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THE FIRST STEP IN PHOTOSYNTHESIS: EVIDENCE FOR ITS ELECTRONIC NATURE

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Communicated by C. B. van Niel, April 27, 1960

Emerson and Arnold¹ nearly thirty years ago showed that the act of photosynthesis is not carried out by one chlorophyll molecule but by the cooperation of several hundred. These results were verified and extended by Gaffron and Wohl,² by Kohn,³ by Tamiya,⁴ and others. Szent-Györgyi⁵ suggested in 1941 that the cooperation was by way of electronic conduction bands in protein, but Evans and Gergely⁶ pointed out that any band in protein would be too high on the energy scale to play a part in photosynthesis. Katz,⁷ in 1949, suggested that chlorophyll in lamellae formed a two-dimensional crystal with a two-dimensional band system for the conduction of electrons and holes. The idea that semiconduction plays a part in photosynthesis was discussed further by Bassham and Calvin⁸ and by a number of others. Arnold and Sherwood⁹ demonstrated that dried chloroplasts act as semiconductors.

In the present paper we give new experimental evidence that in the purple bacteria the first step in photosynthesis appears to be purely *electronic* in nature. We have found in chromatophores a new class of reversible spectral changes that are the same from 300°K down to 1°K. At 1°K no ordinary chemical reaction can take place, and the fact that the spectral changes are the same over such a wide range of temperatures shows that no energy of activation is involved. We have evidence that the process indicated by these spectral changes immediately precedes the oxidation-reduction of the cytochromes that has been studied by a number of workers since Duysens' initial observation.¹⁰ And finally we have an electrical experiment on dried chromatophores, showing that upon illumination the positive and negative electric charges are spatially separated.

Materials and Methods.—*Rhodospseudomonas spheroides*, wild type strain 2.4.1 (from Prof. C. B. van Niel's laboratory) and the carotenoidless¹¹ mutant strain UV-33 (obtained from Dr. W. R. Sistrom) were cultivated anaerobically in the light in a medium described by Cohen-Bazire *et al.*¹² Films of dried chromatophores were prepared as follows: The cells were suspended in distilled water and disrupted sonically; the sonic extract was clarified by centrifuging at 20,000 × *g*. Chromatophores in the extract were collected and washed twice with distilled water by successive 90-min centrifugations at 100,000 × *g*. The final aqueous suspension, dried on a glass plate, formed an optically clear film, stable at least for months and having the same absorption spectrum as the intact cells.

Spectral changes in these films were observed with a Beckman DK-1 spectro-

photometer, modified to admit exciting light to the films and simultaneously to measure their absorption spectra. The monochromatic measuring beam passed through the chromatophore film and on to the detector. Simultaneously a beam of exciting light, perpendicular to the measuring beam, could be projected onto the film. By means of color filters, the detector was made to respond to the measuring beam and not to the exciting beam. Thus by using infrared exciting light and a blue filter in the measuring beam, changes in absorbancy could be measured over the range 340–670 $m\mu$. By passing the exciting light through a $CuSO_4$ solution and placing a red filter in the measuring beam, the absorbancy could be measured from 640 to 2,500 $m\mu$. For some applications the transmitted light was measured with an external photomultiplier used with a Sanborn amplifier-recorder or a Brown potentiometer.

For measurement at low temperature a conventional cryostat was constructed, consisting of a Dewar flask containing liquid helium jacketed by a Dewar flask containing liquid nitrogen. The helium chamber was pumped to a low pressure, in order to attain a temperature of 1°K. The bottom of this arrangement, containing a chromatophore film immersed in liquid helium, projected into the light path of the spectrophotometer.

FIG. 1.—Difference spectrum, light minus dark, for dried chromatophores of wild type *R. spheroides* at room temperature (300° K). The ordinate is normalized for a chromatophore film whose optical density at 590 $m\mu$ is 1.0.

Delayed light, from chromatophore films in a modified Becquerel phosphoroscope, was measured by a photomultiplier, vibrating reed electrometer, and Brown potentiometer.

