UCRL-16492

University of California

Ernest O. Lawrence Radiation Laboratory

QUANTUM CONVERSION IN PHOTOSYNTHESIS: ELECTRON PARAMAGNETIC RESONANCE INVESTIGATIONS

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Research and Development

UCRL-16492
UC-4 Chemistry
TID-4500 (45th Ed.)

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

QUANTUM CONVERSION IN PHOTOSYNTHESIS: ELECTRON PARAMAGNETIC RESONANCE INVESTIGATIONS

R. H. Ruby

January 1966

Printed in USA. Price $3.00. Available from the Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, U. S. Department of Commerce, Springfield, Virginia.

UCRL-16492

Quantum Conversion in Photosynthesis: Electron Paramagnetic Resonance Investigations.

R. H. Ruby

Lawrence Radiation Laboratory, University of California, Berkeley*

Introduction

Primary quantum conversion is the mechanism by which photosynthetic systems convert electromagnetic energy into the chemical potential required to supply energy for the chemistry which these systems perform. Many investigations of this process, the central physical-chemical problem in photosynthesis, have been made using the physical technique of Electron Paramagnetic Resonance (EPR). This paper is an attempt to determine what contribution to our understanding of the energy conversion mechanism has been achieved by that work.

This paper is largely structured as an assembling of information presented in the literature and the offering of some additional results obtained in our laboratory. One of the motives was the hope of achieving some perspective, and perhaps the prospect of some inspiration, leading to a clear cut idea of any additional information which we might hope to obtain.

We limit ourselves mainly to results in biological photosynthetic systems, excluding examination of model systems such as triplet state or charge-transfer studies in synthetic organic materials.

For information on the technique of EPR we refer the reader to the following references: EPR in general (1-6), with reference to biological

* The preparation of this paper was sponsored by the U.S. Atomic Energy Commission.

systems (7-9), and to previous application in photosynthetic systems (10-11). The survey is not exhaustive, ending in the summer of 1964, and perhaps reflects certain emphasis on the part of the author. Where an investigator in this field has participated in several similar efforts, one is chosen as representative; especially when one publication adequately describes a particular experimental approach.

In our own work we reflect the belief that there exists a common physical-chemical description of all biological photosynthetic systems by the choice of a single system, a purple bacterium, for our studies.

Photosynthesis

Photosynthesis in green plants is the synthesis of organic compounds and the liberation of molecular oxygen from H_2O, CO_2 and other inorganic compounds in conjunction with the absorption of light energy by plant pigments, especially chlorophyll. In bacteria O_2 is not liberated, but the light's energy is used to drive chemical reactions necessary for growth and life. We choose to call primary quantum conversion that process between the absorption of light by the pigments and the appearance of the first chemically defined species in thermal equilibrium with its environment. The energy is stored chemically by phosphorylation and the efficiency of the related chemistry has been discussed by Bassham (12). In a recent discussion (13) a consideration of the efficiency requirements for the quantum conversion process leads to the conclusion that it must be about 90% efficient. This most impressive attribute of primary quantum conversion has led to the development by several authors (14-16) of a particular model for the process. The steps in the energy conversion in this model are: 1)the absorption of electromagnetic energy by pigment molecules, 2)the electronic excitation energy is transferred by radiationless dipole transfer (an exciton) between

pigment molecules to a special site, 3)at this special site a charge separation occurs resulting in the primary oxidant and reductant which may participate in the subsequent chemistry. Step 3 must occur in such a way that there is no loss of energy due to the recombination of these charges. There have been several calculations (76-78) of the consistency of an exciton picture with the optical absorption observations in photosynthetic systems. These calculations, based on the structure and efficiency observed in photosynthetic systems in various approximations of radiationless dipole transfer, are in agreement in the conclusion that the exciton mechanism can account for the properties of the photosynthetic system. There seems, however, to be no direct unequivocal evidence that the formation of excitons is in fact involved.

The technique of EPR could be applicable in two ways. One is the direct observation of a paramagnetic triplet energy state formed in the process of exciton energy transfer. No such observations have been reported in the literature. The second is the observation of an unpaired electron spin produced in the act of charge separation. It is from this view that much of the published work on EPR in photosynthesis has been interpreted.

Photosynthetic materials may be demonstrated to possess a morphological and molecular structure which is consistent with the suggested mechanism for the primary quantum conversion process (17-20). Discussions of the rather central role of chlorophyll and the addition of the accessory pigments in photosynthesis are contained in references (21) and (14). Optical absorption experiments in photosynthetic systems constitutes a formidable body of literature, some of which is discussed in references (14-16).

EPR Observations

Let us consider the types of information one can seek in attempting to identify the photoinduced EPR signal which is observed, characterize its properties, and interpret these observations in terms of some mechanism of primary quantum conversion. The environment of an electron's angular momentum (spin) and its associated magnetic dipole moment may be described in several terms; the physical structure in which it is located, its interaction with other chemical entities in that structure, and its relation to the physiological morphology of the photosynthetic unit.

Parameters which describe the EPR signal are the size, shape and position in field of the microwave resonance absorption curve. Further, the signal size may have characteristic time dependencies such as the spin-lattice relaxation time (T_1) or the transient response time to a light pulse (τ).

We measure the size of the absorption maximum as twice the maximum amplitude of the first derivative of the resonance absorption curve; this is the signal (S). The width of the absorption curve (ΔH) is the separation in magnetic field between the points of maximum slope, and as usual, the field position (H_o) of the resonance absorption maximum is given in terms of the g-value; $g\beta H = h\nu$, where h = planck's const., ν is the frequency of the microwave radiation, and β is the Bohr magneton.

One type of variable affecting the EPR signal is a change in the chemical environment as evidenced in mutant types of systems, or as produced by chemically altering the system. The latter may be a change in the chemical pathways achieved by the introduction of poisons, changes in the oxidation-reduction potential by an externally introduced redox couple, or the removal

of pigments participating in the quantum conversion process. We may control some of the physical variables which affect the system such as the intensity and spectral distribution of the irradiating light and the temperature.

In presenting the information on biological materials from the literature, we have organized it in the following sequence with reference to variables affecting the EPR signal size:

1. Changes in chemical environment - mutants, poisons, removal of pigments, organic oxidants and reductants, and redox potential.

2. Temperature.

3. Light dependence of the EPR signal - intensity dependence, spectral response, transient response, and quantum yield.

Following this, we present experiments which provide information about signal parameters such as shape, g-value and spin-lattice relaxation time.

Exploratory

In the time elapsed since the first report of the observation of unpaired electrons in photosynthetic systems by Commoner (23), photoinduced spins have been studied in a number of biological systems. The samples used involve intact biological systems, fragments of biological systems and pigments extracted from biological systems. The appearance in the literature of the various EPR studies paralleled the partial description of the function, structure, and chemical composition of the fragments by other methods mentioned briefly in the introduction. As these fragments were studied, it appeared that certain of them represented subunits of the photosynthetic systems which were self-contained in their ability to perform basic operations of the whole biological systems. A survey of the EPR studies on these various components and on the whole system is presented in Tables I and II.

Table I lists studies on whole and fragmented biological photosynthetic systems; while Table II lists studies in systems composed of pigments extracted from biological systems, often in combination with other relevant organic molecules.

The EPR signals observed in these studies divide into two categories. The first category has a line width of about 10 gauss, a transient response to light of the order of 1 sec. or faster, and has been thought to be associated relatively closely with the primary quantum conversion process. The second category has a line width of about 20 gauss, a transient response to light of the order of seconds and longer (often much longer, sometimes referred to as the dark signal) and is thought to be more closely associated with products of chemistry occuring subsequent to the primary quantum conversion process. This paper is primarily concerned with signals of the first category, and it is to this category we apply the term "light induced signals".

The studies of fragments of photosynthetic systems continues with the philosophy that if their physical description can be obtained, the more complicated whole systems may also be described in similar terms.

In the Tables, we conform to the common nomenclature for these fragments. A discussion of the preparation, nomenclature, and biological significance of these various fragments is given in Appendix I.

A recurrent difficulty in these studies is that in any given experiment the results of attempted measurements on physical and chemical parameters of the system are highly interdependent. Often in the literature, the experimental conditions are insufficiently described to allow easy comparison of the results of different investigators, if indeed they even used the same biological system in which to measure a given parameter. Thus,

interpretation of differences or similarities between experiments by different authors must be made with some reserve.

Mutants

In mutant strains of algae and bacteria, the difference between the EPR spectra of the mutant type and of the wild type is interpreted as being correlated with an observed difference in photosynthetic behavior of the systems.

Allen (24) reports two light induced signals in Chlorella. In a mutant which lacks Chlorophyll-b, one of these signals is missing and thus this signal is interpreted as due to the presence of Chl.b in the wild type. In (22) a mutant of Chlorella which contains Chl. but is not photosynthetic showed a very small light induced signal.

Similarly, Androes (26) observes a mutant of Chlamydomonas which lacks Chl. and produces no light induced EPR signal. As Chl. is synthesized in the light by this mutant, the light induced EPR signal grows in, Fig. 11. It is observed that the rate of increase of EPR signal is greatest when O_2 evolution begins to decrease.

A carotenoidless mutant of Rhodospeudomonas sp., reported in the same paper, yields a light induced signal which is essentially the same as in the wild type. Recently Clayton (65) has obtained a mutant strain of Rhodo.sp. which is unable to grow photosynthetically although it contains its normal compliment of BChl. This species produces no light induced EPR signal.

Levine (34), using several mutant types of Chlamydomonas, concluded from a comparison of the EPR signals and the ability of the systems to perform the Hill reaction that the light induced signal with the broader line width and slow kinetics is associated with the ability of the systems to perform O_2 evolution.

Weaver (45) investigates two types of mutant strains of Scenedesmus. One is unable to assimilate CO_2 and exhibits only the slow broad signal, while the other is unable to perform the Hill reaction and exhibits only the narrow rapid signal. This agrees with the conclusion above. A similar observation of a quite small EPR signal in etiolated as compared with fully green leaves was made by Commoner (79). The signal increases upon greening.

Poisons

The addition of DCMU (3,3,4-dichlorophenyl-1,1-dimethylurea) to plants and algae results in a large increase of the light induced EPR signal. This compound inhibits oxygen evolution in plants (52). Kok (33) observed this effect in Anacystis. Weaver (46) used this method to increase the signal to noise ratio of the steady state light induced signal in Chlamydomonas. In the latter experiments a similar observation was made if the sample was stored in anaerobic conditions.

Pigment Removal

Some chlorophyll present in a photosynthetic system may be removed without greatly changing the production of light induced EPR signals; the implication is that the active chlorophyll is only a small amount in a special physical environment.

Beinert (49) removed the Chl.a from sonicated red algae by acetone extraction. They found a light induced EPR signal in the remaining structure which they identify with the light induced EPR signal generally seen by other authors. They attribute the production of this signal to the presence of a pigment which absorbs at λ=7000 Å (P_{700}).

By selective chemical oxidation with K_2IrCl_6 of chromatophores from R. rubrum, R. spheroides and Chromatium, Loach (35) has achieved the removal of approximately 95% of the absorbance in the near infra-red while leaving the photoactive pigments. The light induced EPR signals in these materials was left pretty much unchanged.

Clayton (53) has investigated the optical absorption changes in R. spheroides chromatophores by converting some of the BChl. to pheophytin in the presence of light and oxygen, and removing this pheophytin with detergent. He observes a photobleaching of a pigment absorbing at λ=8700 Å (P_{870}). EPR has not been done on this system.

Organic Oxidants and Reductants

An example of assigned redox relationships of species in the proposed electron transport pathway in photosynthesis is shown in Fig. 2, Calvin (28). Only the step involving $h\nu_1$ is assumed to apply to photosynthetic bacterial systems in which oxygen is not a product.

The nature of the primary oxidized species produced by the light can, in principle, be determined by the use of externally introduced organic oxidants or reductants. If organic dyes, which when oxidized (or reduced) have a free radical nature with a well-characterized hyperfine structure, can act as donor or acceptor for electrons at a known location in the electron pathway, then the appearance (or disappearance) of this associated hyperfine structure during the process of photosynthesis may provide the identification we seek.

In this laboratory we have attempted to use methyl viologen, which has a characteristic hyperfine structure in the reduced form (54), in this role. However, when added to quantasomes, the desired effect was not observed. The limits in our sensitivity make this, at best, a marginal experiment.

With similar motivation, Heise (32) has added the reducing agents $DPIPH_2$ and TMPD to R. rubrum chromatophores and has observed changes in the decay rate of the light induced signals. They interpret this as consistent with the light induced formation of $BChl^+$.

