*ARE CHLOROPLASTS SEMICONDUCTORS?**

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INTRODUCTION

Chlorophyll in photosynthetic organisms is found in small solid bodies known as "grana." In 1941 Szent-Gyorgyi[1] suggested that the protein in the grana might act as a semiconductor, the energy used in photosynthesis being carried by electrons in the conduction band. Eight years later Katz[2] elaborated the scheme and suggested that the electrons may move through a two-dimensional chlorophyll "crystal." In 1955 Bassham and Calvin[3] discussed the same idea and pointed out that an advantage of the semiconductor arrangement would be the physical separation of the points at which reducing agent and oxidizing agent are formed. A second advantage would be that it gives a simple answer to the question of how energy is transferred for long distances in the grana.[4]

The present paper describes two new types of experiments made with dried chloroplasts, the results of which may be described in terms of semiconduction. First, dried chloroplasts give glow curves (light emission on heating) very much like those made with crystals. Second, the resistance of the chloroplasts varies with the temperature in the manner of a semiconductor.

MATERIALS AND METHODS

Fresh leaves of tobacco, spinach, beets, or turnips were washed and dried, and the large mid-ribs were removed. This material (50–100 gm.) was ground in a Waring Blendor with 75–125 cc. of cold 0.1 *M* phosphate buffer (pH 7.0) for 30 seconds. The mixture was filtered through several layers of cheesecloth and centrifuged for $1^1/_2$ minutes at 146 × *G* and the supernatant then centrifuged for 7 minutes at 910 × *G*. The chloroplasts were washed twice with cold distilled water and suspended in a small amount of water. This suspension was then painted onto disks of aluminum, copper, or stainless steel (5 cm. in diameter) and dried with a stream of air. The disks were kept in a desiccator over Drierite until used.

For measurement of the glow curves, the disks were clamped to the top of an aluminum rod 5 cm. in diameter and 15 cm. long that contained a 500-watt heater. Connecting the heater to a Variac permitted the temperature of the rod to be raised at any rate desired. Temperature was measured by a thermocouple located in the aluminum a few millimeters below the disk. The rod could be cooled by dipping the lower half into water, dry-ice mixture, or liquid nitrogen. The upper end of the rod was fastened in a light-tight housing containing a photomultiplier. In the light path between the disk and the photomultiplier there were a shutter, a space for colored glass filters, and a glass cell containing a 1-cm. thickness of flowing water. The flowing water prevented temperature radiation from the disk and rod from warming the photocathode and changing the dark current.

Both the photomultipliers used in this work (RCA No. 6217 and Du Mont K1292) gave the same curves; both tubes were used at dry-ice temperature. The anode current was allowed to flow to ground through 10^9 ohms. A vibrating reed was

used to measure the voltage developed across the resistance. For the curves given in this paper, 1 mv. of signal is approximately 10^6 light quanta per second emitted into 2π solid angle by the disks.

For the resistance measurements, the same disk and aluminum rod assembly was used. A piece of brass (19 × 19 × 6.5 mm.) was placed on the surface of the dried chloroplast film, and the resistance between the brass block and the metal disk measured with a "Hi-Meg" bridge. This instrument is essentially a Wheatstone bridge, one arm of which is a very high Victoreen resistor. The unbalance is detected with a vacuum-tube electrometer.

RESULTS

Glow Curves.—Figure 1 gives the intensity of the light emitted by one of the dried chloroplast samples as a function of temperature, as the temperature of the disk is raised at the rate of 14° C. per minute. The sample had been illuminated at room temperature before the experiment but had not been heated before. If the disk is cooled in the dark and reheated, no light is emitted. If, however, the disk is cooled and reilluminated, light is emitted upon heating. The second glow curve is much smaller than that given in Figure 1. Each time the experiment is repeated the glow curve becomes lower and assumes a different shape. The change from one glow curve to the next becomes less with each repetition, until after four or five heatings the change is very slight. Samples stabilized in this way were used for Figures 2 and 3.

Figure 2 is a chart of the glow curves obtained after different periods of illumination by a 500-watt projection lamp placed 30 cm. from the disk. A Pyrex cell with 3 cm. of water was used to remove the infrared. In each experiment, measurement of the glow curve was started within 1 or 2 minutes after the illumination ceased.

