* Part of this report is taken from a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree in the Graduate School, University of Wisconsin, Madison.

† Present address: Hercules Research Center, Wilmington, Delaware 19899.

[1] Goldberg, R. J., *J. Phys. Chem.*, **57**, 194 (1953).

[2] Williams, J. W., K. E. Van Holde, R. L. Baldwin, and H. Fujita, *Chem. Revs.*, **58**, 715 (1958).

[3] Fujita, H., *Mathematical Theory of Sedimentation Analysis* (New York: Academic Press, 1962), chap. 5.

[4] Lansing, W. D., and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

[5] For unequal partial specific volumes of the solute components one measures a quantity $\bar{M}_1$ (Van Holde, K. E., and R. L. Baldwin, *J. Phys. Chem.*, **62**, 734 (1958)), where

$$\bar{M}_1 = \frac{(c_b - c_a).2\,RT}{c_s\,(1 - \bar{v}_w\rho)\,\omega^2\,(b^2 - a^2)}.$$

Here, $\bar{v}_w$ = weight average partial specific volume

$$M_1 = \frac{\sum_q^q c_i M_i \phi_i\,(1 - \bar{v}_i\rho)}{\sum_q^q c_i\,\phi_i\,(1 - \bar{v}_i\rho)}$$

$\phi_i = \partial n/\partial c_i$ = refractive index increment for each solute species i

$\bar{v}_i$ = partial specific volume of each solute species

$(1 - \bar{v}_w\rho) = \sum c_i(1 - \bar{v}_i\rho)/c$

ρ = density of the solution.

[6] Stille, J. K., *Introduction to Polymer Chemistry* (New York: John Wiley and Sons, 1962), p. 39.

[7] Hexner, P. D., R. D. Boyle, and J. W. Beams, *J. Phys. Chem.*, **66**, 1948 (1962).

[8] Squire, P. G., and C. H. Li, *J. Am. Chem. Soc.*, **83**, 3521 (1961).

[9] Adams, E. T., Jr., Ph.D. thesis, University of Wisconsin, 1962.

[10] Jeffrey, P. D., and J. Coates, *Nature*, **197**, 1104 (1963).

[11] Adams, E. T., Jr., and H. Fujita, in *Ultracentrifugal Analysis in Theory and Experiment*, ed. J. W. Williams (New York: Academic Press, 1963), p. 119.

[12] Ludwig, M. L., M. J. Hunter, and J. L. Oncley, paper presented at the 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April 5–14, 1960; Oncley, J. L., personal communication.

[13] Tiselius, A., *Z. physik. Chem.*, **124**, 449 (1926).

[14] Svedberg, T., and K. O. Pedersen, *The Ultracentrifuge* (Oxford: Clarendon Press, 1940), pp. 54–56.

[15] Fujita, H., *J. Chem. Phys.*, **32**, 1739 (1960).

---

*TRANSIENT EPR AND ABSORBANCE CHANGES IN PHOTOSYNTHETIC BACTERIA**

BY R. H. RUBY, I. D. KUNTZ, JR.,† AND M. CALVIN

LAWRENCE RADIATION LABORATORY, DEPARTMENT OF PHYSICS, AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated January 17, 1964

Light-induced electron paramagnetic resonance (EPR) signals have been observed in photosynthetic systems for many years. These observations have been discussed in several recent review papers.[1, 2] A positive identification of the signal with a definite molecular species has proved difficult on the basis of EPR properties alone. Thus, correlations of these properties with other physical measurements

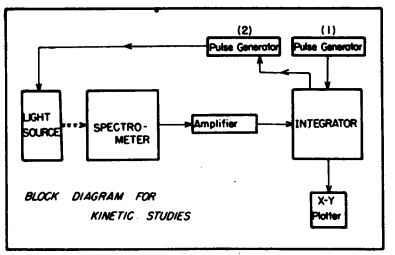

FIG. 1.—Block diagram for kinetic measurements. Operation is described in text. The appropriate spectrometer (optical or EPR) was inserted as desired.

have been sought. Independent studies of absorbance changes in photosynthetic bacteria[3, 4, 7] have led to the tentative detection of bacteriochlorophyll positive ion, thereby leading to the working hypothesis that the EPR signal might be $BChl^+$.[1] This view was strengthened by recent work showing that the bacterial spin signal could be produced by chemical oxidation.[5] This paper, by means of kinetic studies of both spectroscopic systems, presents evidence which is not consistent with the above hypothesis.