Oxidation - Reduction Potential

Measurement of the redox potential for oxidation of the species producing the light induced EPR signal is performed by introducing an external redox couple into the system and attempting to vary the oxidation level of components in the electron transport chain in a controlled fashion.

Increases in redox potential reduce the magnitude of the light induced signal. Table III shows the oxidation potential for the midpoint in a chemical titration of the light induced signal as obtained by several investigators.

Figure 3 illustrates the type of results obtained in such a titration. In R. rubrum, Calvin (28), changes in redox potential, using ferricyanide, induced dark EPR signals identical to those produced upon illumination. A complementarity between the chemically produced and light-induced signals was observed.

Loach (35) in continuing these experiments has observed that at high potentials a large dark signal results in chromatophores. Further, the same author observes a titration curve in quantasomes that implicates two dependent light induced one-electron transfer acts resulting in EPR signals.

Temperature

By sufficiently reducing the temperature of the sample, those processes contributing to the EPR signal which require spacial migration of chemical

species may be excluded. Experiments have been carried out in the temperature range 1 to 273°K. These experiments may be divided into two categories, those which examine the light induced EPR signal magnitude, and those which examine the transient response of the EPR signal to a pulse of light intensity. The latter experiments will be presented in a later section of this paper.

The temperature dependence of the light induced EPR signal in photosynthetic systems has been investigated primarily by workers associated with Calvin. The observation of light induced signals at temperatures less than 273°K has been reported for samples of R. rubrum, in whole cells by Sogo (39) and quantasomes by Androes (25). The temperature range covered in these reports was 113°K to 300°K. The results of Sogo (38) in whole cells of R. rubrum are shown in Fig. 4.

We have extended the investigations on dried films of R. rubrum chromatophores to liquid nitrogen (77°K) and liquid helium (1-4.2°K) temperatures. As at the higher temperatures, a reversible light induced signal, apparently described by the same parameters of line shape, width, and g-value, was found. Detailed measurements of these parameters and of the signal magnitude will be made in order to determine if the signals observed at these temperatures may be associated with some part of those signals observed in biologically functioning systems. The careful measurement of the signal magnitude as a function of temperature is complicated by the ease with which the spin system is power saturated.

Light Intensity Dependence

The EPR signal magnitude as a function of the illuminating intensity has been examined by several authors.

Commoner (10) and later, Treharne (44) demonstrate that in Chlorella the light induced signal with rapid kinetics has a different dependence on light intensity than the broader signal with slow kinetics. Further, the latter author shows that the light induced signal (rapid kinetics) in chloroplast fragments has a light intensity dependence differing from that of whole cells, and has a roughly linear relation to the $\log_{10}$ of the intensity over the range from 25 to 25,000 ft. candles (illumination from a tungsten lamp).

We have observed a similar property in the light induced signals in R. rubrum chromatophores, as shown in Fig. 5.

Sogo (38) has observed that the "functional relationship between the number of unpaired electrons and the light intensity is dependent on temperature."

Wavelength Dependence

Photoproduction of spins by light absorbed in distinct absorption bands should show up as structural features in the dependence of the light induced EPR signal on the wavelength of light used to irradiate the sample (action spectra).

Table IV presents some materials in which action spectra have been measured and the references. These action spectra have the common feature that a broad peak occurs approximately at the wavelength of the optical absorption maximum of chlorophyll in that material. In addition, several references, Allen (22), Levine (34) and Weaver (46), show data which indicate the resolution of structure on this peak. This structure, in some cases, has been interpreted as a demonstration of the formation of two distinct paramagnetic entities by light absorbed in two distinctly different pigments, e.g. Chl_a and Chl_b, Allen (22).

Many of the action spectra published suffer a distortion of their shape resulting from the use of light intensities capable of saturating the photoresponse, or sample concentrations which are too high and result in self-absorption effects.

Demonstrations of the effect of light intensity on the shape of the action spectrum have been given by Weaver (46) and Ruby (56). Weaver examined the light intensity dependence of the light EPR signal at several wavelengths in whole cells of Chlamydomonas reinhardi, where the signal to noise ratic was enhanced by the addition of DCMU. They extrapolated the linear region of these curves to a high value of light intensity for comparison and achieved better resolution of peaks in the action spectrum, Fig. 6. Ruby, using a sample of R. rubrum chromatophores, operated with the lowest light intensity consistent with a workable signal to noise ratio, and also achieved better resolution of the peak at 8800 Å, Fig. 7. The width of the peak is still broader than that of the optical absorption peak occuring at the same wavelength, and masks evidence of possible contributions to the EPR signal resulting from light absorbed in the subsidiary optical absorption peak at 8000 Å.

Tollin (43), in comparing optical emission and fluorescence spectra with EPR action spectrum, concludes that the action spectrum peak is shifted when too high a sample density is used.

Androes (25) shows that an increase in sample concentration can produce a distortion of the action spectrum, giving the appearance of two resolved peaks. Heise (57) corroborated this result.

Another way of examining the participation of two separate light absorbing entities in the production of light induced EPR signals is presented by Kok (38). Instead of seeking structure in the action spectrum, these

authors investigated the combinatorial behavior of light at two different wavelengths in producing EPR signals.

The wavelengths used were 6350 Å, largely absorbed by the accessory pigment phycocyanin, and 7130 Å, largely absorbed by Chl_a. The combination of these two wavelengths produces a signal which is smaller than if only 7130 Å light is present. This is interpreted as a competition between a process which photochemically oxidizes the pigment species P_{700}, forming an EPR signal, as the result of the absorption of light at 7130 Å, and the reduction of this species by products formed as a result of light absorbed at 6350 Å, which latter process is associated with O_2 evolution.

Transient Response

The transient response of the EPR signal in biological materials to a square pulse of light intensity has been investigated by Sogo (38)(39), Tollin (43), Androes (25), Commoner (31), and Ruby (37).

In their paper, Sogo (38) examined a number of materials in the temperate range from 300°K to 113°K. Their values for the rise and decay times observed at 300°K are listed in Table I. In this discussion, we will concentrate on information known about R. rubrum and Spinach.

The results of Tollin (43) in spinach chloroplasts at the temperatures of 298°K and 133°K are shown in Fig. 8. We note that at the lower temperature the signal is irreversibly induced by the light. In his discussion of similar work investigating the effect of H_2O, Androes (25) concludes that "the presence of diffusing water molecules or some molecule carried by the water, or both, is necessary to return the unpaired electrons which produce the (f,n) EPR to a diamagnetic state." A similar observation is made by Holmogorov (63) in work on crystals of Chlorophyll.

The earliest work on R. rubrum whole cells was that of Sogo (38). Their results are shown in Fig. 9. We note that there is a reversible light induced signal present at temperatures down to 113°K, and that the decay of the signals is faster at the lower temperatures. The rise times in these experiments were instrument limited. Experiments with similar results were performed by Androes (25) on chromatophores.

In the work of Ruby, the transient response of the EPR signal in chromatophores was directly compared to the transient response of several prominent optical absorption changes using the same experimental arrangement for each measurement. The results are shown in Fig. 10. The EPR kinetics are the same, within experimental error, as those of the optical absorption change at λ-4330 Å. Based on the identification of the optical absorption change at 8650 Å as associated with the photo-induced oxidation of BChl (58)(59), and the observation that the molecular species absorbing at 4330 Å and at 8650 Å are different (demonstrated by the difference in their kinetics), then the EPR signal is not produced by the $BChl^+$ radical, as often stated.

Obtaining meaningful quantitative values for the rise and decay rates is made difficult by their dependence on such factors as redox potential, pH, temperature, light intensity, the physiological state of the organisms, and the preparation and storage of the chromatophores.

In a continuation of these experiments, the rise and decay times of dried films of chromatophores have been measured at the temperatures 300°K, 77°K, and 4.2°K. The kinetics are similar to the kinetics of chromatophores in aqueous solution, Fig. 11, and may be fit by the same type of curves, with the appearance of two rise components at $T < 77°K$. It has been observed by Cope (60) that the decay may be equally well fit by an expression of the type $-ds/dt = me^{ns}$ where s is the signal, t is the time, and m and n are constants.

The values of the time constants for exponential fits are:

T	τ rise	τ decay
300	3.4s	19.5s
77	.69, 0.96	.54, .11
4.2	.31, 0.28	.71, .070

Again, these numbers are dependent on the factors of redox potential, pH, etc. In addition, however, a new difficulty may arise. It is possible that a long spin lattice relaxation time may interfere with this measurement. We discuss this in the section on the spin-lattice relaxation time.

Commoner (31) investigated the growth kinetics of R. rubrum whole cells in response to a pulse of white light at $\approx$ 300°K. They report a delay in changes of the EPR signal after the light is turned on or off, and interpret this as an indication that "unpaired electrons associated with the EPR signal are not due to a light-excited state of the primary absorber in photosynthesis." Better data than that published by them was presented at the First International Conference on Resonance in Biology, 1964 (unpub).

Quantum Yield

We have measured the quantum yield of spins produced in R. rubrum chromatophores by light of wavelength λ = 8800 Å to be of the order of magnitude of unity. This is an easily accepted result; however, in view of the many difficulties inherent in the absolute measurement of the light intensity and spin concentration, the acceptance of this result demands the verification of an independent investigator. The details of the method of measurement are inscribed in Appendix 2.

Sogo (61) has reported a quantum yield of 0.03 in spinach chloroplast. Schleyer commented at the First International Conference on Resonance in

Biology, 1964 (unpub) that he observes a quantum yield in whole cells of chromatium which is of the order of 10^{-4}. Thus there is an indication that the measured quantum yield is larger in the less physiologically intact systems.

g Value

The g value describes the magnetic interaction of the magnetic dipole moment of the unpaired electron angular momentum with its environment and with the applied magnetic field. If the physical environment of the unpaired electron is known sufficiently well, the g value may be calculated and comparison with the measured g value can help serve as identification of the unpaired electron under observation.

The lack of a specific description for the environment of the unpaired electrons observed in photosynthetic systems has precluded the calculation of g values for these systems. g values have been measured for many of the biological systems; these are listed in Table I. These values are close to that for a free electron, g = 2.0023, a characteristic of free radicals. The information we seek is contained in the difference (Δg) between the measured values and the free spin value. This difference is often in the fourth decimal place and measurement to this place is frequently made difficult by poor signal to noise ratios and by observed asymmetries in the line shape. A discussion of measurement and some interpretation of g values is given by Blois et.al. (9). The g value may be dependent on temperature, and may also be found to be dependent on other conditions in the sample.

The g value can serve as identification to distinguish between several independent EPR absorption lines observed in the same material. A precise measurement and statement of the g value is desired for EPR absorption lines

being discussed in the literature. However, there is no guarantee that lines of the same g value in different materials arise from unpaired electrons in identical environments. Thus while the observed g values can be used as convenient labels for light induced EPR absorption lines in photosynthetic systems, a practice which has merits, reservation should be had about comparisons of g values between photosynthetic systems until sufficient information on the physical environment is available.

Line Shape

The shape of the microwave absorption spectrum is also a result of the interaction of the observed unpaired electron with its environment and, in principle, the shape may be predicted by theoretical calculation. A comparison with theory would provide further identification of the nature of the observed unpaired electrons in photosynthetic systems. Unfortunately, even in relatively simple physical systems such a calculation is difficult. Thus in photosynthetic systems we can only seek particular features of line shapes which are readily interpretable. Such features are resolved hyperfine structure, the demonstration of a particular broadening mechanism contributing to the line width and the contribution of several independent species to the observed signal.

The description of the shape of the absorption curves by either Gaussian or Lorentzian line shapes have been investigated in R. rubrum by Androes (7) and in Chlamydomonas by Weaver (46). The fits of the two curves to their data are shown in Fig. 12 a and b. One interpretation of a gaussian shape is that the observed absorption curve is a distribution of lines, possibly arising from unresolved hyperfine structure.

The observed widths of the absorption curves in photosynthetic systems are listed in Table I. We have noted in our low temperature experiments on

dried films of R. rubrum chromatophores that this width is roughly temperature independent. This observation confirms the statement of Sogo (39) to the effect that the linewidth in R. rubrum is not a result of lifetime broadening. It was also noted by Tollin (51) in mixtures of Chl and quinones that the line shape is independent of microwave power.

There have been no reports of resolved hyperfine structure on the light induced signal with rapid kinetics in any of the photosynthetic systems.