Spectral Changes.—Dried chromatophore films: Spectral changes in illuminated photosynthetic tissues have been described often, e.g. by Smith *et al.*,¹³ by Dyu-sens,¹⁴ and by Kok.¹⁵ At the Brookhaven Symposium on Bioenergetics in the fall of 1959, Chance and Nishimura¹⁶ reported changes in the absorption spectrum of *Chromatium* upon illumination at liquid nitrogen temperature. Their observations prompted us to try films of dried chromatophores.

Dried chromatophore films show rapid, reversible spectral changes upon illumination; the exciting light must be of a color absorbed by the bacteriochlorophyll or the carotenoids. The response occurs unchanged through hundreds of repetitions.

At 1°K the change in absorbancy shows essentially the same spectrum, the same kinetics, and the same dependence on exciting light intensity as at 77°K. The magnitude of the effect changes little from 1 to 300°K. At 1°K this phenomenon could be caused only by the excitation and migration of electrons.

Figure 1 shows the difference spectrum (light minus dark) for a film of wild type

R. spheroides chromatophores at 300°K. Figure 2 shows the difference spectrum at 300°K for the carotenoidless mutant. These difference spectra exhibit waves around all of the bacteriochlorophyll absorption bands (in the wild type these bands are at 375, 590, 800, 850, and 875 $m\mu$). The waves correspond to a shift in the positions of all the bands toward shorter wavelengths, plus a slight diminution and sharpening of the bands. Measurement of scattered light showed that these spectra represent changes in absorption and not in scattering. Other changes, associated with colored

FIG. 2.—Same as Figure 1 for the carotenoidless mutant of *R. spheroides*.

carotenoids, can be discriminated by comparing Figures 1 and 2. Absorption spectra of these films change slightly as the temperature is reduced from 300 to 77°K and to 1°K.¹⁷ The difference spectra show corresponding changes, preserving the appearance of a shift in each bacteriochlorophyll absorption band toward shorter wavelengths.

FIG. 3.—Time course of the change in transmission at 610 $m\mu$ for dried chromatophores of wild type *R. spheroides* at 300°K., exposed to two intensities of exciting light (15 and 340 arbitrary units, respectively). A Sanborn amplifier-recorder was used with a photomultiplier.

FIG. 4.—Same as Figure 3 but at four temperatures. Wavelength 420 $m\mu$; exciting light (250 arbitrary units) on for 1 sec. Beckman DK-1 detecting and recording system (300 and 250°); Sanborn system as in Fig. 3 (77 and 1°). The time scale is written above each curve.

Figure 3 shows the onset and decay of the change in absorption at 300°K (wild type film measured at 610 $m\mu$) for two intensities of exciting light. The kinetics and the dependence upon exciting light intensity are the same at all wavelengths. Figure 4 shows the time course of the change at four temperatures. The slowing

of the decay between 300 and 250°K we attribute to electron trapping, as found in dried chloroplast films by Arnold and Sherwood.¹⁸ At lower temperatures, where electron trapping does not occur, the decay is independent of temperature and follows the second order equation

$$dx/dt = -\alpha x^2$$

as shown by Figure 5.

The steady state value of the change in transmission increases roughly as the square root of the intensity of the exciting light, up to a point at which the exciting light becomes saturating.

FIG. 5.—Kinetics of the decay of the change in transmission for dried chromatophores of wild type *R. spheroides*, when the exciting light is turned off. Wave length 610 $m\mu$; temperature 77°K. The change in transmitted light intensity is denoted x . For second order kinetics ($dx/dt = -\alpha x^2$) a linear relation between $1/x$ and t is required.

FIG. 6.—Effect of hydroxylamine on spectral changes and on photosynthesis in intact carotenoid less mutant *R. spheroides* at 300°K. The change in transmission at 880 $m\mu$ and the rate of photosynthesis are expressed in arbitrary units. Photosynthesis was measured manometrically (gas phase He + 5% CO₂; substrate sodium propionate).

If a film of wild type chromatophores is exposed to ethanol vapor for a few minutes, the changes in Figure 1 that are due to the carotenoids no longer occur. The response to light becomes like that of the carotenoidless mutant, although the absorption spectrum of the film is unchanged. This experiment shows the importance of structure for the optical effect. Ethanol vapor should attack the lipid phase of the chromatophores and disorganize an ensemble of molecules attached to the lipid phase. Carotenoids, having only a nonpolar means of attachment, should become disoriented. The attack should be much weaker on the bacteriochlorophyll ensemble, as this molecule has a polar linkage to protein.