Experimental.—The kinetics studied in this paper are the EPR and absorbance transients induced by a pulse of light. The experimental arrangement used in both experiments is shown in Figure 1. The EPR and optical spectrometers used have been described in earlier publications.[6, 7] An electronically modulated neon lamp was used to induce the photo signals. Its design was kindly made available for our use by Dr. L. Piette of Varian Associates. Most of the details of its

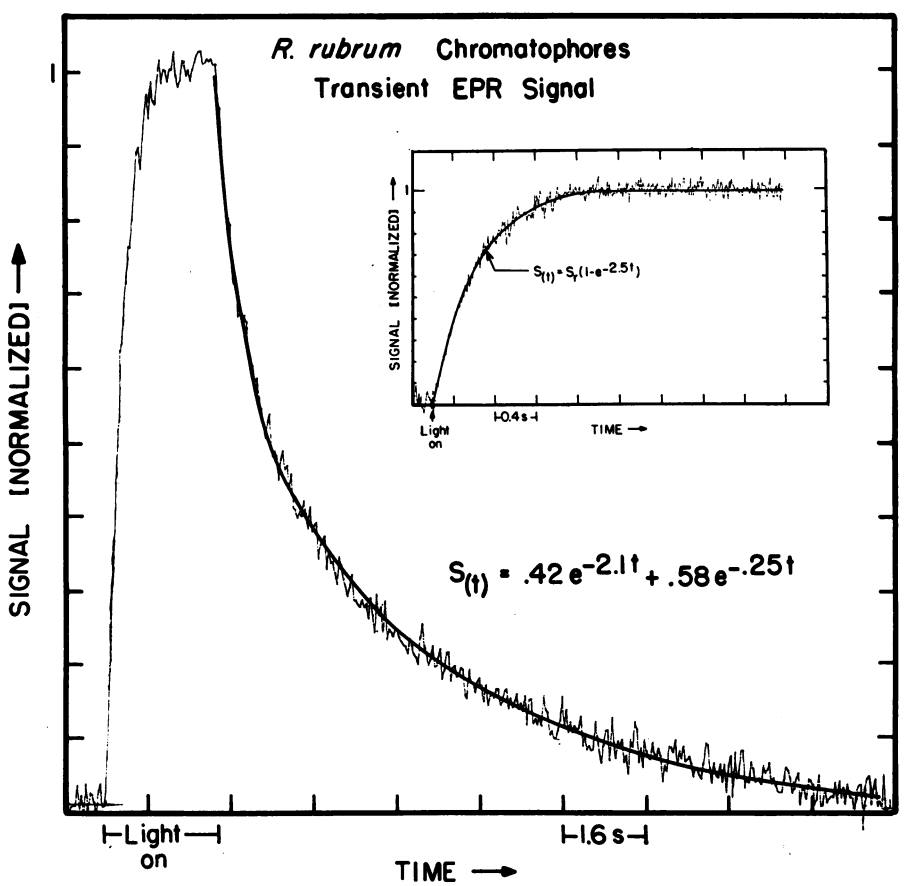

FIG. 2.—Time response of the electron paramagnetic resonance (EPR) signal to light. The insert is the growth of the EPR signal on an expanded time scale. Also shown are exponential curves fitting the data. S_r is the normalized steady-state value of the signal.

