have elapsed and the sites of the observed radicals have still not been identified. There is, as yet, insufficient evidence to

place them unequivocally in the main pathway of quantum conversion, as distinct from putting them in some less important side reaction. What, then, can be said of the photo-induced EPR signals which are observed?

Chlorophyll is definitely the sensitizing pigment for their formation. This is evident from the absence of the photo-signal in etiolated leaves and chlorophyll-less algae mutants, and their appearance with the subsequent greening of these materials (Sec. III.B.5). Apparently carotenoids, the only other pigment always present in photosynthetic systems, is not involved in a fundamental way (Sec. III.B.5).

Given the presence of chlorophyll, the formation of photo-induced signals, as such, requires little else of the living organism. The structure into which the chlorophyll is incorporated in the chromatophore or quantasome as it exists there is not required (Extracts, Sec. III.B.1); nor is the presence of the pigment molecules associated with chlorophyll in the structure of the photosynthetic unit required (chlorophyll crystals, Sec. III.B.2). It should be remarked, however that the presence of water or possibly some electron acceptor is required in both the experiments just cited. The condition of 'life' is obviously not required. To be sure, these signals differ in many respects from those observed in photosynthetic systems in good physiological condition, but they do suggest that at least some of the radicals contributing to the resonances in 'living' photosynthetic systems might be of a physical nature. That is, their formation should be relatively independent of temperature,

which it would not be if they were the intermediates in some chemical reaction. This is, indeed, the case (Fig. 19). In the purple bacteria it appears that at a given temperature two or more radicals are contributing to the observed resonance (multiple decay times; inhomogeneous line behavior, Sec. III.B.5), and that as the temperature is lowered various routes by which these radicals are utilized become blocked. At the lowest temperatures studied they apparently disappear by reversal of the mechanism by which they were formed.

It should be emphasized in this connection that some of the faster optical density changes observed by Witt et al. (1961) in chloroplasts and algae are temperature independent at least down to -150°C . Others occur, but are irreversible at this temperature. Characteristic optical density changes have also been observed to occur reversibly in the bacterium Chromatium at 77°K by Chance and Nishimura (1960) and in the chromatophores of R. spheroides by Arnold and Clayton (1960) at 1°K . Witt et al. correlate the changes they observe with the oxidation of chlorophyll a and a cytochrome. Chance and Nishimura identify the change they observe with the oxidation of a cytochrome. The unpaired electrons which have been observed in the above systems may be associated with these molecular components.

Of the two resonances observed in green algae and chloroplasts, the narrow one with the faster kinetics seems to be more closely associated with the quantum conversion act. This is shown by the fact that the broad resonance is absent in the quantasomes

although present in the chloroplasts before the final stages of sample preparation. Thus, while this broad signal might result from light absorbed by the auxiliary pigments in these systems (e.g., chlorophyll b, Allen et al., 1961) its position in space seems to lie outside the basic photosynthetic unit. The narrow resonance in aerobic systems seems to correspond to the narrow single resonance observed in anaerobic systems.

The fundamental difference between the aerobic and the anaerobic photosynthetic systems is reflected in the EPR experiment in several ways. Neither the six line spectrum due to Mn^{++} ion nor the second broader resonance with slow kinetics is observed in the anaerobic systems. In addition, the low temperature decay kinetics are markedly different, the photosignals in the aerobic systems being produced irreversibly at sufficiently low temperatures. Finally, there is apparently a complicated dependence of the amplitude of the photo-induced EPR signal in whole anaerobic photosynthetic systems on oxygen concentration, light intensity and time. (See Sec. III.B.5 and below.) This dependence is absent in aerobic photosynthetic systems, but it must be remembered that these systems liberate oxygen during photosynthesis.

We have placed the resonance observed in R. rubrum and the narrow resonance observed in green algae and chloroplasts in what may prove to be the basic photosynthetic units for these two different types of systems. Some of the radical species that can be produced at room temperature can be produced at liquid nitrogen tem-

peratures, and may be correlated with the molecules undergoing electronic transitions at these temperatures. Is there evidence for placing the observed radical species directly in the photosynthetic pathway?