Prolonged treatment with acetone, sufficient to extract the carotenoids but not the bacteriochlorophyll, abolishes the entire difference spectrum.

Intact cells: Chromatophores suspended in water show the same absorbancy changes, upon illumination, as dried films. The response to light of intact cells¹⁹ is entirely different; the carotenoidless mutant of *R. spheroides* shows changes only in the regions of cytochrome absorption. The absence of changes near the bacteriochlorophyll maxima suggested that in the healthy intact cells, the electron transport machinery prevents a pool of primary excited electrons from accumulating. Poisons should then permit this accumulation and bring on the corresponding spectral changes. Sodium azide, in the range 0 to 1 *M*, first exaggerates and then abolishes the cytochrome changes in intact carotenoidless mutant *R. spheroides*. Concomitantly the spectral changes seen in dried films appear, becoming maximal at the highest azide concentration. A more delicate experiment, using 0 to 0.03 *M* hydroxylamine, is illustrated in Figure 6. The rise in the optical effect at 880 $m\mu$ exactly parallels the decline in rate of photosynthesis. These spectral changes are abolished by heating for 1 min at 80°C.

In wild type *R. spheroides*, illumination causes large spectral changes in the regions of carotenoid absorption and smaller changes in the cytochrome and bacteriochlorophyll regions. These changes are more rapid than in the films. The role of carotenoids in this system is too poorly understood to warrant speculation.

Delayed light: That purple bacteria emit delayed light has been known for some time.²⁰ It would be of interest to know if dried chromatophores emit delayed light and how the decay of the light corresponds to the decay of the spectral changes discussed above. For this purpose we have constructed a large aperture Becquerel phosphoroscope. We have had great difficulties with the instrument because photomultipliers are very sensitive to light in the 900–1,000 $m\mu$ region, and therefore we have as yet little certain information on the kinetics of the delayed light. Two facts important for the present argument have emerged:

1. The delayed light from dried chromatophores is an order of magnitude brighter than from living bacteria.
2. The intensity of the delayed light, measured 0.005 sec after the exciting flash, is approximately proportional to the intensity of the exciting light. This linear relationship extends to intensities of the exciting light higher than that needed to saturate the spectral changes.

Photoconductivity: Films of dried chromatophores can be shown to be photoconductors by the method used by Arnold and Maclay²¹ for dried chloroplasts. A film of chromatophores on a sapphire plate, provided with graphite electrodes spaced 1 mm apart and each 20 mm long, has a resistance of 10^{17} ohms. By connecting 500 volts to one electrode and a vibrating reed electrometer to the other, the current in the dark and light can be easily measured. Figure 7 gives the results of such an experiment. The vibrating reed is connected so as to measure charge; thus the slope of the line is a measure of the current flowing through the sample. As can be seen a larger current flows while the sample is illuminated. By changing the wavelength of the exciting light it is found that the action spectrum for this photoconductivity has a peak between 800–900 $m\mu$, as is to be expected for bacteriochlorophyll.

Figure 7 also shows that at the instant the exciting light is turned on there is a sudden flow of charge through the sample. When the exciting light is turned off this flow is reversed. This step in the charge, representing a sudden change in the dielectric constant of the chromatophores, also has the action spectrum of bacteriochlorophyll in the 800–900 $m\mu$ region.

Discussion.—As a model of the chromatophore we will use the one proposed by Bergeron:²² The chromatophore is a sphere of 320 Å diameter; the outer shell of 60 Å is made of protein; the inside sphere of 200 Å is lipid. The 600 bacteriochlorophyll molecules in one chromatophore form a monolayer on the boundary between the protein and the lipid. We consider the bacteriochlorophyll monolayer to be a two-dimensional crystal with valence and conduction bands in the plane of the crystal. Franck and Teller²³ have argued that in a chlorophyll crystal where there

FIG. 7.—The photoconductivity of the chromatophore film is shown by the larger slope during the time that the exciting light is on. A sudden step in the charge can be seen at the beginning and at the end of the illumination. The wavelength of the exciting light was 850 $m\mu$.