The Du Mont K1292 photomultiplier is sensitive to light out to about 13,000 A in the infrared. The bottom curve in Figure 2, made with a smoked copper disk in place of the sample, shows black-body radiation at the higher temperatures. The black-body curve is the same on heating or cooling, but the glow curve gives light only on heating.

Figure 3 shows the glow curves obtained after illuminating the sample for 5 minutes with the same light system used for Figure 2. During irradiation the temperature of the sample never varied more than 3° C. from the values given on the curves. Measurement of the glow curves was started immediately after the illumination.

Chloroplasts from the different species gave similar glow curves. The kind of metal used for the disk made no difference.

Resistance Measurements.—Figure 4 gives the resistance of the film of dried chloroplasts as a function of the temperature. Curve *A* was obtained on heating the sample for the first time. The disk had been illuminated at room temperature before the experiment. Curve *B* was made as the sample was cooled to room temperature by dipping the lower half of the rod into cold water. The cooling rate was variable under these conditions. The experiment was exactly repeated, except that the sample was not illuminated, giving curves *C* and *D*.

The sample was illuminated for 10 minutes, to obtain the curves in Figure 5.

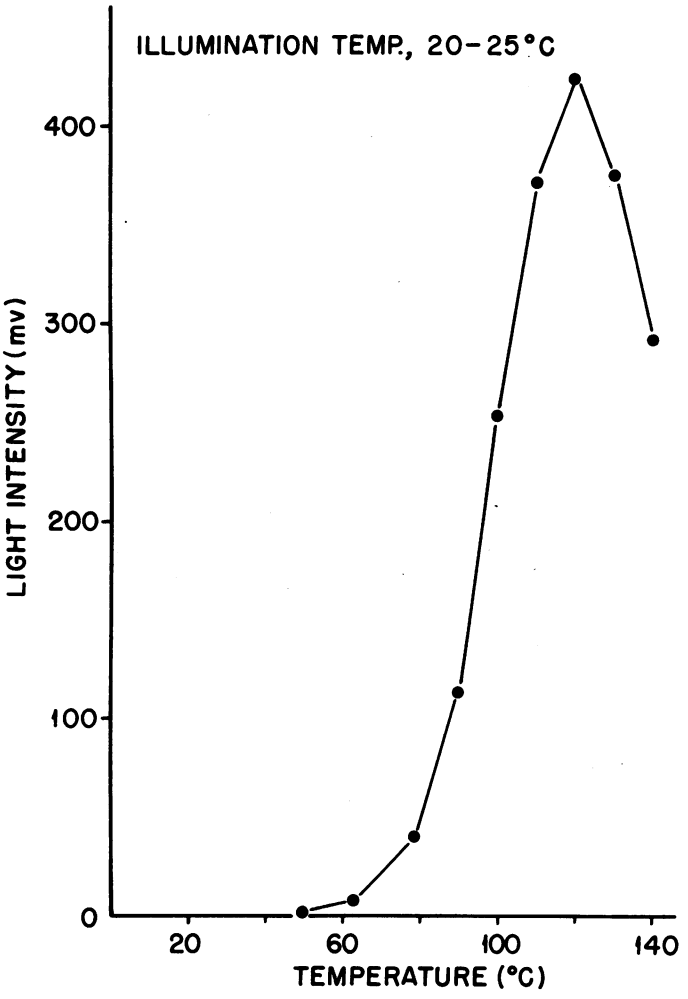

FIG. 1.—Light intensity expressed as a function of the temperature. Integrated light emission up to 140° C. is approximately 8 × 10^{10} light quanta. (Cf. Fig. 5 in J. T. Randall and M. H. F. Wilkins, *Proc. Roy. Soc. London*, *A*, **18s**, 366, 1945.)

Curve *A* was made after illumination. Curve *B* was obtained as the sample was cooled to room temperature.