Several experimental cases lead one to conclude that when photosynthesis is limited or inhibited in any way the photo-induced EPR becomes considerably easier to see. If the radicals exist in several biological pools then the resonance will become easier to see when the kinetics of the system are altered in such a way as to make these pool sizes larger. Thus, the photosynthetic systems in the best physiological condition require the highest light intensities to attain a given EPR amplitude. When the systems are dried or fragmented, the kinetics slow down and a given EPR amplitude is obtained with a much lower light intensity. It was remarked in Sec. III.B. 5 that the presence of oxygen enhanced the amplitude of the EPR observed in R. spheroides. This effect is much more marked as well as being considerably more complicated in R. rubrum and R. spheroides when they are in good physiological condition (in aqueous suspension in a closed system) where it is known that traces of oxygen markedly inhibit anaerobic photosynthetic processes (van Niel, 1941; Clayton, 1955). A photo-induced EPR is observed in oxygen-free samples of these bacteria. When small amounts of oxygen are admitted the resonance amplitude is generally enhanced, but the amplitude behaves in a complicated way as a function of light intensity, oxygen concentration and time. This behavior has not, as yet, been completely elucidated.

(It might be remarked here that the resonance observed in the R. rubrum chromatophores displays none of the complicated behavior observed in the whole cells.)

In the study of the greening of the chlorophyll-less mutant of C. reinhardtii, described above, one has a case in which the photo-induced EPR becomes visible only after photosynthesis, as measured, for example, by oxygen evolution, has become limited. In the early stages of greening one might expect the low chlorophyll content to be the factor limiting photosynthesis. This is the case in the early stages of the greening of etiolated barley leaves (Smith, 1954). After a short induction period in which chlorophyll a is synthesized and the oxygen evolution rate is zero, there follows a period in which the rate of oxygen evolution is linear with chlorophyll a content. Following this period, the rate of photosynthesis, again as measured by the rate of oxygen evolution in etiolated leaves, is limited by other factors (Blaauw-Jansen et al., 1950). A state exists in which the rate at which energy is absorbed is greater than the rate at which it can be used in subsequent reactions. The number of unpaired electrons in the biological pools presumably lying between the point of energy absorption and the limiting reaction, is thus enhanced.

These pools of unpaired electrons are certainly quite near the point of energy absorption (they can be produced in the smallest photosynthetic units which have so far been removed from plant materials and purple bacteria, and at temperatures as low as 77°K) and are quite possibly in the path of energy flow into the photosyn-

thetic cycle. The facts are consistent with, but, of course, do not prove, the later assertion. ¶ The statement regarding photosynthesis which emerges from these considerations is that one or more of the steps following the absorption of the light quantum is of a physical nature. That is, it, or they, are essentially independent of temperature. This is no new statement; it was made soon after the original observation of photo-induced EPR in photosynthetic systems, and has been made more recently in connection with the temperature independent changes in absorption spectra. When made in connection with the absorption changes this assertion has some validity because of the additional information available about the optical absorption spectra of the molecular contents of the photosynthetic apparatus. Here, molecular species thought to lie in the photosynthetic pathway have been tentatively identified with the observed changes in absorption. When made in connection with the EPR observations it has been based on the assumption that the observed radical species lie in the photosynthetic pathway. Although the validity of this assumption has been hard to prove, the growing body of information concerning the radicals is consistent with it (~~Andrees and Calvin, 1962~~). A physical description of the quantum conversion process has been given by Calvin (1961).

Before the EPR experiment can be used to make more concrete and far reaching statements about photosynthesis, the sites of the unpaired electrons ought to be identified. This problem might be approached in several ways.

One possible approach is the study of a wide variety of mutant organisms in which specific known molecules were absent or altered. Success in this approach depends on selecting the right mutant.

