

UCRL 8358

UNIVERSITY OF
CALIFORNIA

*Radiation
Laboratory*

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*

BERKELEY, CALIFORNIA

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

EVIDENCE FOR FREE-RADICAL PRODUCTION

IN PHOTOSYNTHESIZING SYSTEMS

F. Sogo, M. Jost and M. Calvin

July 1958

Berkeley, California

EVIDENCE FOR FREE-RADICAL PRODUCTION
IN PHOTOSYNTHESIZING SYSTEMS*

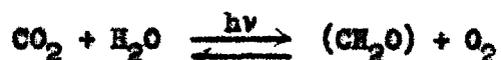
F. Sogo, M. Jost and M. Calvin

July 1958

Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

I. Introduction

The primary quantum conversion process in photosynthesizing systems, which involves the initial transformation of electromagnetic energy into chemical potential, presents a challenging unsolved problem. The overall process of photosynthesis can be represented by:



The energy required to reduce carbon dioxide to carbohydrate and liberate oxygen from water must be derived ultimately from the light quanta which are absorbed by the plant. The thermodynamic requirements are such that the energy of 4 quanta of red light is required to liberate 1 molecule of oxygen in green plants. Quantum requirements of about 4 to 8 have been measured^{1,2,10} for this process indicating that the overall efficiency of

* The work described in this paper was sponsored in part by the United States Atomic Energy Commission and in part by the Department of Chemistry, University of California, Berkeley, California.

the photosynthetic system is very high. If an allowance is made for some degradation of energy in enzymatic or chemical steps along the path of oxygen evolution, then one must ascribe an efficiency of the order of 70% or so for the initial storage of the absorbed light energy in the form of chemical potential. Efficiencies of this magnitude have not been achieved in solar energy converters manufactured in the laboratory. The rate at which the photosynthetic mechanism functions is quite rapid. Flashing light experiments^{3,7} indicate that a time of about .03 sec. is required between the light absorption act and the evolution of molecular oxygen.

A solid-state semiconductor model for the photosynthetic apparatus has been proposed^{3,9} in an effort to provide a mechanism for the primary quantum conversion process which is in accord with a large variety of experimental evidence. This model involves the production of unpaired electrons as intermediate energy carriers between the absorption of light by chlorophyll or an equivalent pigment and the formation of stable chemical oxidizing and reducing agents. A variety of unpaired electrons was postulated including mobile charge carriers, trapped electrons, and chemical free radicals.

In 1956, Commoner, et. al.,⁵ reported the observation of photo-induced unpaired electrons in plant material. In 1957, three papers appeared in the literature. Two, from this laboratory,^{4,8} extended the measurements as a function of temperature and the other, by the St. Louis group,⁶ presented a refinement of their previous room temperature data. The method used in the above work for the observation of the unpaired electrons is that of electron spin resonance.

II. Electron Spin Resonance

The technique of electron spin resonance utilizes the fact that the

electron possesses an angular momentum or spin and a magnetic dipole moment. In most molecules, all of the electrons are paired so that the resultant angular momentum and magnetic moment are zero. When one places a "free" electron or a molecule containing unpaired electrons in a magnetic field, magnetic energy levels are established which are designated by the parallel or anti-parallel orientation of the spin with respect to the applied field. The spacing of the levels is proportional to the applied field and transitions may be induced between the levels by an oscillating electromagnetic field of proper frequency. The observation of the absorption of energy from the oscillating field by the unpaired electrons constitutes the rudiments of electron spin resonance spectroscopy. Perturbations of the local field at the electrons by neighboring magnetic species may influence the resonance frequency and the width of the observed spin resonance absorption, and these factors may be of help in identifying the source of the signal. For "free" electrons in a laboratory field of 3300 gauss, the spin resonance frequency is about 10 Kmc which corresponds to a wavelength of 3 cm.

III. Apparatus

A block diagram of the apparatus used in these experiments is shown in Fig. 1. The optical system consists of a 300 watt tungsten-filament projection lamp with quartz lenses to collect and focus the light. A Kodak synchro-rapid shutter is used to establish the illumination period. The energy spectrum of the incident light is restricted to suitable wavelengths by a 2.5 cm. water filter in conjunction with Corning sharp-cut filters and Federal Engineering band-pass filters. The wavelength range used for the illumination of plant material containing chlorophyll extends from 5800 to

8000 Å and a range of about 7000 to 12000 Å was used for the material containing bacteriochlorophyll.