The effect on the EPR signal of substituting deuterons for protons in the molecules of photosynthetic systems has been examined by several authors. When such substitutions are achieved the observed EPR absorption line is narrowed. Commoner (10) reports a narrowing in Chlorella of the light induced signal from $\Delta H = 9g$ to $\Delta H = 1g$. Smaller (62) reports a similar observation in pigment extracts of Chlorella. Androes (25) attempted the same experiment in R. rubrum chromatophores by exchange, with no effect -- indicating that the protons may not be labile. Kohl, again at the First International Conference on Resonance in Biology, has observed a narrowing in R. rubrum whole cells when the cells are repeatedly grown in D_2O medium. They performed this experiment in a number of photosynthetic systems with the same results.

Spin Lattice Relaxation Time

The calculation of the spin-lattice relaxation time (T_1) from first principles is as difficult as the linewidth calculation. Further, a small amount of paramagnetic impurity can cause the observed times to be orders of magnitude different than the calculated times. Extreme shortening of T_1 may result from the presence of unfunctional paramagnetic transition metal ions in the materials. Thus, as a method of precise identification of the unpaired electron's environment, the measurement of spin-lattice relaxation times will

probably not be very useful. There are no known measurements of T_1 on photosynthetic systems in the literature. However, it is desirable to make such measurements.

Working in chromatophores of Rodopseudomonus spheroides, Androes (26) observes that the carotenoidless mutant species displays a different power saturation behavior than the wild type. This implies that the environment of the observed spin is different in these two species. Similarly, both Allen (22) and Treharne (44) state that in Chlorella the light induced signal with rapid kinetics and the broader signal with slow kinetics also display different power saturation behavior. Thus the measurement of T_1 may help to discriminate between several signals if they occur at the same g value and line width. It has been shown, Portis (66), that information concerning contributions to the line shape may be obtained by observing the power saturation behavior. Androes (25) states that the EPR line in R. rubrum chromatophores exhibits the saturation behavior of an inhomogeneously broadened line.

A long T_1 in a photosynthetic material may interfere with the measurement of the transient response to light. If we assume, as seems likely, that the production of the unpaired electron by the quantum of light is effectively a high temeprature process, then a measurement of the number of electrons by EPR is not valid until they have reached thermal equilibrium with the lattice. Thus, if $T_1 \gg \tau$, it is the spin lattice relaxation time which will actually be observed, rather than the transient response time for the photoproduction of spins.

We have observed that in R. rubrum power saturation may be achieved with modest amounts of microwave power. In dried R. rubrum chromatophores at 300°K, 10 mw incident on a closely coupled cavity with a Q of 2000 will produce partial saturation, and at 4°K, 10^{-2} mw is sufficient. We have made

preliminary measurements on this sample of T_1 using a modification of the method of saturation of the spin system by a microwave pulse, and observation of the recovery to the thermal equilibrium signal as discussed in Scott and Jeffries (67). Instrumental limitations prevented a good measurement of this time, but indications are that T_1 at 4°K is of the order of magnitude 10 to 100 ms. Thus T_1 could easily be interfering with the measurement of the transient response to the light at this temperature.

A discussion of the converse effect is given by Sohma (68), in which he proposes that a short lifetime for radicals can serve as an energy transfer process from the spin system to the lattice and thus effect T_1. He indicates that in this circumstance a measurement of T_1 can be used as a method of measuring the radical lifetime. He assumes in this paper that the radicals when created are in thermal equilibrium with the lattice, which assumption remains to be tested. For spins thus far observed, the lifetimes are long, and this effect should not occur. However, if we locate an extremely kinetically fast ($<$1 ms) EPR signal, which we might expect to arise if the unpaired electron is associated with the primary process, such considerations may have to be made.

Model Systems

By model systems we restrict ourselves to extracts from photosynthetic systems and combinations of relevant biologically obtained molecules which are able to produce light induced EPR signals.

Light induced EPR signals have been produced both in solutions containing Chl and in solutions of mixtures of Chl and other molecules such as quinones, listed in Table II. Signals are also produced in Chl crystals; here there seems to be some effect of H_2O on the signal, Holmogorov (63).

In the mixture of Chl_a and quinones investigated by Tollin and Green (51), a signal larger than that due to Chl alone was observed and was interpreted as arising from the quinone after a one electron transfer act from the quinone to Chl driven by the light.

Allen (47) extracted a Chl complex from Chlorella which would undergo the photoinduced dye reduction with DCPIP and which also produces a light induced signal.

Thus the requirement of a viable photosynthetic system is not necessary for Chl to participate in the production of a light induced EPR signal. The relevance of these signals to the ones observed in photosynthetic systems remains to be shown.

Mauzerall and Feher (50) have successfully produced light induced signals in biologically obtained porphyrin molecules, similar in structure to Chl, as seen in Fig. 13. These experiments were done in solution, and the evidence is strong that it is the porphyrin free radical which is being observed. These authors have proposed (priv. comm.) to continue experiments which, if successful, can lead to a more definite identification of the spin. The resonance absorption curve is thought by these authors to be a distribution of hyperfine lines resulting from interaction of the unpaired electron with the Nitrogen nuclei (I = 1). They propose to resolve this structure by the use of the ENDOR technique (69). If successful in this material, an experiment of this type would then be attempted to provide evidence toward identifying the site of the light induced unpaired electron in photosynthetic systems. It is also hoped that calculations of g value and line width can be performed for the porphyrin system.

Discussion

In reviewing the literature we must come to the conclusion that there has been little success towards identifying specifically the physical entity which produces the EPR signal. In addition doubts must be entertained whether the observed signals are in fact related to the primary energy conversion process, as opposed to chemical reactions at a subsequent time, and whether these signals may be explained in the terms of parameters which describe the physical-chemical system in which the energy conversion takes place.

We may divide the literature into two opposite categories with regard to evidence of a charge separation which produces the signal.

Positive	Negative
Chl requirements*	Comparison of EPR and O.D. Kinetics*
Action Spectrum*	Delay in EPR transient signal
Redox experiments*	
Low Temperature exp.	

We should note that little of the interpretation of the EPR results stands independently of observations made in photosynthetic systems by other methods. Those entries in the list marked by an asterisk depend explicitly on the interpretation of optical observation changes to indicate that BChl is the first reductant (oxidized species) resulting from the primary quantum conversion act. Thus EPR can only serve to corroborate this interpretation. We further note that the positive pieces of evidence are all steady state measurements on the concentration of spins under various conditions, while the negative evidence is based on observation of the transient properties of the light induced signals.

It may be that we lack the sensitivity to observe any component of the signal associated with the primary process. Following the conclusions of Clayton (21) we may make a quick calculation of the expected steady state

concentration of unpaired electron spins produced by the initial act. We assume the concentration of BChl present in chromatophores is 10^{-1}M and that the amount present as an active site for oxidation is of the order of 10^{-2} of that amount. In an EPR spectrometer we have a sample of 0.01 ml presenting the order of 10^{-15} possible active sites. With light intensity of 10^{16} photons/sec^{2}cm incident on the cavity in an area of 1 sq. cm. and with a quantum yield of 1 the steady state number of spins observable would in general be of the order $10^{16}\tau$ where τ is the lifetime of the oxidized BChl. Thus for a lifetime of 1ms we would have a steady state concentration of only 10^{13} spins which is less than the sensitivity of the EPR spectrometer (10^{14} unpaired electrons with a line width of 10 gauss) at room temperature in an aqueous solution. In our experiments the limitation of the number of possible sites reduces the number of expected unpaired electrons so that even with our optimistic assumptions of quantum yield and lifetime the steady state concentration of unpaired electrons associated with the primary is quite possibly unobservable.

It would appear that the main emphasis must be placed on investigating the nature of the light induced signal which appears at low temperature. The line width, g value and kinetics of this signal appear essentially the same as the room temperature signal and yet it appears at temperatures where the usual chemical reactions are not expected to occur and thus ought to represent the primary oxidized species produced by the light. There is insufficient information to merit a discussion of the relevance of this signal to the biologically functional photosynthetic system.

TABLE I

Reversible Light Induced EPR Signal in Photosynthetic Systems

Material	Reference	g-value	H [g]	Line shape	Sample	Photosynthetic Activity Demonstrated	Kinetics [s] Rise	Kinetics [s] Decay
BACTERIA								
Rhodospirillum rubrum	(61) (38)		12		WC		≤ 1	≤1
	(7) (25)	2.003	11.2	Asymmetric Gaussian	Chro		2,15	7,30
	(31)	2.002	10		WC		0.030	0.034
	(37)				Chro		0.4	0.5,4
Rhodopseudomonas spheroides	(38)				WC		≤1	≤1
	(26)	2.0026	11.2±0.4		*WC&Chro			
Chromatium	(38)				WC & Chro		≤1	≤1
					WC			
Chloropseudomanas Ethylicum	(42)	2.002± 0.001	17	Asymmetric	WC & Chro			
HIGHER PLANTS								
Spinacea Oleracea	(30)	2.002± 0.001	8 - 10		Chlo			

TABLE I (page 2)

	(39) (43)				Chlo		0.2	≤1,10
	(25)	2.002	10	Asymmetric	Quant.			
Nicotiana tabacum	(29)	2.002± 0.001	10		Chlo		12	45
Hordeum vulgare	(27)	2.004	1.8	Doublet	Leaves			
	(36)	2.00	8		"			
Triticum vulgare	(27)			Doublet	Leaves			
Sancheria nubilum	(27)				"			
Eucalyptus	(39)				"			
ALGAE								
Chlorella pyrenoidosa	(10) (30)	2.002	8 - 10		WC		10	11,20
	(38)				WC		≤1	1,10
	(23)	2.0030	9		WC	O_2 evol. CO_2 uptake	≈0.3+	0.05
Chlamydomonas reinhardi	(34)	2.0023	8.2		WC	O_2 evol.		
	(46)	2.0025	8.3	Gaussian	WC	O_2 evol.	0.2	6
	(26)					O_2 evol, CO_2 fix.		

TABLE I (page 3)

Anacystis nidulans	(38)			WC		≤1	1,10
	(33)			WC			
Scenedesmus obliquus	(38)			WC		≤1	1,10
	(49)				O_2 evolution		
	(45)	2.0025		WC	CO_2 uptake		
Romaria	(38)					≤1	≤1
Nostoc	(38)					≤1	≤1
Euglena Gracilis	(31)			WC		0.4	
Red Algae	(49)	2.0025± 0.0005	7.2±0.1	Treated Sonicate			
Porphyridium cruentum	(41)			WC			

WC = whole cells

Chro = chromatophores

Chlo = chloroplast

Quant = quantasomes

* Dried Sample

TABLE II

Photo-induced Signals in Relevant Biological Molecules

Material	Sample	g value	ΔH	Line shape	Reference
Chl_a					
$Chl_a + Chl_b$					
Chl + Carotene	Solution	~2.0	6g		(38)
Chlorella Ext.					
R. rubrum Ext.					
$Chl_a + Chl_b$					
β Carotene	Solution	2.014 & 2.003		Two lines	(62)
Chlorella Ext.					
Chl_a	Crystals	2.0027	6 ± 1		(64)
$Chl_a + Chl_b$	Crystals	2.0027	6 ± 1		(63)
Chl_a	Crystals	2	15g		(48)
P-672	Solution				(47)
Uroporphyrin	Solution	2.0021 ± 0.0001	5.4 ± 0.2	Gaussian	(50)
Chl_a + Quinones					(51)

TABLE III

Oxidation-Reduction Titration Mid-points for Light Induced Signal

Material	Reference	E_m	Redox Couple Used	pH	Sample
Treated Red Algae (P 700)	(49)	+0.43	Ferri-Ferrocyanide	7.0	Sonicated, Chl extracted with 68% Acetone
R. rubrum	(28)	+0.44	"	7.3	Chromatophores
Spinach	"	+0.46	"	7.2	Quantasomes
Chlorella Pyrenoidosa extract (P 672)	(47)	+0.45	"	7	P_{672} Chl complex extracted from Chlorella

TABLE IV

Action Spectra of Biological Materials

Material	Reference	Sample
R. rubrum	(55)	WC
	(25)	Chrom.
	(56)	"
Spinach	(43)	Chloro.
	(28)	Quant.
Chlorella pyr.	(43)	WC
	(23)	WC
Chlamydomonas rein.	(34)	WC
	(46)	
Nostoc	(43)	

Appendix 1

Part 1. Systems Studied

The whole systems studied with EPR have been leaves of plants, the whole cells of algae and the whole cells of photosynthetic bacteria. Work has been done on systems which are physiologically in good condition (e.g. in plants where CO_2 fixation and O_2 evolution at near normal rates is demonstrated) and in systems lacking water. The higher green plants and the algae may be broken into large fragments, the 'chloroplast', in a blender. This fragment behaves in most respects as a whole leaf. Literature characterizing the chloroplast is cited in Park and Pon (70).