A complete understanding of the difference between the photo-induced EPR in aerobic and anaerobic photosynthetic systems would aid in the identification of the sites. Additional correlations with the behavior of the observed optical density changes in these systems could well supplement this approach.

An approach more on the physical side is that of electron-nuclear-double resonance. As has been indicated (Sec. III.B.5) the photo-induced resonances display some behavior characteristic of inhomogeneous spin systems. Thus, a double resonance experiment may provide information concerning the radical site. The success of this experiment will depend on the unpaired electrons being coupled isotropically (i.e. through the contact interaction) to some uncommon nucleus in the system. Finding that the unpaired electrons are coupled only to protons will probably not yield a great deal of information about the site.

Some attention has been given to photo-induced EPR in model systems (Tollin et al., 1960; Kearns et al., 1960; Kearns and Calvin, 1961). These systems, whose form is based on the ultrastructure exhibited in electron micrographs of photosynthetic materials, are composed of lamellar layers of electron-donating and electron-accepting molecules. Several parallels with real photosynthetic systems have been found. The models have the ability to separate

positive and negative charge (oxidizing and reducing power) when illuminated. EPR is observed in them. The amplitude of the resonance is photosensitive. That is, upon illumination of the system the amplitude increases or decreases, depending critically upon the electron donating and accepting properties of the molecules involved. The photo-induced paramagnetism is essentially temperature independent. As these systems become better understood and more subtle donor-acceptor combinations are used (e.g. molecules extracted from photosynthetic systems) the conditions under which the unpaired electrons are produced will be so defined as to help identify the sites in the real systems. However, this is certainly a long range project since the sites of the unpaired electrons in the lamellar systems are also, as yet, not known with certainty.

V. APPENDIX

A. Detectors

Two types of detecting elements are used in spectrometers to remove the resonance information from the microwaves incident upon them. One of these is sensitive to the incident microwave power (e.g. bolometers), the other is sensitive to the microwave electric field (e.g. crystal diodes). The first gives a signal proportional to H_1^2 (H_1 is the magnitude of the microwave magnetic field at the site of the sample), the second a signal proportional to H_1 . Thus, the exact shape of the recorded resonance curve and the power saturation curves will depend on the particular type of detector employed.

B. Line Shape Functions

Two resonance line shapes result from the solution of problems concerning nuclear and electronic paramagnetism. These are the Gaussian and the Lorentzian line shapes. The line shape observed in most actual resonance experiments falls someplace between these two forms. They may be defined as follows:

$$\text{Gaussian} \quad g(\omega - \omega_0) = T_2 \pi^{-1} \exp \left[-(\omega - \omega_0)^2 T_2^2 \pi^{-1} \right]$$

$$\text{Lorentzian} \quad g(\omega - \omega_0) = T_2 \pi^{-1} \left[1 + (\omega - \omega_0)^2 T_2^2 \right]^{-1}$$

C. T_2

The parameter T_2 appearing in these expressions is related to the width of the resonance line. If one determines the distance

between points of maximum slope (the peak-to-peak separation in the derivative representation of the absorption line) one finds for

Gaussian $\Delta\nu_{p-p} = (\pi)^{1/2} T_2^{-1}$

Lorentzian $\Delta\nu_{p-p} = 2(\pi)^{-1/2} T_2^{-1}$

As a time characteristic of the resonating system T_2 may be thought of as follows: Magnetic moments precess around the direction of a field H_0 at a frequency given by the resonance condition. If two magnetic moments in the sample are precessing in phase at a given time, then at time T_2 later they will be out of phase, say, by π radians. This results from slightly different precessional frequencies at the different magnetic sites (i.e. different effective values of H_0) and this, of course, fixes the line width.