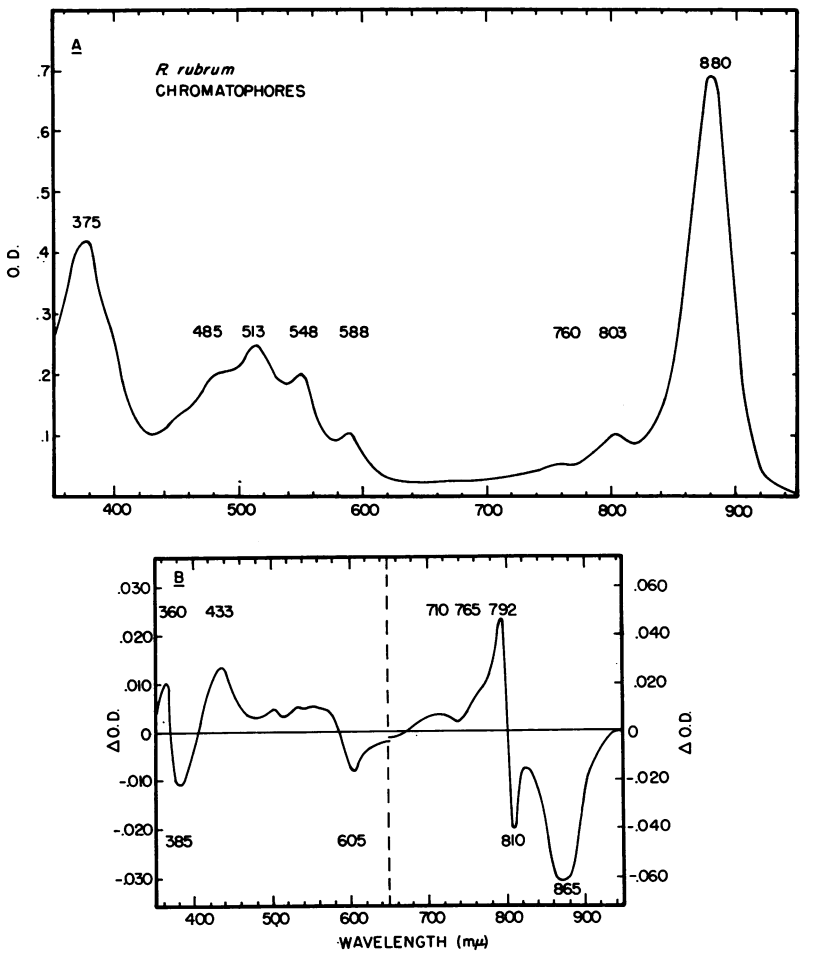

FIG. 3.—(*A*) Absorption spectrum of *R. rubrum* chromatophores suspended in 0.01 *M* phosphate buffer, pH 7.13; 1-cm cuvette. (*B*) Light-induced absorption changes in *R. rubrum* chromatophores, whose absorbance was 2.2 at 880 mμ. Excitation wavelengths were 650–900 mμ for the blue absorption changes, 400–500 mμ for the infrared absorption changes. Note that the absorbance scale below 650 mμ is expanded twofold. For further experimental details, see ref. 7.

circuitry are in the literature.[8] The spectral output was primarily in the wavelength region between 580 and 720 mμ. Rise and decay times were of the order of 10 μsec. The signal resulting from a single flash was too noisy to allow the determination of the full time course of its growth and decay. To improve the signal-to-noise ratio, the integration technique of Klein and Barton[9] was used. We used the commercial integrator, the Computer of Average Transients Model 400 (Mnemotron Corp., Pearl River, New York). The over-all rise time with each spectrometer was less than 1 msec.

The sequence of events was as follows: The repetition rate was determined by a pulse generator which initiated a sweep of the integrator. The integrator triggered the lamp after a fixed delay period. The second pulse generator determined the duration of the flash. The observation time of the integrator could be set for any fraction of the repetition time. Typical flash durations were 2 sec, repetition times 16 sec, and integrator sweep time ranged from 1 to 16 sec. Suitable signal-to-noise ratios were obtained after 50 to 500 events.

Chromatophores were prepared from *Rhodospirillum rubrum* (originally supplied by R. Y. Stanier, #1.1.1.). The samples were harvested after 5 days of growth in modified Hutner's

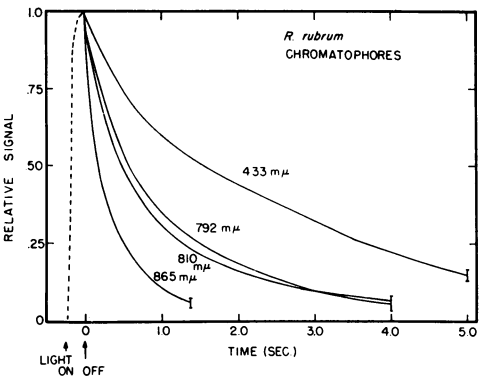

FIG. 4.—Typical time response of light-induced absorbance changes in *R. rubrum* chromatophores. For purposes of comparison, signal heights are normalized, and all signals are shown as positive 0.10^{-4} *M* KuFe $(CN)_6$ present, E_h = +0.35 volt; 0.01 *M* phosphate buffer, pH 7.4; O.D. at 880 mμ, 0.70. Further details are given in ref. 7.

medium,[10] using malate as substrate. The chromatophores were prepared as outlined in the paper of Loach *et al.*[5] The sample was buffered at a pH of 7.5 with 0.1 *N* glycylglycine.

To minimize differences in the experimental conditions, the same sample contained in a Varian EPR aqueous sample cell was used in both spectrometers. Typical optical densities at 880 mμ were 1.5.

The following factors were found to affect rise and/or decay rates: redox potential, pH, temperature, light intensity, and the physiological state of the organisms and the preparation and storage of the chromatophores. These were held at the following values for both the EPR and absorbance experiments: +0.30 volts, 7.5 pH units, 22 ± 2°, 10^{16} photons/cm²/sec, 5-day growth, and variable storage. The light intensity was measured with a photodiode which was calibrated against a U.S. National Bureau of Standards lamp.