Smaller fragments are particles obtained from spinach chloroplast or whole bacteria by rupturing the cell walls by mechanical shear or intense sonic fields.

The particles obtained from the purple bacterium are called "chromatophores" (71). These are roughly spherical particles approximately 200 Å in diameter, which have the same optical absorption spectrum as the whole cell and will perform photophosphorylation reactions. The entity of the chromatophore is not well characterized. Following the Zeitgeist apparent at the Symposium on Bacterial Photosynthesis 1963, (E) which is well stated by Kamen in his summary remarks (72), we describe in Part 2 of this appendix our procedure for preparing chromatophores, including a biochemical assay for photosynthetic activity based on photophosphorylation.

The particle obtained from spinach chloroplast by sonic rupture is the "quantasome" and is prepared following the procedure of Park and Pon (70). Again these particles have the optical absorption spectrum of the parent structure. They will evolve O_2 in the Hill reaction, and when

combined with the water-soluble protein leached out in the preparative procedure, they will fix CO_2.

Part 2. Chromatophores

Rodospirillum rubrum (Strain no. 1.1.1, originally supplied by R. Y. Stanier, University of California at Berkeley in 19) is grown anaerobically in modified Hutner's medium (73). The illumination for growth is provided by flourescent lights with an intensity of 400 candle power at the sides of 1 liter culture flasks. Five days after innoculation, the bacteria cells are collected by centrifugation at 5000 xg. The precipitate is resuspended in 0.1 M glycyl-glycine buffer (pH7.5) using a minimum volume of buffer. The suspension is sonicated for 2-1/2 minutes in a Biosonik apparatus at a dial setting of 50. The cell debris is removed by centrifugation for 30 minutes at 20,000 rpm (Spinco Model L - 40 head). The chromatophores are collected from the supernatent by centrifugation for 50 minutes at 40,000 rpm. The resultant pellet is washed once by resuspension in fresh buffer and again centrifuging for 50 minutes at 40,000 rpm. The final chromatophore sample is resuspended in buffer and passed through a coarse sintered-glass filter to insure homogeneity. The optical density of the chromatophores is measured directly in the EPR spectrometer aqueous cell (path length 0.03 cm). The bacteria chlorophyll concentration is $0.493 \times O.D._{880}$ mg/ml.

The photosynthetic activity is assayed by the ability of the chromatophore to perform photophosphorylation. The light induced esterification of added ADP by cell free preparation in the presence of Mg^{++} ions and suitable reducing agents is discussed by Frenkel (74).

Two methods of assay were used. Both utilize the enzyme hexokinase to catalyse the conversion of glucose to glucose-6-phosphate, employing ATP and generating ADP. The first is the chromatographic method, which follows the amount of glucose ^{14}C-6-phosphate produced from glucose ^{14}C. Separation is achieved by killing the system with 80% EtOH after 5 minutes illumination at 2,600 candle power, spotting on "Ederol" chromatographic paper, developing for 40 hours in butanol: propionic acid: water, drying and counting the ^{14}C activity of the separated spots. We achieved a rate of 20 umP /mgBChl hr for our chromatophores.

The second, and more convenient, method is the spectroscopic detection of reduced TPN. The glucose-6-phosphate production is coupled to a TPN reducing reaction by the enzyme Glucose-6-phosphate dehydrogenase (74). The assay media is as follows:

Reagent	Amount
Mg^{++}	10uM
Glucose	1
$=PO_4$	35
Succinate	10
ADP	8
Hexokinase	- non-rate limiting
TPN	3

A 2 ml solution of chromatophores, Glucose-6-phosphate dehydrogenase and media is made up, illuminated for 5 minutes at 2,600 candle power in a Warburg bath, and a spectroscopic determination reduced TPN in comparison to a dark control is made. This method requires additional investigation.

Appendix 2

Method of Quantum Yield Determination

The quantum yield is determined from the initial slope of the transient response of the photoinduced spins to a light pulse of known intensity. We require the precise knowledge of two parameters, the rate at which photons are being absorbed by the sample, and the number of unpaired electrons represented by a given signal recorded on the chart paper.

The measurement of the light intensity incident upon the front of the cavity was made with a Hoffman 120C photodiode. The photodiode was calibrated in the following manner: A reader RBL-500 thermopile was calibrated using a radiation standard (C 424) from the Bureau of Standards (operated at the specified voltage and current from a regulated DC power supply); the photodiode was then compared directly to the thermopile as a function of wavelength by measuring the output of a Bausch and Lomb monochrometer (illuminated by a 500 watt projection lamp which was powered by a regulated DC power supply operated at 99 volts). The sensitivity of the photodiode as a function of wavelength is shown in Fig. 14. The linearity of the response of the photodiode to the intensity of light in the range of intensities to be used in the experiment was checked.

The rate at which photons were absorbed by the sample, in the varian cavity geometry was calculated from the expression:

$$I_{abs} = I_o \; W \; A \; F \; r \; K \quad \text{(photons/sec)}, \quad \text{where}$$

I_o = intensity incident upon front of cavity.

W = fraction of light transmitted by cavity window, (=1/2).

A = area of sample irradiated, (=1.6 cm^2).

F = fraction of light absorbed by the sample, calculated from the measured optical density.

r = correction for light absorbed upon multiple reflection of light from the cavity walls.

K = conversion factor from milliwatts to photons/sec at λ=8800 Å (=4.5×10^{15}).

The number of light induced spins with rapid kinetics was determined by comparison with a standard of Cr^{3+} spins in MgO powder imbedded in polyethelene. The number of spins in the standard was determined by the chemistry of preparation, and checked against a standard of powdered phosphorous doped silicon imbedded in polyethelene (75). We compared the area under the EPR absorption curves for the difference between the steady state signal for R. rubrum in the light and in the dark to that of the Cr^{3+} signal as follows:

$$S_o = Cr \left[\frac{\text{Area } \underline{\text{R. rubrum}} \text{ (light-Dark)}}{\text{Area Cr}} \right] C \left(\frac{G_{Rr}}{G_{Cr}} \right) \left(\frac{H_{M_{Rr}}}{H_{M_{Cr}}} \right), \text{ where}$$

Cr = the number of chromium spins.

Area = area under the EPR absorption curve obtained by twice numerically integrating the displayed derivative on the chart paper.

G = total gain of spectrometer system

H_M = field modulation amplitude

C = correction factor for the distribution of aqueous sample over the cavity volume.

We obtained results of $\approx 5 \times 10^{14}$ spins in a sample of O.D. = 1,5. During the period of time required to make the quantum yield measurement, the steady state number of light induced spin changes (ostensibly due to changes in the physiological condition of the sample) necessitating the use of an average value in the final calculation.

The initial slope of a transient light response measurement, as represented by Fig. 10, was obtained and the quantum yield calculated by:

$$Q = \frac{\text{Slope}\Big|_{t=0}}{I_{abs}}$$

which had the value of 1.3 in a representative sample.

The possible errors in a measurement of this type are manifold. Alterations in experimental conditions can materially affect the measured value of the quantum yield. For example, microwave power saturation and field modulation broadening will distort the observed line shape, a saturating light intensity for spin production will affect the apparent efficiency of quantum conversion, and too large a sample concentration results in a steep gradient of light intensity through the sample with a concomittant concentration gradient and an effect on the quantum yield if it is concentration dependent.

In order to preserve the signal to noise ratio and to keep the experimental observation time to a reasonable value, we chose to operate at less than ideal conditions. While we feel that the magnitude of these effects does not badly prejudice the results, we prefer to state the accuracy of the measurement as a somewhat subjective level of confidence at a factor of 2.

Appendix III

GLOSSARY OF TERMS

ADP - Adenosine diphosphate

ATP - Adenosine triphosphate

Chloroplast - see Appendix I

Chromatophore - see Appendix I

Etiolated - Green plants, yellow in appearance, grown in the dark and lacking their normal Chlorophyll content.

Hill reaction - Liberation of O_2 from green plants or fragments, in the presence of light, simultaneous with the reduction of an organic dye as a substitute for CO_2 fixation by the photosynthetic system.

Photophosphorylation - Storage of the radiant energy of sunlight in the energy rich bonds of ATP formed from ADP and inorganic phosphate.

Quantasome - see Appendix I.

REFERENCES

(A) "Free Radicals in Biological Systems", Blois et al., eds., Academic Press, New York, 1961.

(B) "Light and Life," McElroy and Glass eds., John Hopkins Press, Baltimore, 1961.

(C) "Biophysical Science - A Study Program", Oncley et al., eds., John Wiley & Sons, New York, 1960.

(D) "The Photochemical Apparatus: Its Structure and Function", Fuller et al., eds., Brookhaven National Laboratory, 1959.

(E) "Bacterial Photosynthesis", Gest, San Pietro and Vernon eds., Antioch Press, 1963.

---

1. Ingram, D.F.E., "Spectroscopy at Radio and Microwave Frequencies," Butterworths, London, 1956.
2. Pake, G.E., "Paramagnetic Resonance," W. A. Benjamin, Inc., New York, 1960.
3. Low, W., "Paramagnetic Resonance in Solids," Academic Press, New York, 1960.
4. Ingram, D.J.E., "Free Radicals as Studied by Electron Spin Resonance," Butterworths, London, 1956.
5. Varian associated staff, "NMR and EPR Spectroscopy," Pergamon Press, New York, 1960.
6. Slichter, C.P. "Principles of Magnetic Resonance," Harper & Row, N.Y., 1963.
7. Androes. G.M. and Calvin, M., Biophy. J., 2, 217, 1962. EPR in Biology.
8. Smaller, B., "Advances in Biological and Medical Physics," 1963. EPR Studies of Biological Interest.
9. Blois, M.S. et al., Ref. A, p. 117. Precision g-value Measurements on Free Radicals of Biological Interest.

10. Commoner, B., Ref. B, p. 356. ESR Studies of Photosynthetic Systems.

11. Calvin, M. & Androes, G.M., Science, 138-3543, 867, 1963. Primary Quantum Conversion in Photosynthesis.

12. Bassham J.A., Adv. In Enzymology, 25, 39 (1963). Photosynthesis: Energetics and Related Topics.

13. Ruby, R.H., Kuntz, I.D., Jr., & Calvin, M., 50th Anniv. of the Society of Biol. Chem., France, p. 75 (1964).

14. Lumry, R. & Spikes, J.D., Rad. Res. Supp., 2, 539 (1960). Collection and Utilization of Energy in Photosynthesis.

15. Rabinowitch, E., Faraday Soc. Disc., 27 (1959), Aberdeen U. Press. "Energy Transfer with Special Reference to Biological Systems" Primary Photochemical and Photophysical Processes in Photosynthesis.

16. Kasha, M., Ref. C, p. 162. Relation between Exciton Bands and Conduction Bands in Molecular Lamellar Systems.

17. Biggins, J. & Park, R., Science, 144, 1009 (1964). Size & Composition of the Quantasome.

18. Cohen-Bazire, Ref. E, p. 89. Some Observations on the Organization of the Photosynthetic Apparatus in Purple & Green Bacteria.

19. Sauer, K. & Calvin, M., J. Mol. Biol., 4 451 (1962). Molecular Orientation in Quantasomes. I. Electric Dichroism & Electric Birefringence of Quantasomes from Spinach Chloroplasts.

20. Goedheer, J.C., Biochim. and Biophys. Acta, 16, 471 (1955). Orientation of Pigment Molecules in the Chloroplast.

21. Clayton, R.K., Photochem. & Photobiol., 1, 201-323 (1962). Primary Reactions in Bacterial Photosynthesis - I, II, III.

22. Allen, M.B., Piette, L.R. & Murchio, J.C., Biochim. & Biophys. Acta., 60, 539 (1962). Free Radicals in Photosynthetic Reactions: I. EPR Signals from Illuminated Chlorella Pyrenoidosa.

23. Commoner, B., Townsend, J., and Pake, G., Nature 4432, 689(1954) Free Radicals in Biological Systems.

24. Allen, M.B., Piette, L.R. & Murchio, J.C., Biochim. & Biophys. Res. Comm., 4, 271 (1961). Observation of Two Photoreactions in Photosynthesis.

25. Androes, G.M., Singleton, M.F. & Calvin, M., Proc. Nat. Acad. Sci., 48, 1022 (1962). EPR in Chromatophores from Rodospirillum Rubrum and in Quantasomes from Spinach Chloroplasts.