D. Q

The cavity Q enters as an important parameter in the spectrometer sensitivity. The cavity serves to concentrate the microwave field (i.e. to increase the magnitude of H_1). This it does by establishing a standing microwave pattern in the cavity. There are energy losses in the cavity walls and in the devices by which the cavity is coupled into the spectrometer, as well as in the sample itself. The loaded cavity Q is defined as the ratio of the energy stored to the energy dissipated, and is a measure of how efficiently the sample concentrates H_1 . In general, the greater the efficiency the greater the sensitivity.

E. Inserting Water into Cavity

The energy losses in the sample are usually very much smaller than those in the cavity walls and coupling devices. However, when highly polar substances such as water are inserted into the cavity this situation may change. The dipole moment of the polar substance interacts strongly with the microwave electric field, and energy dissipation is enhanced. This can drastically reduce spectrometer sensitivity. If the polar substance could be placed in a region in the cavity of zero electric field, then the electric interaction would be zero and the sensitivity would remain unimpaired. This can be partially achieved. Reference to Fig. 4 shows that in the plane of maximum H_1 (where we would like to place the sample) the field E_1 is zero. A thin planar cell (≈ 0.25 mm thick) positioned in this plane allows the insertion of water into the cavity with little loss in spectrometer sensitivity.

F. Illuminating the Sample

Light is admitted into the cavity usually in one of two ways. (1) The hole in the cavity wall is at the end of a short section of tubing (of the same dimensions as the hole) which to the microwaves looks like a waveguide beyond cut-off. This terminology refers to the fact that microwaves of a given wavelength, λ_g , will not travel down waveguides which are too small. The limiting dimensions are related to λ_g . The tube, then, through which light is admitted to the cavity is much too small for waveguide propagation,

and the standing wave pattern in the cavity is not seriously altered.

(2) The hole in the wall is composed of a system of narrow slits oriented so as not to interrupt the flow of current in the cavity walls, and each with width ^{much} ~~very~~ smaller than λ_g . In this case, the system of slits looks like a solid wall to the microwaves, but the wavelength of the light is very much smaller than the width of the slits, so it passes easily through.

REFERENCES

Texts

NMR

Abragam, A. (1961). 'The Principles of Nuclear Magnetism', Oxford University Press, London.

Andrew, E. R. (1955). 'Nuclear Magnetic Resonance', Cambridge University Press, London.

EPR

Ingram, D.J.E. (1955). 'Spectroscopy at Radio and Microwave Frequencies', Butterworths Scientific Publications, London.

Ingram, D.J.E. (1958). 'Free Radicals as Studied by Electron Spin Resonance', Butterworths Scientific Publications, London.

Allen, M. B., Piette, L. H. and Murchio, J. C. (1961). Biochem. Biophys. Res. Comm. 4, 271.

Anderson, A.F.H. (1960). Unpublished results.

Androes, G. M., Singleton, M. F. and Calvin, M. (1962). To be published.

Arnold, W. and Clayton, R. K. (1960). Proc. Nat. Acad. Sci. U.S. 46, 769.

Beinert, H. and Lee, W. (1961). Biochem. Biophys. Res. Comm. 5, 40.

Bennett, J. E., Gibson, J. F. and Ingram, D.J.E. (1957). Proc. Roy. Soc. (L) A 240, 67.

Blaauw-Jansen, G., Komen, J. G. and Thomas, J. B. (1950). Biochim. Biophys. Acta, 5, 179.

Blois, M.S., Brown, H. W. and Maling, J. E. (1961). In 'Free Radicals in Biological Systems' (M.S. Blois et al., eds.), p. 117, Academic Press, New York.

Bubnov, N. N., Krasnovskii, A. A., Umrikhina, A. V., Tsepalov, V. F. and Shliapintokh, V. Ia. (1960). Biofizika, 5, 121.

Calvin, M. (1961). J. Theoret. Biol. 1, 258.

Chance, B. and Nishimura, M. (1960). Proc. Nat. Acad. Sci. U.S. 46, 19.