FIG. 8.—Schematic diagram of the electronic band system of the chromatophore. *Ch* represents the chlorophyll crystal viewed edge on. The two directions of electron and hole motion are normal to the paper. *CB* is the conduction band, and *VB* is the valence band, *A* is the first substance to become reduced, by accepting an electron from the conduction band. *B* is the first substance to be oxidized, by accepting a hole from the valence band.

is interaction between the molecules there should be large changes in the absorption spectrum. The small chlorophyll crystals prepared by Jacobs *et al.*²⁴ do show the expected shift to the red. We point out that the type of crystals that we propose differs in three ways from the foregoing crystals:

1. They are only one molecule thick in one direction.
2. They are made of only a few hundred molecules.
3. The orientation of the bacteriochlorophyll is partly determined by the protein and lipid and may be very different than in an ordinary crystal.

Figure 8 is an energy diagram for this model; the ordinate represents energy of the electron. The abscissa is the direction from lipid to protein. The space labeled *Ch* represents the bacteriochlorophyll crystal viewed edge on, so that the two direc-

tions of electron and hole motion must be thought of as normal to the paper. *CB* is the conduction band, and *VB* is the valence band. "*A*" is the first substance to become reduced, by accepting an electron from the conduction band. "*B*" is the first substance to be oxidized, by accepting a hole from the valence band. The placing of *A* and *B* in the protein is completely arbitrary.

The illumination of the chromatophore transfers electrons from the valence band to the conduction band; the return transfer has a certain probability of emitting light and is the mechanism for the production of delayed light.

When the electron transport system is not taking electrons and holes from the bacteriochlorophyll, the electric field surrounding the separated electrons and holes modifies the energy levels of the bacteriochlorophyll to produce the spectral changes near its absorption bands. It is clear, since all five of these bands show the same shift toward shorter wavelengths, that the ground state is the one that is changed. The ground state is lowered by the electric field. Furthermore, the effect of the electric field saturates as the number of electrons and holes is increased. Disregarding electron trapping, the kinetics of the spectral changes are of second order, as expected for the recombination of electrons and holes in a semiconductor.

When the bacteria are doing photosynthesis, the number of electrons and holes is reduced so that the spectral changes in bacteriochlorophyll are very small or absent. The appearance of these spectral changes as the enzyme machinery is poisoned provides the most compelling evidence, short of a determination of quantum yield, that we are dealing with a step in photosynthesis and not with a collateral effect.

The very high electrical resistance shown by the dried films is due to the difficulty the electrons have in going from one chromatophore to another through two layers of protein. The increased current during illumination then represents a sharp drop in the resistance within each chromatophore. The abrupt change in dielectric constant upon illumination is the strongest argument possible that light actually separates the positive and negative charges in the chromatophore, and that within one chromatophore they are then free to move under the action of the external electric field. The fields we have used, of the order of 5000 volts per cm, are far too small to cause ionization. The return of charge when the light is turned off is simply the recombination of electrons and holes.

We give no discussion of the role of the carotenoid pigments as we do not understand the part they play in photosynthesis.

The fact that the reversible spectral changes are still found at 1°K means that they are purely electronic in nature. We believe that the first step in photosynthesis, in purple bacteria, is the simple act of separating an electron and a hole.

We expect that further experiments will show a similar process operating in green plant photosynthesis and probably in the visual process as well.

Summary.—The first step in photosynthesis appears to be the separation of an electron and a hole in a chlorophyll semiconductor. This statement rests on the following evidence:

1. In chromatophores of photosynthetic bacteria the chlorophyll shows, upon illumination, a reversible shift in every absorption band toward shorter wavelengths.

2. This shift is essentially the same from 300 to 1°K.

3. The process responsible for this shift precedes the operation of the enzymatic electron transport system. To the extent that enzymes can remove the primary excited electrons, the shift is diminished.

4. The sudden change in dielectric constant of dried chromatophores upon illumination shows that electrons and holes are spatially separated.

During the course of this work we have had help from many people, but three in particular must be mentioned: Dr. L. D. Roberts, Physics Division, Oak Ridge National Laboratory; Dr. Michael Kasha of the Department of Chemistry, Florida State University; and Dr. S. F. Carson, Biology Division, Oak Ridge National Laboratory.

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

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