26. Androes, G.M., Singleton, M.F. & Calvin, M., Biochim. & Biophys. Acta, 66, 180 (1962). Photo Induced EPR in Mutant Photosynthetic Species Lacking Carotenoids or Chlorophyll.

27. Bubnov, N.N., Krasnovskiy, A.A., Umrikhine, A.V., Tsepalov, V.R. & Shlyapintokh, V., UCRL Trans. 560, Biofizika, 5, 121, (1960). Electron Paramagnetic Resonance Spectra Observed when Leaves of Plants are Illuminated, and the Photoreduction Reactions in Chlorophyll and its Analogues.

28. Calvin, M. & Androes, G., Science, 138, 867 (1962). Primary Quantum Conversion in Photosynthesis.

29. Commoner, B., Heise, J.J. & Townsend, J., Proc. N.A.S., 42, 710 (1956). Light-induced Paramagnetism in Chloroplasts.

30. Commoner, B., Heise, J.J., Lippincott, B.B., Norberg, R.E., Passoneau, J.V. & Townsend, J., Science, 126, 57 (1957). Biological Activity of Free Radicals.

31. Commoner, B., Kohl, D.H. & Townsend, J., Proc. N.A.S., 50, 638 (1963). Fast Kinetics of Unpaired Electrons in Photosynthetic Systems.

32. Heise, J.H. & Vernon, L.P., Ref. E, p. 343. Effect of Reduced 2,6-Dichlorophenolindophenol and N,N,N',N'-tetra methyl-p-phenylenediamine on the Light Induced Electron Spin Resonance Signal Observed with Rhodospirillum rubrum.

33. Kok, B. & Beinert, H., Biochem. & Biophys. Res. Comm., 9, 349 (1962). The Light Induced EPR Signal of Photocatalyst P-700. II. Two Light Effects.

34. Levine, R.P. & Piette, L.H., Biophys. J., 2, 369 (1962). An Investigation of ESR in Wild Type Chlamydomonas reinhardi & Mutant Strains Having Impaired Photosynthesis.

35. Loach, P.A., Androes, G.M., Maksim, A.F. & Calvin, M., Photochem. & Photobiol., 2, 443 (1963). Variation in EPR Signals of Photosynthetic Systems with the Redox Level of Their Environment.

36. Nicolau, C. (Switz) Arch. Sci., 13, 278 (1960). Research by EPR on Photosynthesis in Green Plants.

37. Ruby, R.H., Kuntz, I.D. Jr, & Calvin, M., Proc. N.A.S., 51, 515 (1964). Transient EPR and Absorbance Changes in Photosynthetic Bacteria.

38. Sogo, P., Jost, M. & Calvin, M., Rad. Res., Supp., 1,511 (1959). Evidence for Free-Radical production in Photosynthesizing Systems.

39. Sogo, P., Pon, N. & Calvin, M., Proc. N.A.S., 43, 387 (1957). Photo Spin Resonance in Chlorophyll Containing Plant Material.

40. Storey, W.H., Monita, C.M. & Cadena, D.G., Nature, 195, 963 (1962). Kinetics & Oxygen Requirements of a Light-induced ESR Signal from Whole Spinach Chloroplasts.

41. Weaver, E., Carnegie Inst. Yearbook, 1961. Electron Paramagnetic Resonance Studies on Chlamydomonas.

42. Sybesma, C. & Heise, J.J., Ref. E, p. 351. The Light Induced ESR Signals Observed in the Green Bacterium Chloropseudomonas ethylicum.

43. Tollin, G., Sogo, P. & Calvin, M., Ann. N.Y. Acad. Sci., 74, 310 (1958). Energy Transfer in Ordered and Unordered Photochemical Systems.

44. Treharne, R.W. & Vernon, L.P., Biochem. & Biophys. Res. Comm., 8, 481 (1962). Light Saturation Studies on the ESR Signals Observed with Chlorella Pyrenoidosa.

45. Weaver, E.C. & Bishop, N.I., Science, 140, 3571 (1963). Separation of EPR signals by means of Photosynthetic Mutants of Scenedesmus.

46. Weaver, E.C. & Weaver, H.E., Photochem. & Photobiol., 2, 325 (1963). EPR Studies on Chlamydomonas Reinhardi.

47. Allen, M.B. & Murchio, J.C., Biochem. & Biophys. Res. Comm., 11, 2, 115 (1963). Formation of a Stable Free Radical in an Illuminated Chl Complex.

48. Anderson, A.F.H. & Calvin, M., Nature, 199, 4890, 214 (1963). ESR of Crystalline Chl A & Crude Mixtures of Chl A with Normally Associated Pigments.

49. Beinert, H., Kok, B. & Hoch, G., Biochem. & Biophys. Res. Comm., 7, 219 (1962). Light Induced Electron Paramagnetic Resonance Signal of Photocatalyst P-700.

50. Mauzerall, D. & Feher, G., Biochim. & Biophys. Acta, 79, 430 (1964). A Study of the Photo Induced Porphyrin Free Radical by Electron Spin Resonance.

51. Tollin, G. & Green, G., Biochim. & Biophys. Acta, 60, 524 (1962).

Light Induced Single Electron Transfer Reactions between Chl_a & Quinones in Solution: I. Some General Features of Kinetics & Mechanism.

52. Bishop, N.J., Biochim. & Biophys. Acta, 27, 205 (1958). The Influence of the Herbicide DCMU on the Oxygen-evolving System of Photosynthesis.

53. Clayton, R.K., Ref. E, p. 377. Photochemical Reaction Centers in Photosynthetic Tissues.

54. Johnson, C. J., Jr. and Gutowsky, H. S., J. Chem. Phys. 39, 58 (1963). High Resolution ESR Spectra of Photochemically Generated Free Radicals: The Viologens.

55. Calvin, M., Ref. B, p. 346. Some Photochemical and Photophysical Reactions of Chlorophyll and its Relatives.

56. Ruby, R.H. & Calvin, M., Ref. E, p. 335. Primary Quantum Conversion: ESR Evidence.

57. Heise, J.J., "Electron Spin Resonance Studies of Free Radicals in Photosynthetic Systems", Dissertation, Washington Univ., St. Louis, Mo. (1962).

58. Clayton, R.K., Photochem. & Photobiol., 1, 201 (1962). Primary Reactions in Bacterial Photosynthesis - I The Nature of Light-Induced Absorbancy Changes in Chromatophores; Evidence for a special Bacteriochlorophyll Component.

59. Duysens, L.N.M., Ref. D, p. 325. Reversible Oxidations of Pigments in Bacterial Chromatophores.

60. Cope, F.W., Proc. N.A.S., 51, 810 (1964). Elovich Decay of Free Radicals in a Photosynthetic System as Evidence for Electron Transport Across an Interfacial Activation Energy Barrier.

61. Sogo, P.B., Carter, L.A. & Calvin, M., Ref. A, p. 311. Electron Spin Resonance Studies on Photosynthetic Materials.

62. Smaller, B., Ref. A, p. 315. Photo-Induced Free Spin Species in Plant Pigments.

63. Homolgorov, V. & Terenin, A.N., Naturwissenschaften, 48, 158 (1961). Light Induced ESR in Crystalline Chlorophyll, Due to Water Vapor Sorption.

64. Brody, S.S., Newell, G. & Castner, T., J. Phys- Chem., 64, 55 (1960). Paramagnetic Resonance of Chlorophyll Crystals & Solutions.

65. Clayton, R.K. & Sistrom, W.R., Proc. N.A.S., 52, 67 (1964). The Importance of Reaction Centers for the Photochemistry of Photosynthesis.

66. Portis, A.M., Phys. Rev., 91, 1071 (1953). Electronic Structure of F Centers: Saturation of the Electron Spin Resonance.

67. Scott, P.L. & Jeffries, C.D., Phys. Rev., 127, 32 (1962). Spin-Lattice Relaxation in Some Rare-Earth Satls at Helium Temperatures; Observation of the Phonon Bottleneck.

68. Sohma, J., J. Chem. Phys., 37, 2151 (1962). Effect of Lifetime of Free Radicals in Chemical Reaction of ESR Relaxation.

69. Feher, G., Phys. Rev., 114, 1219 (1959). Electron Spin Resonance Experiments on Donors in Silicon. I Electronic Structure of Donors by the Electron Nuclear Double Resonance Technique.

70. Park, R.B. & Pon, N.G., J. Mol. Biol., 6, 195 (1963). Chemical Composition and the Substructure of Lamellae Isolated from Spinacea Oleracea Chloroplasts.

71. Frenkel, A.W. & Hickman, D.D., J. Biophys. Biochem. Cytology, 6, 285 (1959). Structure & Photochemical Activity of Chlorophyll Particles from Rhodospirrillum rubrum.

72. Kamen, M.D., Ref. E, p. 445. Extemporaneous Remarks by way of a Summary.

73. Cohen-Bazire, G., Sistrom, W.R. & Stanier, R.Y., J. Cellular Comp. Physiol., 49, 25 (1957).
Kinetic Studies of Pigment Synthesis by Non-Sulfur Purple Bacteria.

74. Frenkel, A.W., Ref. D., p. 276.
Light-Induced Reactions of Chromatophores of Rhodospirillum rubrum.

75. Gere, E.A., Bell Telephone Technical Memorandum 38139-7.
A Calibration Marker for Paramagnetic Resonance.

76. Arnold, W., and Oppenheimer, J.R. J. Gen Physiology 33, 423, (1950)
Internal Conversion in the Photosynthetic Mechanism of Blue Green Algae.

77. Takeyama, N. Experentia 15, 289, (1962)
On the Diffusion Model of Localized Exciton in the Photosynthetic System

78. Bay, Z., and Pearlstein, R.M. Proc. N.A.S. 50, 1071, (1963)
Theory of Energy Transfer in the Photosynthetic Unit.

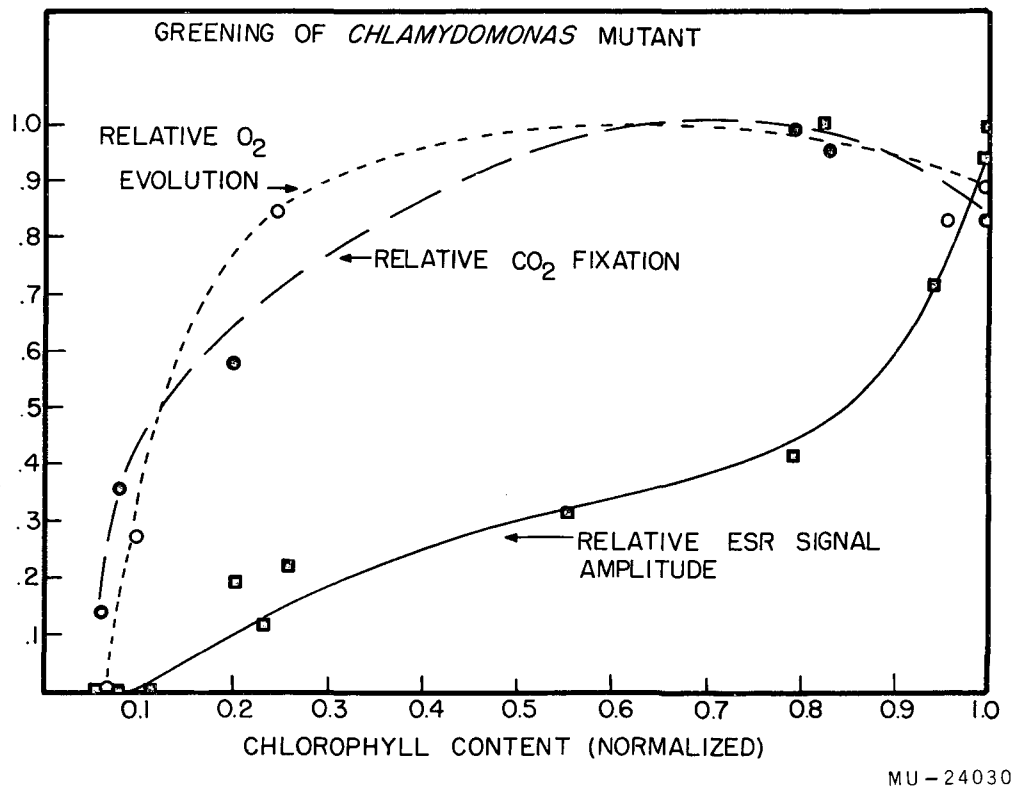


Fig. 1. The EPR amplitude, O_2 evolution rate and $C^{14}O_2$ fixation rate as a function of chlorophyll (a + b) content during the greening of a yellow mutant of Chlamydomonas reinhardi.