Chorney, W., Scully, N. J., Crespi, H. L. and Katz, J. J. (1960). Biochim. Biophys. Acta, 37, 280.

Clayton, R. K. (1955). Archiv Mikrobiol. 22, 180.

Cole, T., Heller, C. and Lambe, J. (1961). J. Chem. Phys. 34, 1447.

Commoner, B. (1961). In 'Light and Life' (W. D. McElroy and B. Glass, eds.), p. 356, Johns Hopkins Press, Baltimore.

Commoner, B., Townsend, J. and Pake, G. E. (1954). Nature, 174, 689.

Commoner, B., Heise, J. J. and Townsend, J. (1956). Proc. Nat. Acad. Sci. U.S. 42, 710.

Commoner, B., Heise, J. J., Lippincott, B. B., Norberg, R. E., Passonneau, J. V. and Townsend, J. (1957). Science, 126, 57.

Cummerow, R. L. and Halliday, D. (1946). Phys. Rev. 70, 433.

Eastman, J., Andrees, G. and Calvin, M. (1962). Nature, in press.

Feher, G. (1958). In 'Proceedings of Kamerlingh Onnes Conference on Low Temperature Physics, Leiden', p. 83, supplement to Physica, Sept. 1958.

- Feher, G. (1959). Phys. Rev. 114, 1219.
- Feher, G. and Kip, A. F. (1955). Phys. Rev. 98, 337.
- Fletcher, R. C., Yager, W. A., Pearson, G. L. and Merritt, F. R. (1954).
Phys. Rev. 95, 844.
- Frenkel, A. W. and Hickman, D. D. (1959). J. Biophys. Biochem.
Cytology, 6, 285.
- Gibson, J. F. and Ingram, D.J.E. (1957). Nature, 180, 29.
- Griffiths, M., Siström, W., Cohen-Bazire, G. and Stanier, R. Y.
(1955). Nature, 176, 1211.
- Holmogorov, V. and Terenin, A. II. (1961). Naturwissenschaften,
48, 158.
- Hutchinson, C. A. and Mangum, B. W. (1958). J. Chem. Phys.
29, 952.
- Ingram, D.J.E. and Bennett, J. E. (1955). Discussions, Faraday
Soc. 19, 140.
- Jacobs, E. and Holt, A. S. (1954). Amer. J. Bot. 41, 710.
- Kearns, D. R. and Calvin, M. (1961). J. Am. Chem. Soc. 83, 2110.
- Kearns, D. R., Tollin, G. and Calvin, M. (1960). J. Chem. Phys.
32, 1020.
- Kohnlein, W. and Muller, A. (1961). In 'Free Radicals in Biological
Systems' (M.S. Blois et al., eds.), p. 113, Academic Press, New York.
- Kok, B. (1959). Plant Physiol. 34, 184.
- Leifson, O. S. and Jeffries, C. D. (1961). Phys. Rev. 112, 1781.

- Malmstrom, B. G. and Vanngard, T. (1960). J. Mol. Biol. 2, 118.
- Melville, H. W. and Burnett, G. M. (1953). In 'Techniques of Organic Chemistry' (S. L. Friess and A. Weissberger, eds.), Vol. VIII, p. 138, Interscience Publishers, New York.
- Milner, H. W., French, C. S., Koenig, M.L.G. and Lawrence, N.S. (1950). Arch. Biochem. 28, 193.
- Park, R. B. (1962). J. Chem. Ed., in press.
- Park, R. B. and Pon, N. G. (1961). J. Mol. Biol. 3, 1.
- Portis, A. M. (1953). Phys. Rev. 91, 1071.
- Roberts, E. M. and Koski, W. S. (1960). J. Am. Chem. Soc. 82, 3006.
- Shields, H. and Gordy, W. (1959). Proc. Nat. Acad. Sci. U.S. 45, 269.
- Singer, L. S. and Kommandeur, J. (1961). J. Chem. Phys. 34, 133.
- Smith, J.H.C. (1954). Plant Physiol. 29, 143.
- Sogo, P., Pon, N. G. and Calvin, M. (1957). Proc. Nat. Acad. Sci. U.S. 43, 387.
- Sogo, P., Jost, M. and Calvin, M. (1959). Rad. Res. Suppl. 1, 311.
- Sogo, P., Carter, L. A. and Calvin, M. (1961). In 'Free Radicals in Biological Systems' (M.S. Blois et al., eds.), p. 311, Academic Press, New York.
- Ollin, G., Kearns, D. R. and Calvin, M. (1960). J. Chem. Phys. 32, 1013.
- van der Waals, T. H. and de Groot, M. S. (1959). Mol. Phys. 2, 333.
- van der Waals, T. H. and de Groot, M. S. (1960). Mol. Phys. 3, 190.
- van Niel, C. B. (1941). Adv. Enzymol. 1, 263.
- Wagoner, G. (1960). Phys. Rev. 118, 647.