The EPR signal, $S_{(t)}$, was measured at the point of maximum slope of the absorption curve, and is proportional to the number of observable unpaired electrons. The response of this signal to the light pulse is shown in Figure 2. Also shown is an example of the growth of the signal when the light is turned on (when an expanded time scale is used). The growth may be described by the expression

$$S_t = S_r\,(1 - e^{k_r t}) \tag{1}$$

and the decay curve by the expression

$$S_{(t)} = S_d e^{-k_d t} + S_d' e^{-k_d' t}, \quad \text{with} \quad S_r = S_d + S_d'. \tag{2}$$

S_r is proportional to the steady-state of photoproduced spins, S_d and S_d' are proportional to the fraction of photoproduced spins decaying by parallel paths with unimolecular rate constants k_d and k_d'. k_r is approximately the unimolecular rate constant for spin production.

A typical absorption spectrum and a light-minus-dark difference spectrum for the chromatophores used are shown in Figure 3. We are here concerned with the major light-minus-dark bands at 433 mμ, 792 mμ, 810 mμ, and 865 mμ. The responses of these signals to light are shown in Figure 4. The shapes of these curves can be expressed by equations of the form of (1) and (2) above.[7] In both the EPR and the optical absorption measurements the decay rates were found to be approximately independent of the light intensity. This, together with the simple exponential behavior (Fig. 2 and ref. 7), indicates that these processes obey first-order kinetics.

To demonstrate the relation between the two spectroscopic observations, the time response (course) of the signals from the same sample have been plotted together (Fig. 5). The steady-state magnitude of each signal was normalized to unity.

Conclusions.—The following conclusions may be drawn from this evidence: (1) The rise and decay kinetics of the spin signal are the same as the kinetics of the 433-mμ absorbance changes, within experimental error. Of the major absorbance changes, only the one at 433 mμ shows this close agreement. We thus assign the observed EPR signal to the molecular species which produces the 433 mμ optical change. (2) The molecules responsible for the absorbance change at 433 mμ are not the same as those molecules responsible for the absorbance change at 865 mμ because of the much slower decay rate of the 433 mμ band, as was earlier reported.[7]

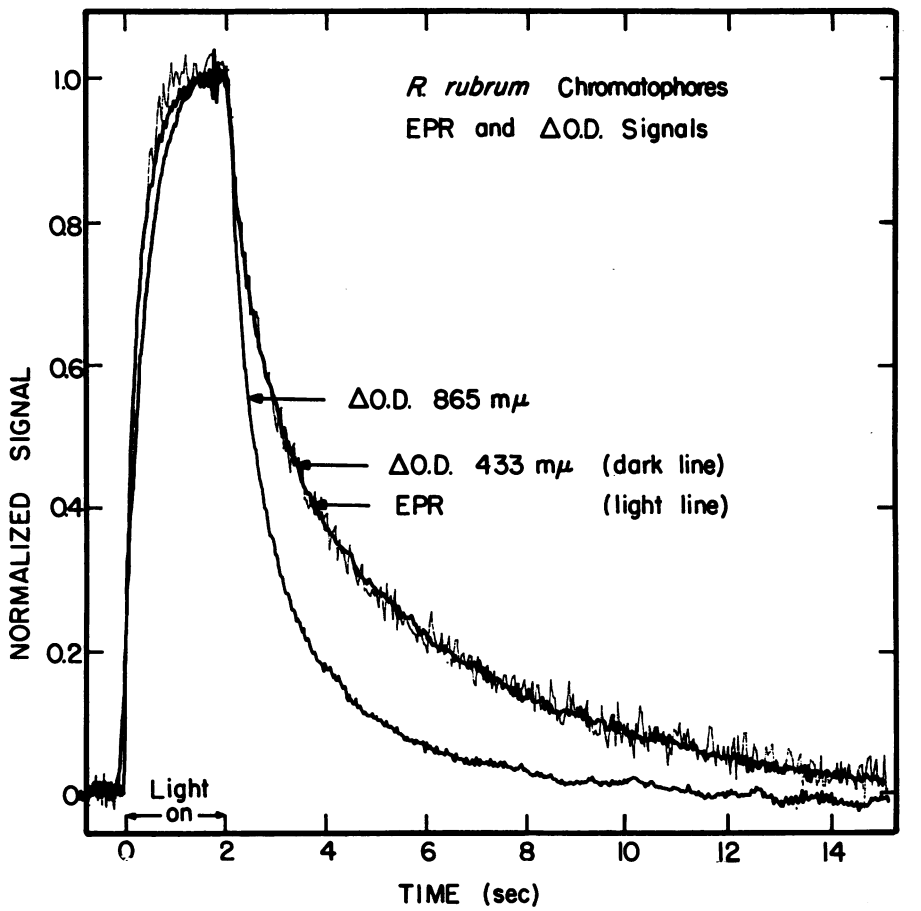

FIG. 5.—Comparison of EPR and ΔO.D. signals from the same sample of *R. rubrum* chromatophores. Experimental conditions are given in the text.