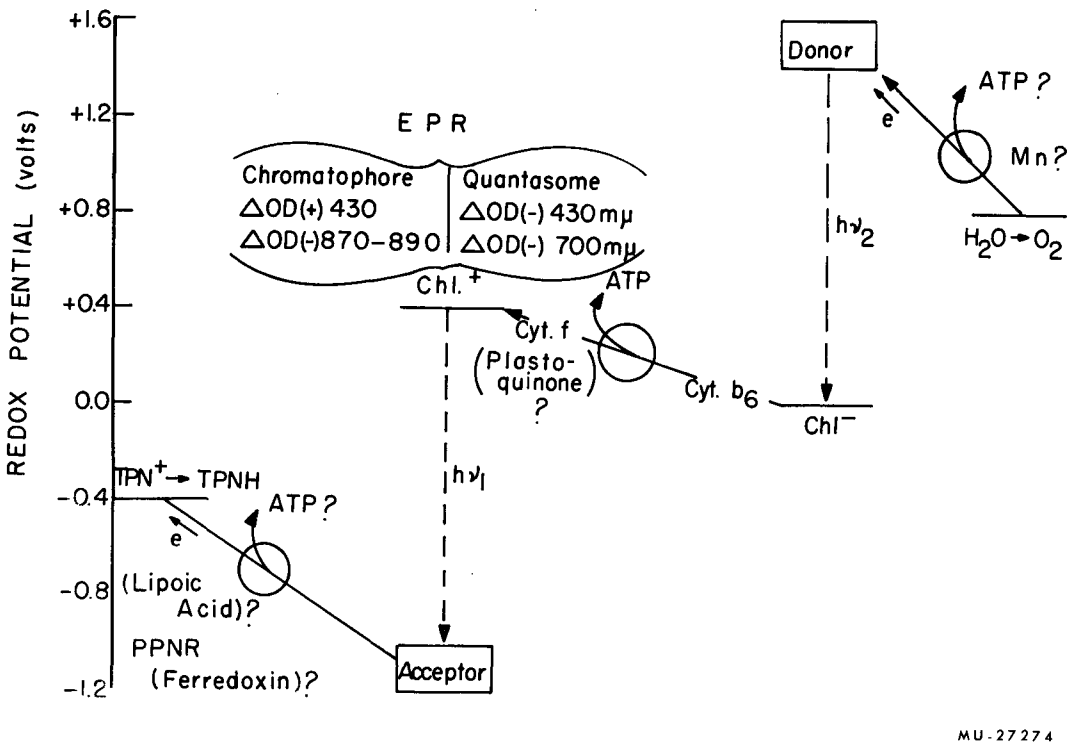


Fig. 2. Schematic diagram showing the approximate redox relationships of some species proposed as involved in the primary quantum conversion act or acts.

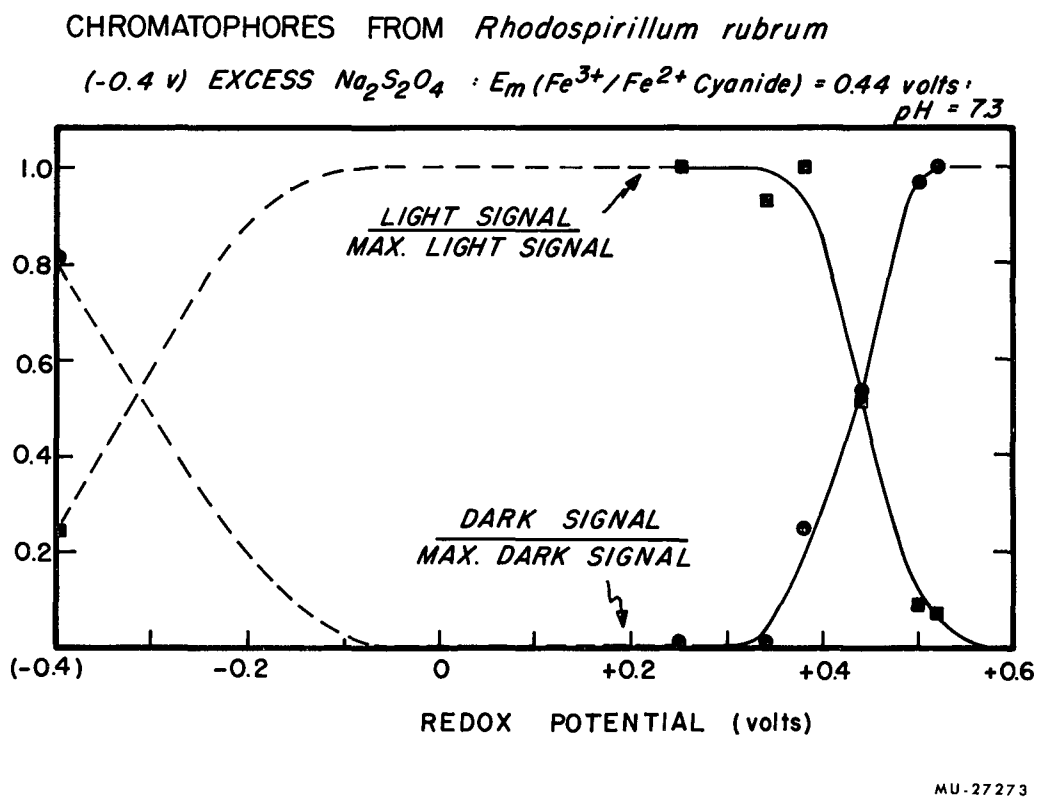
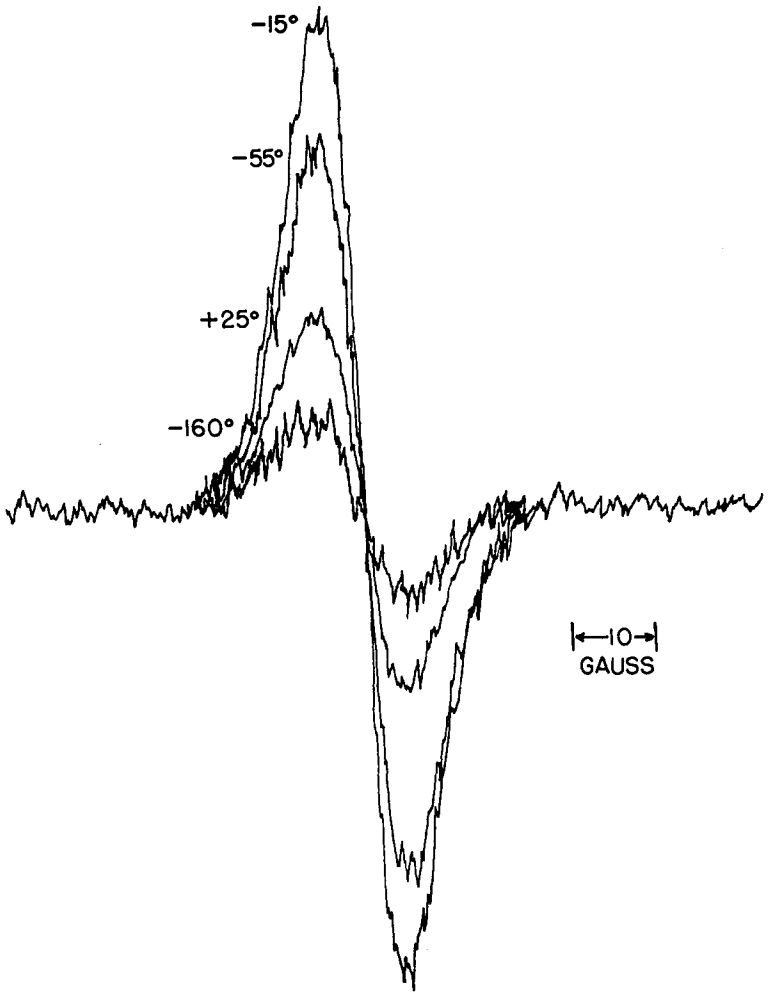


Fig. 3. Redox titration of the chemically induced and photo induced EPR signal in chromatophores from Rhodospirillum rubrum ($-O_2$; pH=7.3). Potential calculated: E_m (0.02 mole of Fe^{3+}/Fe^{2+} cyanide) = +0.44 volts.



ESR SIGNALS FROM *RHODOSPIRILLUM RUBRUM*
5 MINUTES CONTINUOUS ILLUMINATION

MU-15137

Fig. 4. ESR signals from Rhodospirillum rubrum 5 minutes continuous illumination.

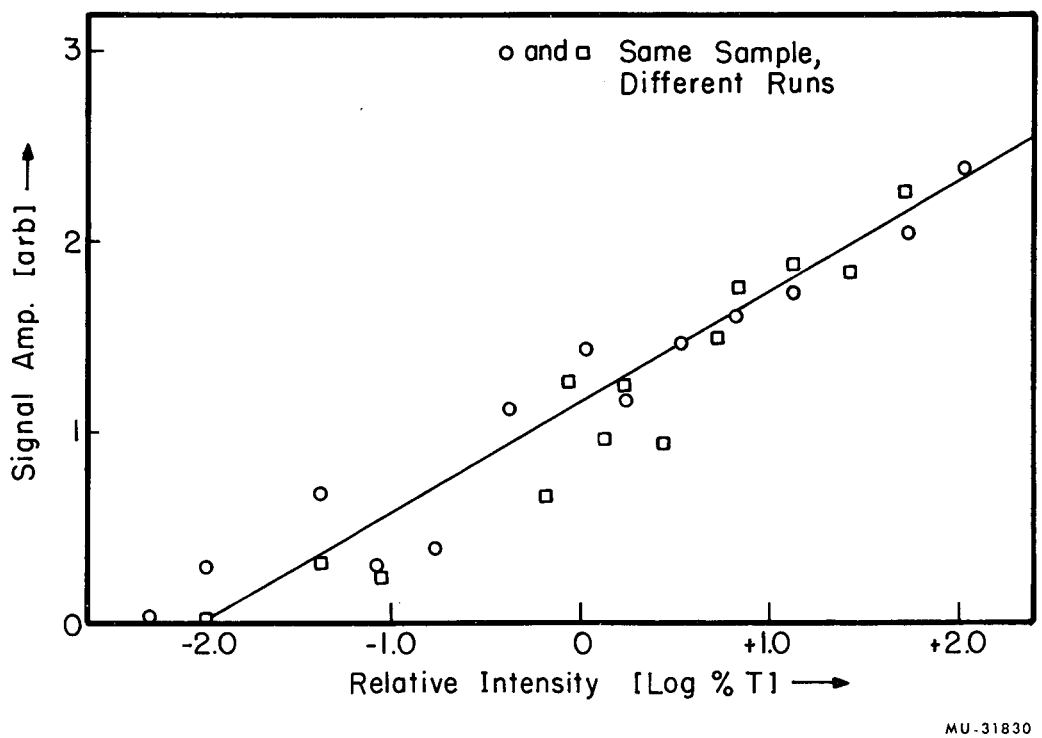


Fig. 5. Intensity dependence of the light induced signal produced by white light in R. rubrum chromatophores. The maximum intensity was ≅ 3000 cp. Reduced intensities were obtained by inserting neutral density filters, and were plotted as the log % transmission of the filters. T = 300°K, O.D. = 0.79, $-O_2$, pH = +7.2.

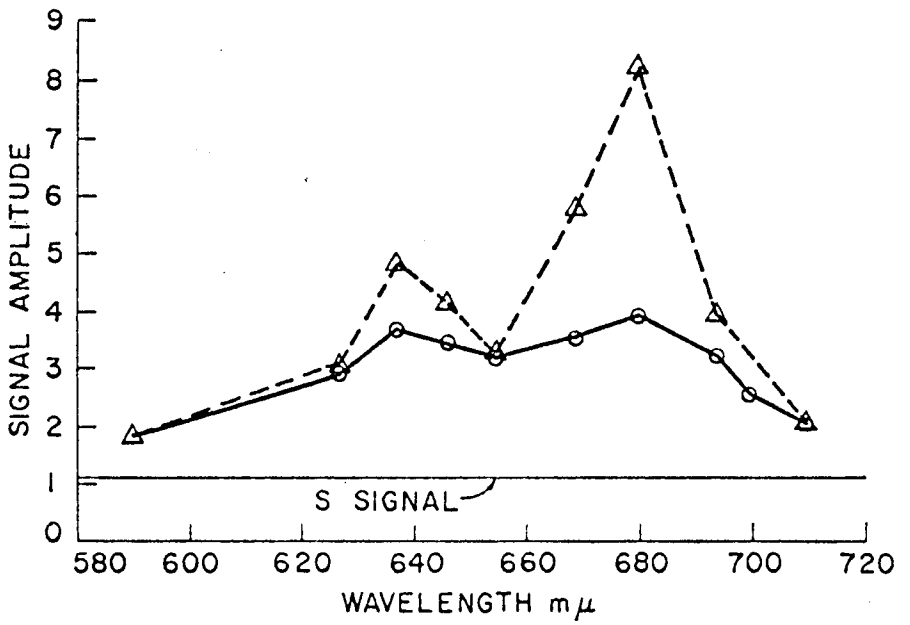


Fig. 6. Action spectrum for R signal amplitude, plotted (1) by measuring the peak-to-peak amplitude at a constant light intensity for each wavelength (O------O); (2) signal amplitude as it would be at the same light intensity if the slope had remained constant at its steepest point (Δ------Δ).