Weaver, H. E. (1961). Private communication.

Wertz, J. E. and Vivo, J. (1955). J. Chem. Phys. 23, 2441.

White, H. (1934). 'Introduction to Atomic Spectra', p. 215,
McGraw-Hill, New York.

Witt, H. T., Muller, A. and Rumberg, B. (1961). Nature, 192, 967.

This is the latest of several relevant papers by Witt and co-workers.

References to previous work are contained in the reference given.

Yamazaki, I., Mason, H. S. and Piette, L. (1960). J. Biol. Chem.
235, 2444.

Zavoisky, E. (1945). J. Phys. U.S.S.R. 9, 211.

FIGURE CAPTIONS

- Fig. 1 Diagrammatic representation of the behavior of a population of free electrons in an external magnetic field.
- Fig. 2 Field modulation applied to a resonance absorption, showing the origin of the derivative signal.
- Fig. 3 Transmission spectrometer employing field modulation and phase sensitive detection. The microwave magnetic field, H_1 , in the cavity is perpendicular to H_0 , the applied magnetic field.
- Fig. 4 Top: The magnitude of the electric, ----, and magnetic, ———, microwave fields as a function of distance along the center line of the cavity. C is related to the dielectric constant of the medium, and is such that $C|E_1|_{\max} = |H_1|_{\max}$. Bottom: The cavity (a rectangular transmission type, TE_{103}) showing the sample positions and the field directions at the positions of the maxima in E_1 and H_1 . H_1 and E_1 reverse directions sinusoidally in time.
- Fig. 5 The EPR spectrum of Mn^{++} (at 200 molar ppm) in MgO .
- Fig. 6 The EPR spectra of a series of chlorine-substituted semiquinones. (After Wertz and Vivo, 1955).
- Fig. 7. Idealized power saturation behavior.

- Fig. 8 The effect of electron exchange on the EPR spectrum of 10^{-4} M fluoranil semiquinone in 90% tetrahydrofuran-10% acetonitrile. The spectra were produced by reacting 0.005 M NaI with 0.005 (top), 0.100 (middle) and 0.900 (bottom) M fluoranil at -75°C . The scale magnitude of one gauss is indicated. (Eastman et al., 1961).
- Fig. 9. The EPR spectrum of Cu^{++} in solid ceruloplasmin. $T = 77^{\circ}\text{K}$. Magnetic field increases from left to right. (After Malmstrom and Vanngard, 1960).
- Fig. 10 g-Value as a function of the number of aromatic rings for some substituted benzosemiquinones. (After Blois et al. 1961).
- Fig. 11 Characteristics of an inhomogeneously broadened line. At left: The observed electron spin resonance line shape. One observes the envelope of many narrow resonance lines, each with slightly different resonant frequency, ω_i . At right: The energy level system which produces the observed resonance. Double resonance effects are observed in the electron resonance ($\Delta m_s = \Delta m_j = \pm 1$) when the nuclear transitions ($\Delta m_I = \pm 1$) are induced. (After Feher, 1958).
- Fig. 12 Variation of the amplitude of the EPR of phosphorus impurities in silicon as a function of the frequency of the 'nuclear-transition' radiofrequency field. (After Feher, 1959).