The electron spin resonance spectrometer operates at a frequency of about 9.6 Kmc and utilizes magnetic field modulation at 200 cps and lock-in detection to present the first derivative of the absorption. A balanced double-bolometer bridge is used to balance out low-frequency noise components in the klystron output. Automatic frequency stabilization of the klystron on the frequency of the resonant sample cavity is achieved by frequency modulation of the klystron at 10 kc and lock-in detection. A cylindrical transmission cavity operating in the TE_{011} mode is used. A small hole in the cavity wall with a diameter of about 1/4 inch admits the light, and a sleeve acting as a waveguide-beyond-cutoff prevents the leakage of appreciable microwave energy.

The sample may be placed in the cavity in two ways. A quartz dewar jacket which is open at both ends is placed in the cavity. The sample may then be put into a small glass tube and suspended in the dewar. The temperature of this type of sample is varied by blowing cold, dry nitrogen gas through the dewar. An alternative method consists of painting a thick aqueous suspension of the sample on a 5/32 inch diameter silver-plated copper rod and suspending the rod in the dewar. Temperature variation of this sample is achieved by immersing the end of the rod in a suitable coolant and relying on the thermal conductivity of the rod to carry the heat from the sample. The second method has the advantage of a high surface area to volume ratio so that most of the sample is available for light excitation even with optically dense materials.

IV. Results

A summary of the results is given in Table I. The wet, packed samples of

algae used in these experiments were prepared by centrifugation from the nutrient medium. The purple bacteria were filtered prior to centrifugation to remove inorganic crystals which exhibited a large electron spin resonance signal. Since the response time of the recorder used in these measurements is 1 second, the time constants having this value must be considered to be instrument limited.

Representative light and dark signals from *Rhodospirillum rubrum* are shown in Fig. 2. The line width between points of maximum slope of the light-induced signals is about 12 gauss and is independent of temperature down to at least -170°C . Fig. 3 shows the steady state signal height as a function of relative light intensity after about a 5 minute illumination period. It appears that the functional relationship between the number of unpaired electrons and the light intensity is dependent upon the temperature. A superposition of rise and decay curves at four temperatures is shown in Fig. 4.

The signals from spinach chloroplasts, *Chlorella*, *Scenedesmus*, *Nostoc*, *Anacystis*, and *Rhodospirillum rubrum* exhibit a progressive lengthening of decay times and a gradual decrease in signal intensity as the temperature is lowered from 25°C to -170°C . By contrast, as the purple bacteria are cooled, their signals present a lengthening of decay times only to a temperature of about -50°C and a peak of signal intensity at about -15°C . Below -50° , a progressively larger percentage of the decay is very fast (1 sec.) until at a temperature of about -100° , all of the decay is 1 sec. or faster. When light is allowed to shine continuously on the purple bacteria as the temperature is changed from $+25^{\circ}$ to -100° , the resulting dark signal is very large and has a long decay time of the order of hours. The behavior of the low temperature signal in the green material does not depend upon illumination conditions as the sample is cooled.

V. Discussion

The absence of resolvable hyperfine structure and the lack of a definitive change in line shape as a function of temperature prevents us from making a simple identification of the different spin centers which make up the resultant signal observed. The appearance of multiple rise times at intermediate temperatures indicates the formation of more than one kind of spin center while the multiple decays differing by orders of magnitude indicate a set of parallel processes leading to non-radicals.

We have examined a number of chemical mixtures and relatively pure chemicals under similar conditions to those described above for the structured plant elements. These included purified chlorophyll a and mixtures of chlorophyll a and chlorophyll b as well as mixtures of chlorophyll with carotene. In addition, we have examined mixtures from crude methanolic extracts of both the green organisms, such as Chlorella, and the purple organisms, such as Rhodospirillum rubrum.

We have seen both dark signals and photo-induced signals at room temperature but these signals (both dark and photo-induced) were markedly dependent upon the presence of oxygen, both during the preparation of the sample and during its measurement in the cavity. Oxygen enhanced both the dark signal and the photo-induced signal. Replacement of oxygen in the cavity by nitrogen reduced both the dark signal and the photo-induced signal in the extracts. This is in contrast to the behavior of the structured elements such as chloroplasts and whole bacteria whose signals were not sharply dependent upon the nature of the ambient gas in the cavity.