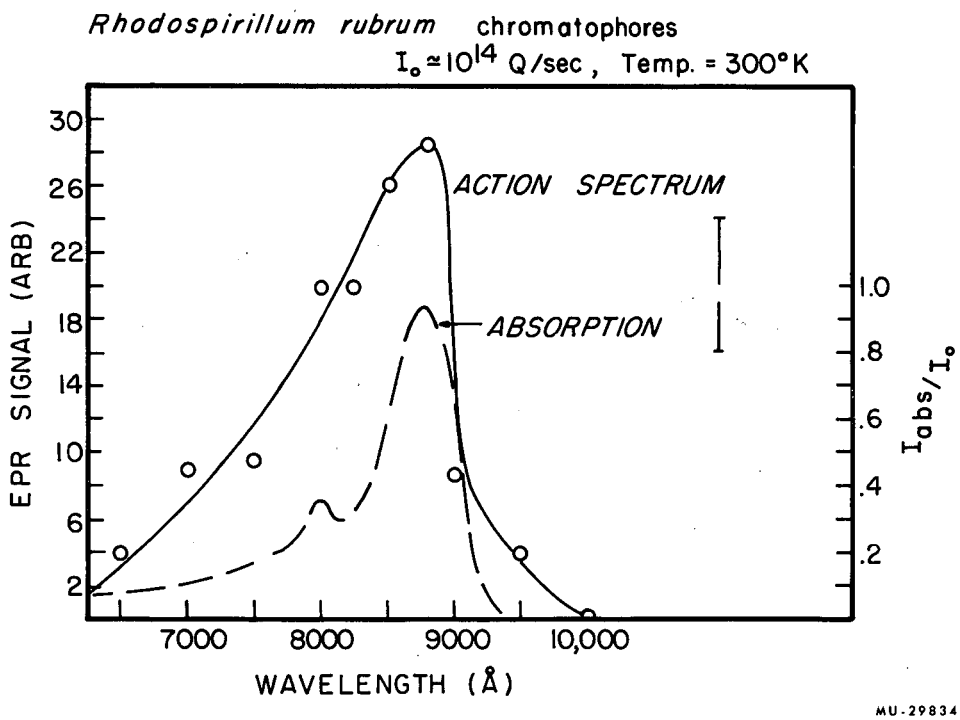
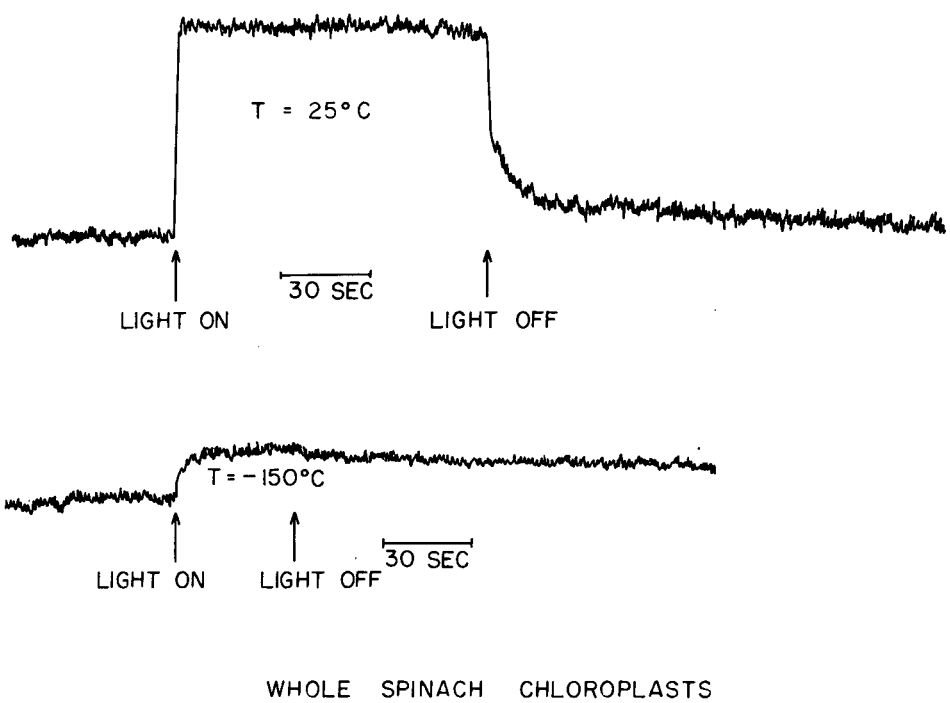
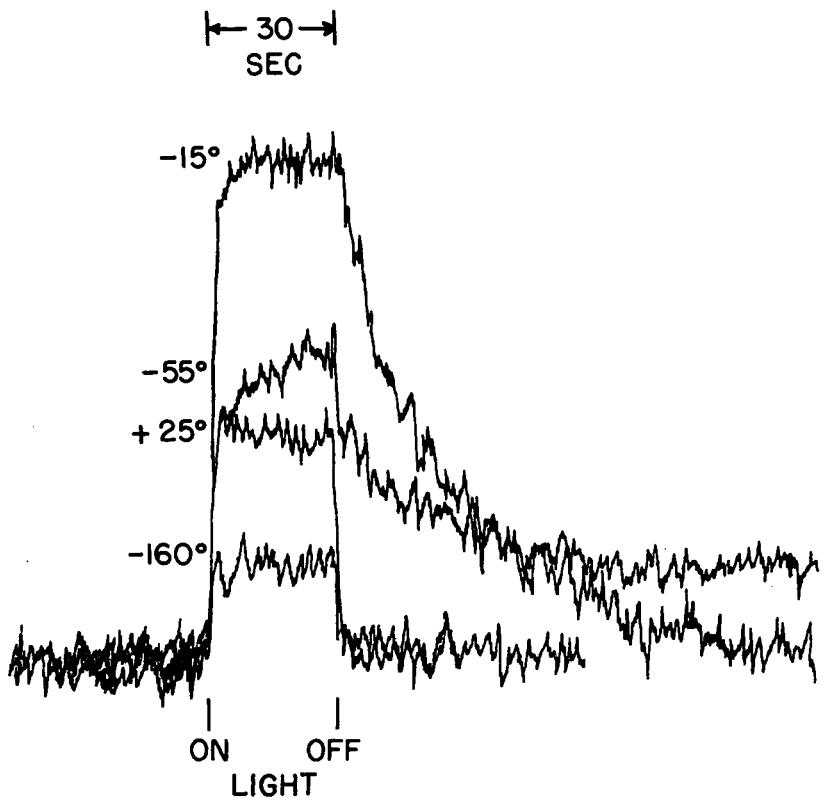


Fig. 7. Action spectrum of chromatophores from R. rubrum, taken at constant quanta/sec-cm^2 incident on the sample. The absorption spectrum is shown in dotted lines for comparison.



MU-14535

Fig. 8. Growth and decay curves of whole spinach chloroplasts at T = 25° C and -150° C.



RISE AND DECAY OF ESR SIGNALS
FROM *RHODOSPIRILLUM RUBRUM*

MU-15138

Fig. 9. Rise and decay of ESR signals from Rhodospirillum rubrum.