- Fig. 13 Relative energies of the components of the lowest triplet state of naphthalene as a function of magnetic field. Heavy lines: Magnetic field along x axis. Thin lines: Magnetic field along z axis. The dashed transitions indicate those observed by Hutchinson and Mangum (1958). The solid transitions observed by van der Waals and de Groot (1959). (After van der Waals and de Groot, 1959).
- Fig. 14 The EPR spectra of $\Delta m_s = \pm 2$ transitions of naphthalene in a rigid glass. (a) H_1 parallel to H_0 . (b) H_1 perpendicular to H_0 . $T = 77^\circ K$. (After van der Waals and de Groot, 1960).
- Fig. 15 The light induced EPR amplitude in an acetone extract of spinach chloroplasts as a function of the pressure of water vapor present. The extract was evaporated to dryness and evacuated before admission of water vapor.
- Fig. 16 Light induced EPR in 'crystalline' chlorophyll (a + b) in vacuo in the presence of water vapor. (a) dark, (b) response to light, (c) superimposed dark and light signals. (After Holmogorov and Terenin, 1961).
- Fig. 17. Action spectrum of Rhodospirillum rubrum chromatophores. The action spectra were performed on different samples on different days. They cannot be compared in absolute magnitude since this varied unpredictably from sample to sample. However, the shape of either curve is reproducible (e.g. the top action spectrum always peaked at $\sim 800 m\mu$ and at $\sim 880 m\mu$). For the action spectra the monochromator half-intensity band width was $15 m\mu$.

- Fig. 18 Power saturation of the EPR in the wild and a mutant type of Rhodospseudomonas spheroides. Dried films at room temperature.
- Fig. 19 Shape, amplitude and rise and decay kinetics of the EPR signal in Rhodospirillum rubrum as a function of temperature. Dried film.
- Fig. 20 Line shape analysis of the EPR spectrum of Rhodospirillum rubrum in aqueous suspension at room temperature.
- Fig. 21 Light induced EPR signals in C. pyrenoidosa at two different wavelengths of illumination. (After Allen et al., 1961).
- Fig. 22 The EPR amplitude, O₂ evolution rate and C¹⁴O₂ fixation rate as a function of chlorophyll (a + b) content during the greening of a yellow mutant of Chlamydomonas reinhardi.

$$E_2 - E_1 = \mu_0 \cdot g_0 \cdot H_0 = \Delta E = h \cdot \nu = h \cdot c / \lambda$$

$$\lambda = \frac{h \cdot c}{\mu_0 \cdot g_0 \cdot H_0} \quad \mu_0 = 0.927 \cdot 10^{-20} \quad H_0 = 3,300 \quad \lambda \sim 3,2 \text{ cm}$$

MU-15462

Fig. 1.

MU-12660

Fig. 2.

MU-12659

Fig. 3.

MU-25362

Fig. 4.

MU-17661

Fig. 5.

MU-15934

Fig. 6.

MU-23970

Fig. 7.

MU-23917

Fig. 8.

MU-26839

Fig. 9.

MU-26840

Fig. 10.

MU-26841

Fig. 11.

P^{31} DOPED Si (5×10^{16} P/cm³)
 $T = 1.25^\circ K$, $H_0 \approx 3000$ GAUSS

MU-25361

Fig. 12.

MU-26842

Fig. 13.

MU-26843

Fig. 14.

MU-21327

Fig. 15.

MU-26844

Fig. 16.

Fig. 17.

MU-23969

Fig. 18.

MU-26882

Fig. 19.

MU - 23961

Fig. 20

CHLORELLA PYRENOIDOSA
(Allen, Piette and Murchio)

MU-26845

Fig. 21.

Fig. 22.

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