That a relationship between optical density changes and EPR signals existed was apparent from the experiments of Clayton[3] and Loach *et al.*[5] In these experiments all the light-induced optical absorbance changes and the EPR signal were removed upon oxidation and were replaced by a "dark" signal of the same magnitude. However, no choice among the optical signals could be made, to identify the source of the EPR signal.

The spectral position of the 865 mμ band with respect to the *in vivo* absorption of BChl and its interaction with redox reagents has been used to identify this change with a one-electron photoinduced oxidation of BChl in the organized environment.[3-5] Based on this identification of the optical density change at 865 mμ and the above kinetic data, oxidized bacteriochlorophyll in the organized environment of the chromatophore is not the site of the unpaired electron producing the observed electron paramagnetic resonance signal. Such a molecular species ($BChl^+$) in solution would be expected to show an EPR signal characteristic of a free radical. That we do not see one could be explained by an interaction between this electron and its environment which broadens the resonance line. Such an interaction may

raise from delocalization, among several BChl molecules, of the charge associated with the oxidized BChl. A delocalization of this sort can be used as the conduction system for separation of charge in the primary quantum conversion act.

We would like to express our appreciation for the technical assistance given by Miss Ann Maksim and invaluable discussion with Dr. M. P. Klein.

* The work described in this paper was sponsored in part by the U.S. Atomic Energy Commission.

† National Science Foundation fellow, 1962–1964.

[1] Calvin, M., and G. M. Androes, *Science*, **138**, 867 (1962).

[2] Commoner, B., in *Light and Life*, ed. W. D. McElroy and B. Glass (Baltimore: Johns Hopkins Press, 1961), p. 356.

[3] Clayton, R. K., *Photochem. and Photobiol.*, **1**, 201 (1962).

[4] Duysens, L. N. M., in *The Photochemical Apparatus—Its Structure and Function*, Brookhaven Symposia in Biology, No. 11 (1958), p. 325.

[5] Loach, P. A., G. M. Androes, A. F. Maksim, and M. Calvin, *Photochem. and Photobiol.*, **2**, 443 (1963).

[6] Androes, G. M., *Adv. Bot. Research*, **1**, 326 (1963).

[7] Kuntz, I. D., Jr., P. A. Loach, and M. Calvin, *Biophys. J.*, in press.

[8] Bell, W. E., A. L. Bloom, and J. Lynch, *Rev. Sci. Inst.*, **32**, 688 (1961).

[9] Klein, M. P., and G. W. Barton, *Rev. Sci. Inst.*, **34**, 754 (1963).

[10] Cohen-Bazire, W., R. Sistrom, and R. Y. Stanier, *J. Cell. Comp. Physiol.*, **49**, 29 (1957).

THE DISTRIBUTION OF BUOYANT DENSITY OF HUMAN ERYTHROCYTES IN BOVINE ALBUMIN SOLUTIONS

BY ROBERT C. LEIF AND JEROME VINOGRAD

GATES AND CRELLIN LABORATORIES OF CHEMISTRY* AND THE NORMAN W. CHURCH LABORATORY OF CHEMICAL BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated by Norman Davidson, January 2, 1964

Several reports have appeared in the literature of the variation of "density" among different types of dispersed cells and of physical separations based upon this property.[1, 2] Two general separation procedures have been used. *Packed cell methods:* cells are centrifuged from a suspension to form a viscous mass of packed cells, which is separated layerwise. *Neutral density separations:* cells are centrifuged in a dense liquid medium and are segregated into two fractions, one denser and one lighter than the suspending medium. With such methods the order of density of certain types of blood cells has been established.[3–5] It has also been shown that erythrocytes are heterogeneous in density and that young erythrocytes are less dense than old.[6–9]

The above procedures are not suitable for the quantitative investigation of the density distribution in cell populations. The packed cell mass is difficult to fractionate. The neutral density procedure yields only two fractions in each experiment. These restrictions may be overcome by centrifuging the cells in a buoyant density gradient.[2, 10, 11]

The present communication describes a method for the quantitative fractionation of erythrocytes in a linear density gradient of bovine serum albumin. It is shown