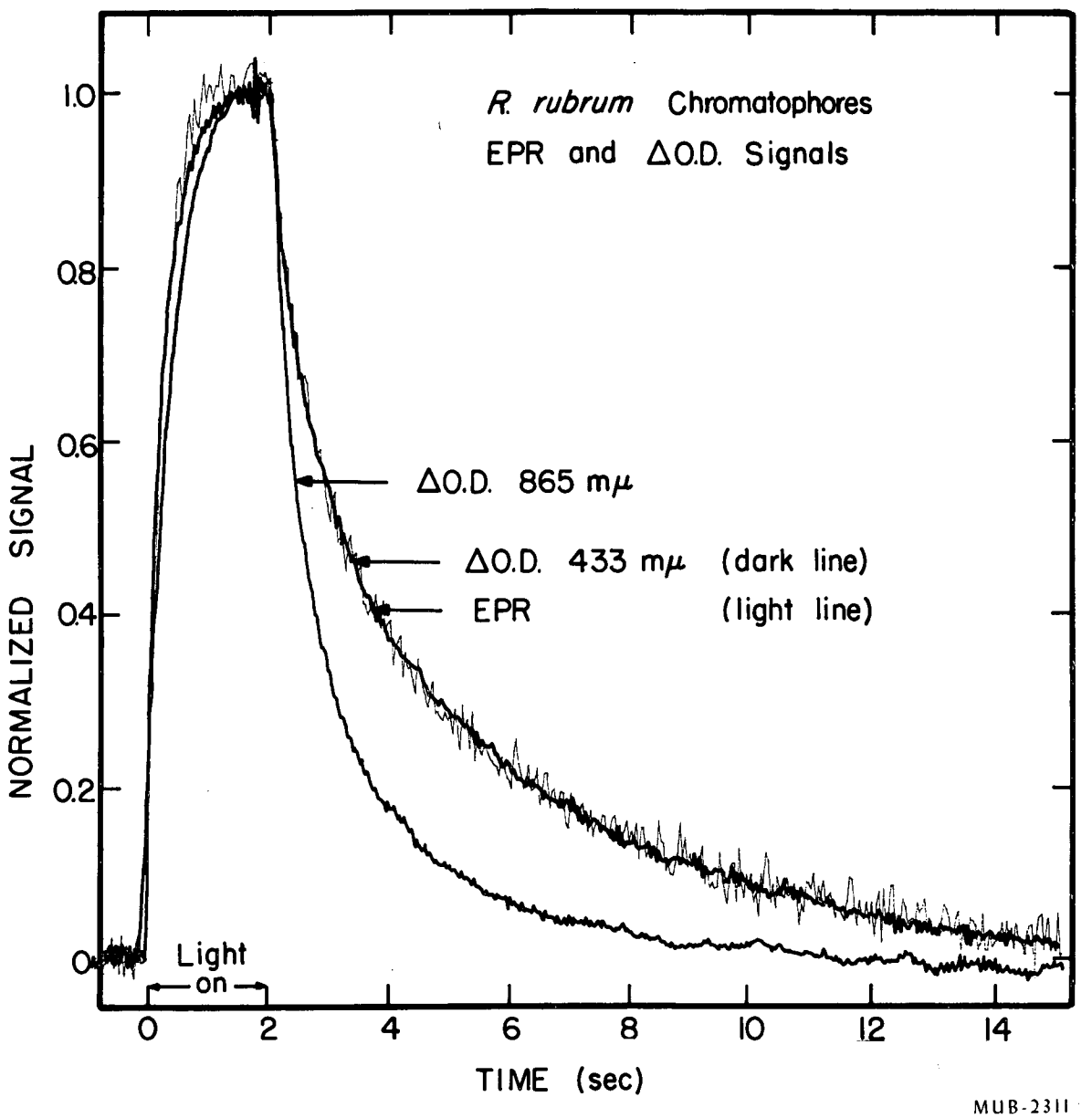
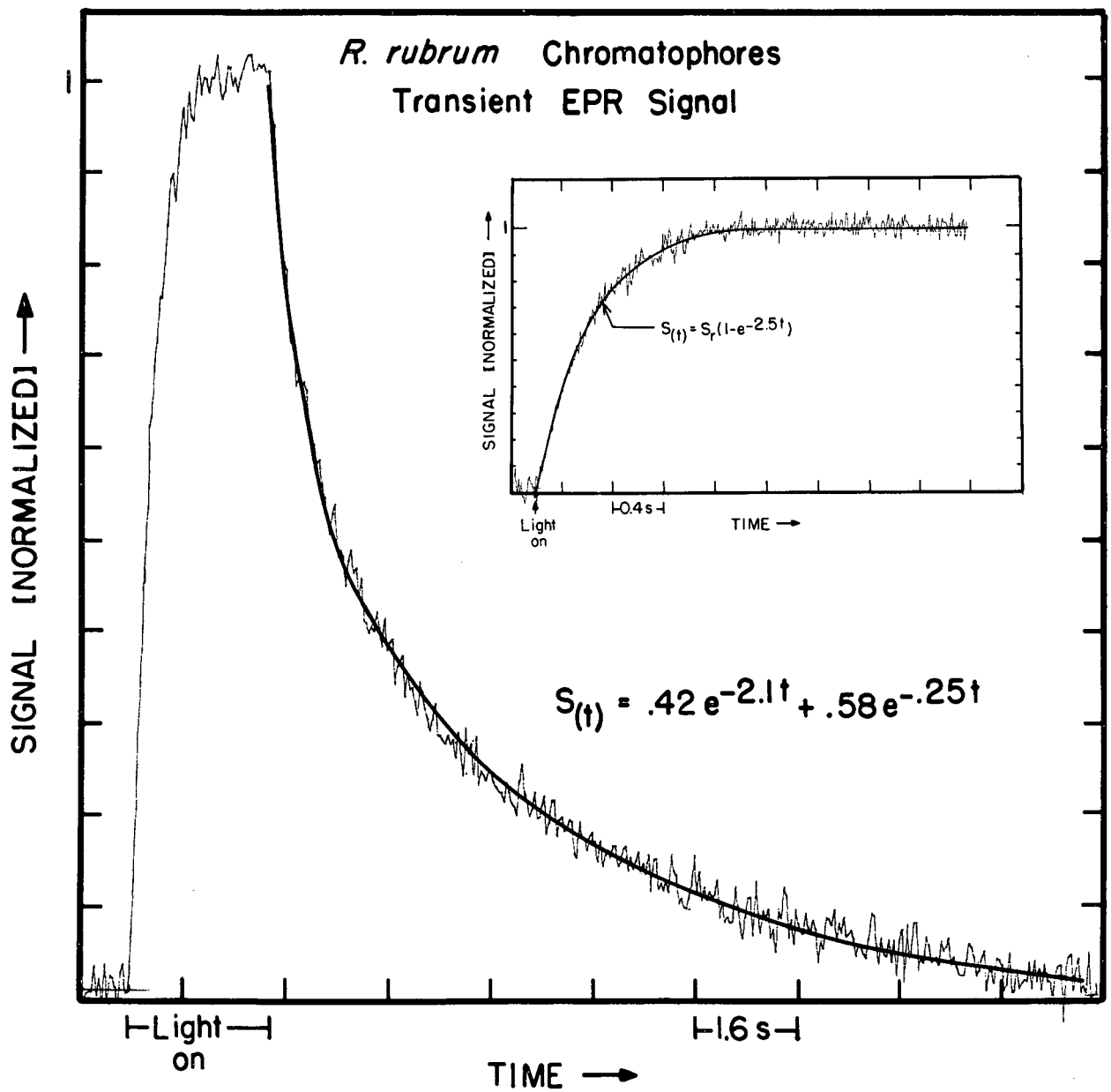
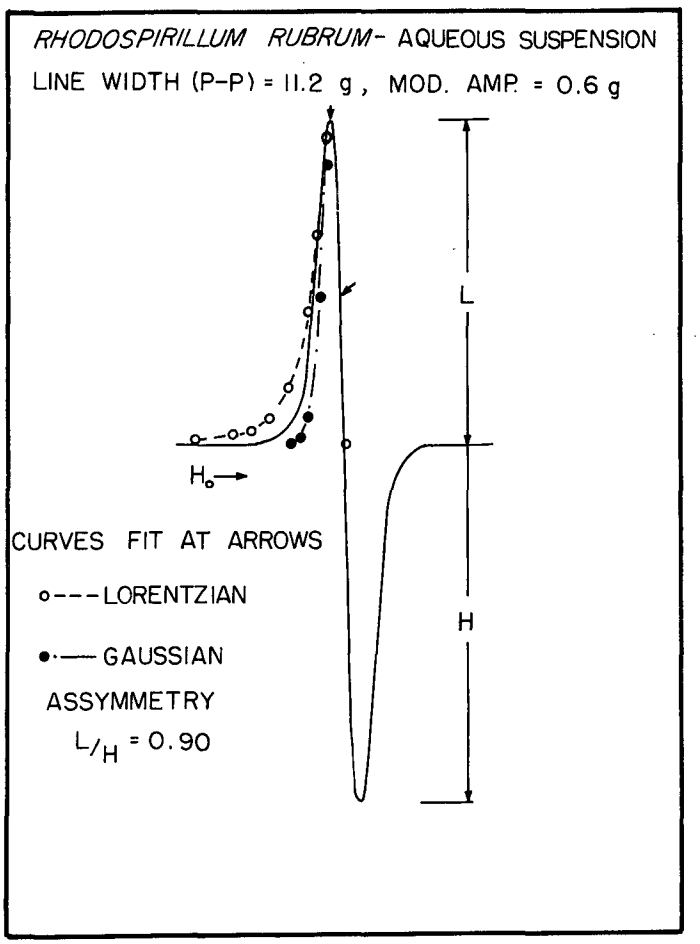


Fig. 10. Comparison of EPR and Δ O. D. signals from the same sample of R. rubrum chromatophores. E_n = +0.30, pH = 7.5, Temp. = 22 ± 2°, light intensity in a bandwidth between 5800Å and 7200 Å is 10^{16} photons/cm^2 sec., and chromatophores prepared from a 5 day growth.



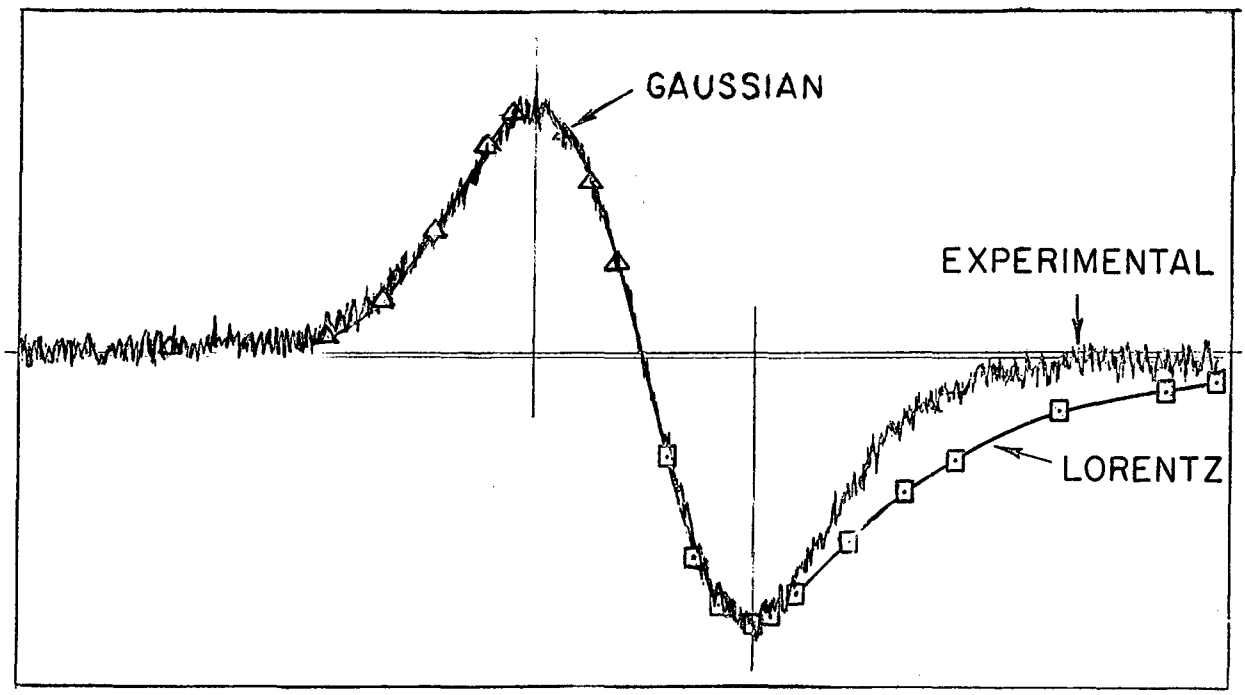
MUB-2312

Fig. 11. Time response of EPR signal to light. The insert is the growth of the EPR signal on an expanded time scale. Also shown are exponential curves fitting the data. S_t is the normalized steady-state value of the signal.



MU-23961

Fig. 12a. Line shape analysis of the EPR spectrum of R. rubrum in aqueous suspension at room temperature.



MUB-9322

Fig. 12b. Comparison of R (= Rapid) signal, obtained from material in which no slow signal was present, with calculated Gaussian and Lorentzian curves.

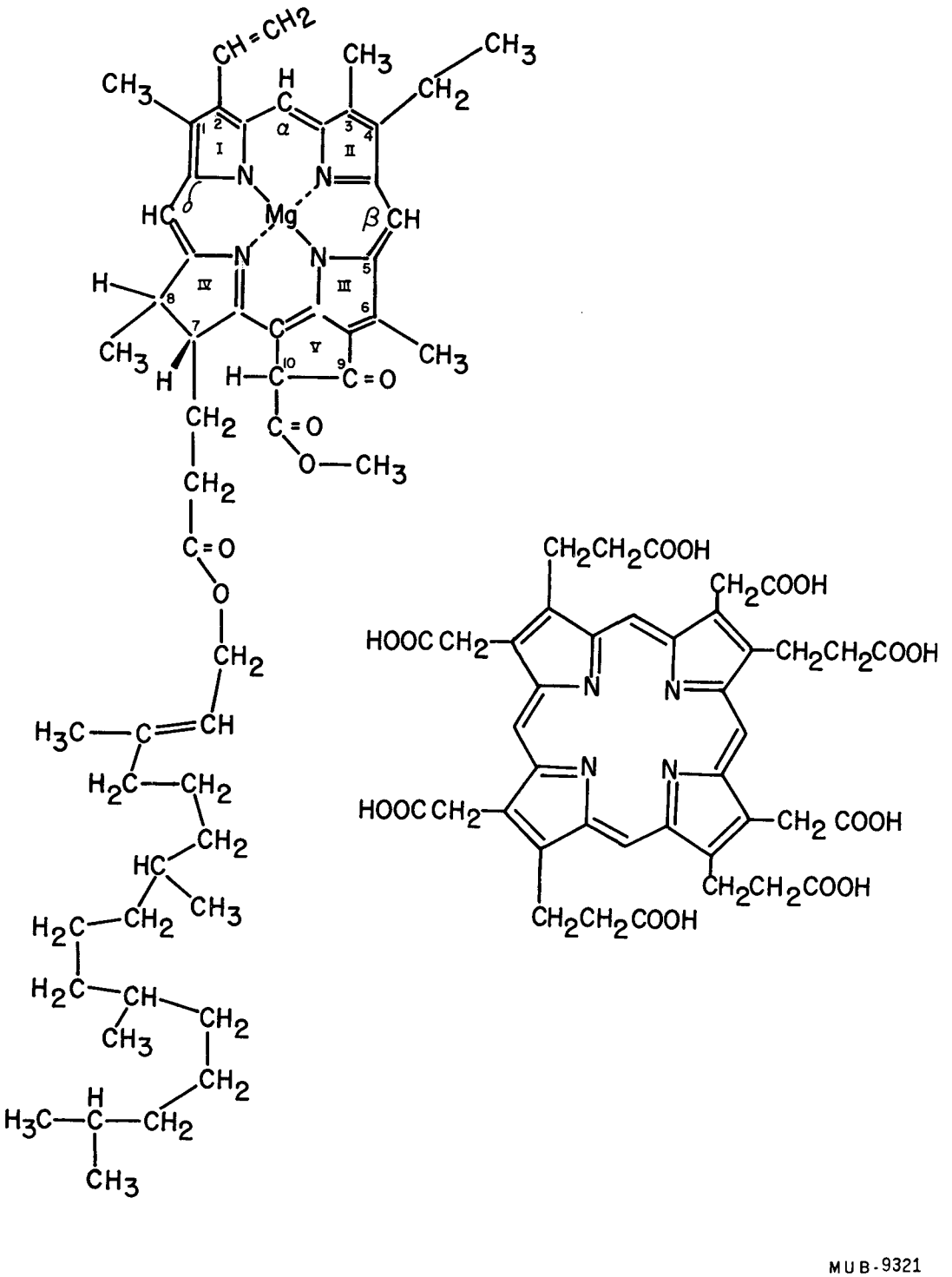


Fig. 13. A comparison of the structures of molecules of Chlorophyll and Uroporphyrin.

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.