Another major point of difference between the two types of material was their sensitivity to temperature. The chemicals and extracts failed to produce

significant photo-induced signals at low temperatures, whereas, as described above, the structured elements not only produced them but produced them at the rapid rates comparable to those at room temperatures. In contrast, the photo-induced spin signals in the chemicals and extracts, which were obtainable only at the higher (room) temperatures, were relatively slow, particularly in decay.

Still another feature which distinguishes between the two types of spin signals is the line width which appears approximately twice as broad in the structured materials as it is in the chemicals and extracts (12/6 gauss*).

It would appear that the nature of the spin signals induced in the chemicals and extracts does indeed represent chemical, or photochemical, transformations (that is, transformations involving the displacement of atomic nuclei), while the induction of at least some of the spin signals in the structured elements does not involve such displacements but rather can be best described in terms of electron and hole migration and trapping.

* Calibrated with nitrosyldisulfonate.

References

1. Bassham, J. A., Shibata, K., and Calvin, M., Biochim. Biophys. Acta 17, 332 (1955).
2. Brackett, F. S., Olson, R. A., and Crickard, R. G., J. Gen. Physiol. 36, 529 (1953).
3. Bradley, D. F., and Calvin, M., Proc. Natl. Acad. Sci. U.S. 41, 563 (1955).
4. Calvin, M., and Sogo, P. B., Science 125, 499 (1957).
5. Commner, B., Heise, J. J., and Townsend, J., Proc. Natl. Acad. Sci. U.S. 42, 710 (1956).
6. Commner, B., Heise, J. J., Townsend, J., et al., Science 126, 57 (1957).
7. Emerson, R., and Arnold, W., J. Gen. Physiol. 16, 191, (1932).
8. Sogo, P. B., Pon, N. G., and Calvin, M., Proc. Natl. Acad. Sci. U.S. 43, 387 (1957).
9. Tollin, G., Sogo, P. B., and Calvin, M., New York Academy of Sciences Conference on Photoreception, Jan. 31 to Feb. 1, 1958, in press.
10. Warburg, O., Kruppahl, G., Buchholz, W., and Schroder, W., Z. Naturf. 8 (b), 675 (1953).

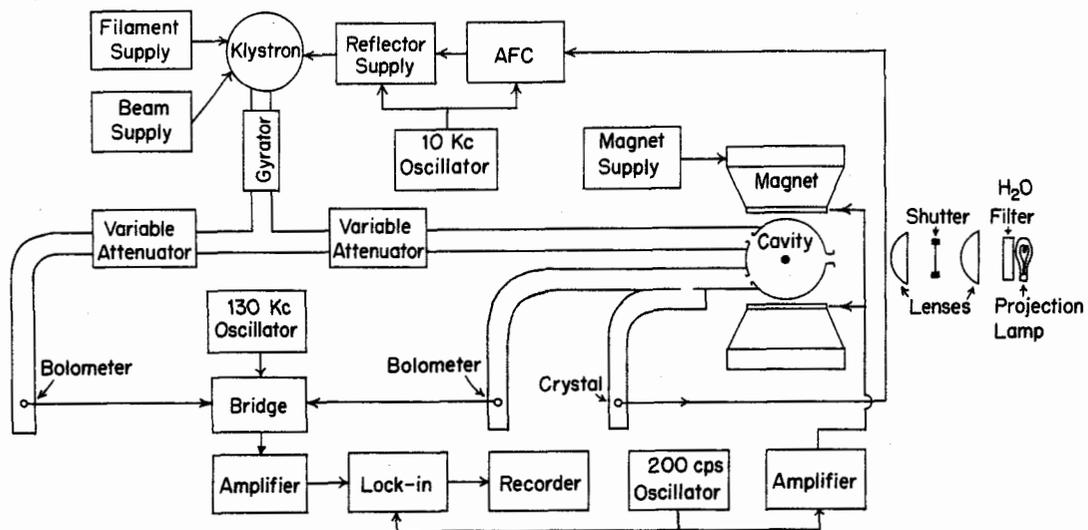
Table 1

RISE AND DECAY TIMES FOR THE PHOTO-INDUCED
ESR SIGNAL IN PHOTOSYNTHETIC MATERIAL

Material	Temp. (°C)	Rise Times	Decay Times
Spinach Chloroplasts Chlorella Scenedesmus	25	1 sec	1 sec (50%) 10 sec (50%)
	-40	1 sec (75%) 10 sec (25%)	1 sec (33%) 10 sec (33%) 5 min (33%)
	-140	1 sec	10 sec (20%) very long (80%)
Romaria, Nostoc Anacystis	25	1 sec	1 sec
	-160	1 sec	10 sec (25%) very long (75%)
Wet Chromatium chromatophores	25	1 sec	2 sec (50%) 15 sec (50%)
	-40	1 sec	5 sec very long
	-140	1 sec	1 sec
Rhodospirillum rubrum	25	1 sec	1 sec
Rhodopseudomonas spheroides Chromatium	-15	1 sec	15 sec to mins
	-55	1 sec (50%) 7 sec (50%)	1 sec (33%) 10 sec (33%) 1 min (33%)
	-120 to -170	1 sec	1 sec

MU-15488

Table I



MU-15489

Fig. 1. Block diagram of esr spectrometer and optical system.

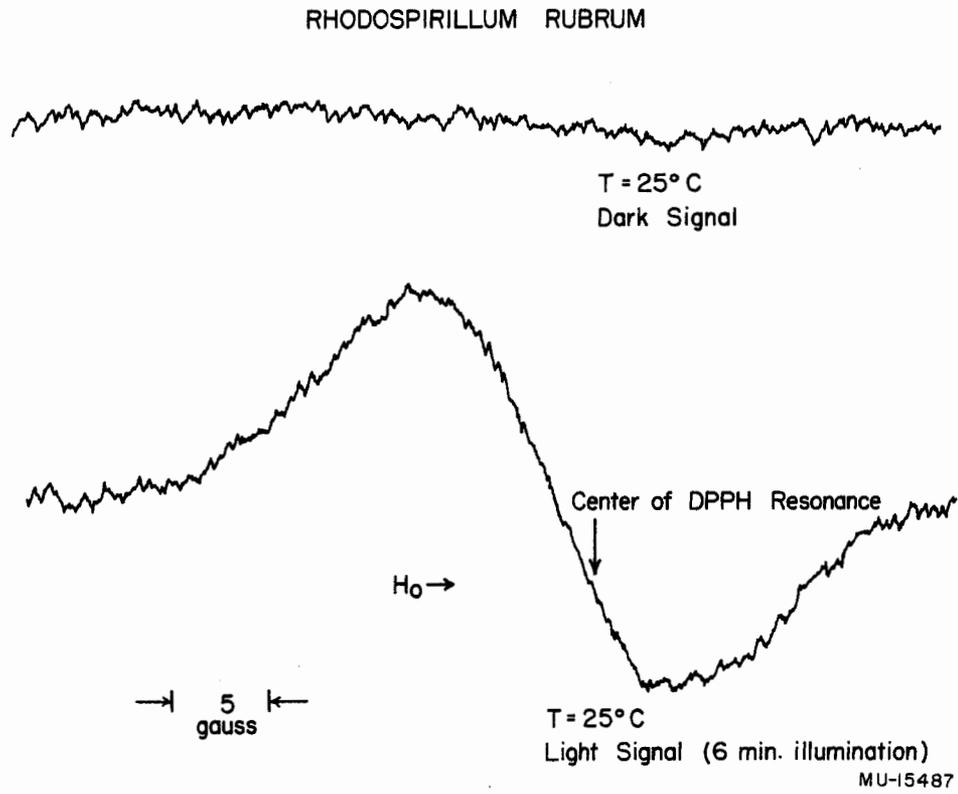


Fig. 2a. Light and dark signals from Rhodospirillum Rubrum at 25°C.

RHODOSPIRILLUM RUBRUM



T = -170°C
Dark Signal
(Taken to -170°C in dark)

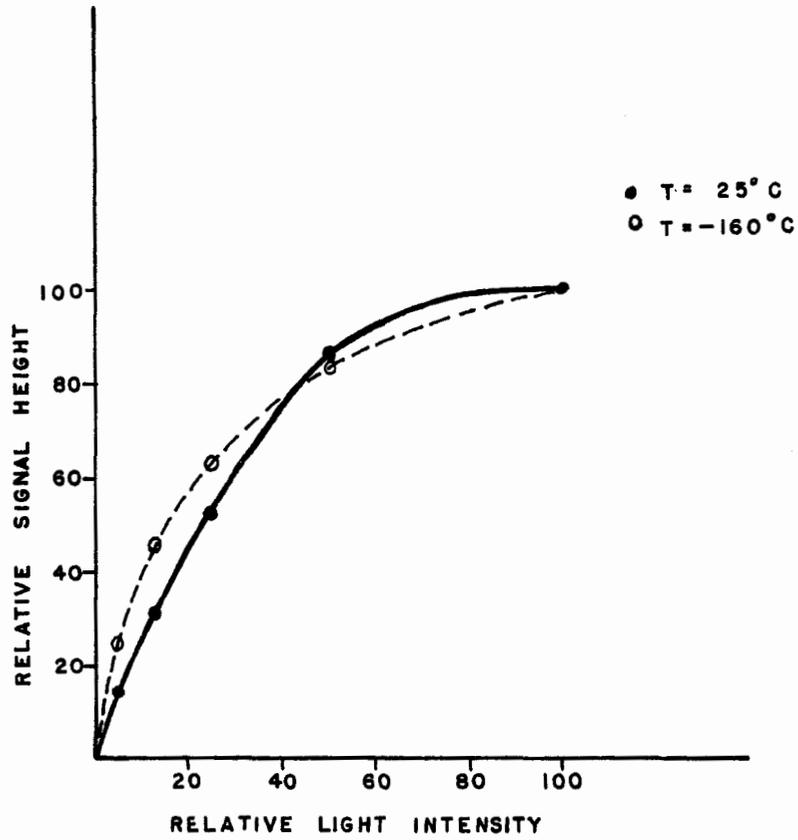


→ | 5 | ←
gauss

T = -170°C
Light Signal
(Taken to -170°C in dark)

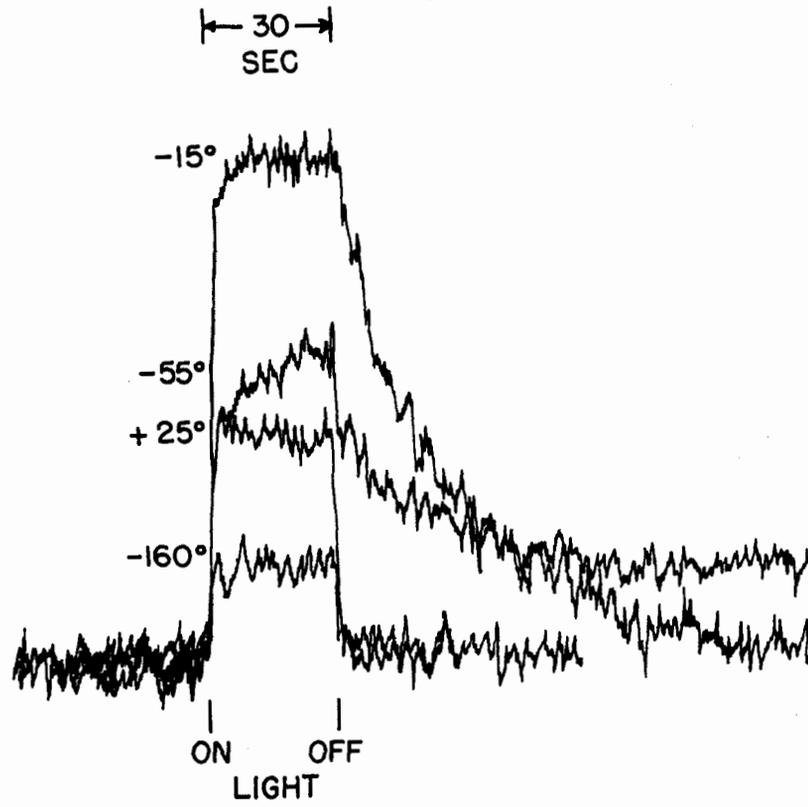
MU-15486

Fig. 2b. Light and dark signals from Rhodospirillum Rubrum at -170°C.



MU-15490

Fig. 3. Signal height vs. light intensity for *Rhodospirillum rubrum*.



MU-15138

Fig. 4. Rise and decay of ESR signals from *Rhodospirillum Rubrum*.