DISCUSSION

The experiments described as glow curves show clearly that when the samples of dried chloroplasts were illuminated, a part of the light energy was stored. The stored energy could later be released by heating. Since heating causes the emission, the involvement of an activation energy is implied. A simple experiment shows not a single activation energy but rather a distribution of activation energies. A sample of dried chloroplasts, after being illuminated, was allowed to stand at room temperature in the dark for several hours before the glow curve was made. The intensity of the light emitted at 30°–40° C. was greatly reduced, whereas the light

emitted at 100°–110° C. was not changed from the value it would have had if the glow curve had been made immediately after the illumination. Had only a single activation energy been involved, the ordinates would have been reduced by the same factor at all temperatures.

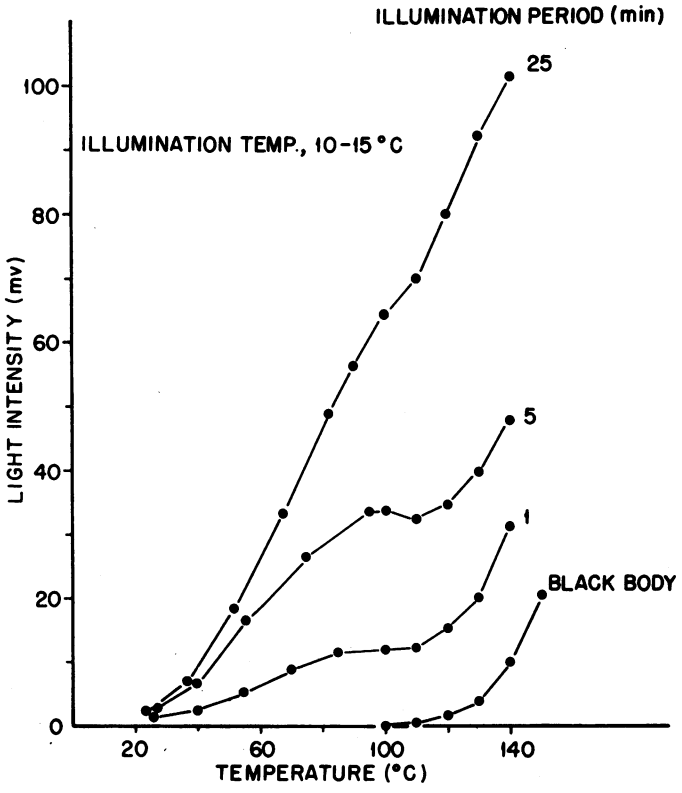

FIG. 2.—Light emission for a sample of "stabilized" chloroplasts as a function of the temperature (rising at 14° C. per minute). The curves show that the amount of light energy stored is not proportional to the time of illumination. Saturation is beginning to show.

An energy storage in crystals analogous to that in the chloroplasts has been known since 1602 from the work of the alchemist Vincenzo Cascariolo.[5] Any of a large number of inorganic crystals, after irradiation with ultraviolet light or X-rays, will emit visible light on being heated. Figure 6 is a schematic representation of the energy levels in the crystal. The absorption of a quantum of ultraviolet light transfers an electron from the filled band into the empty conduction band. As it travels through the conduction band, the electron can be trapped, at certain points in the crystal, by giving up a part of its energy. Once the electron is trapped, it remains at that point in the crystal until a thermal fluctuation supplies the energy needed for it to reach the conduction band. Again, in the conduction band, the electron can be retrapped or it can emit light by falling into a vacant hole in the filled band. The light emission takes place at only certain points in the crystal known as "luminescence centers."

A theory of glow curves has been given by Randall and Wilkins,[6] the equations they derive completely describe the results obtained with chloroplasts.

Experiments made with Corning filter No. 2404 between the 500-watt lamp and the sample showed that the chloroplasts stored almost as much energy as when the whole spectrum was used. Since the filter transmitted only light of wave-lengths longer than 6300 A, and the water cell only those shorter than 14,500 A, the light effective in storing the energy must have a wave-length between these limits. Glow curves made with the No. 2404 filter between the sample and the RCA No. 6217 photomultiplier (long-wave-length limit of sensitivity about 8000 A) show that the wave-length of the emitted light is between 6300–8000 A. These two experiments leave little doubt that it is chlorophyll that absorbs and emits the light in the glow curves.

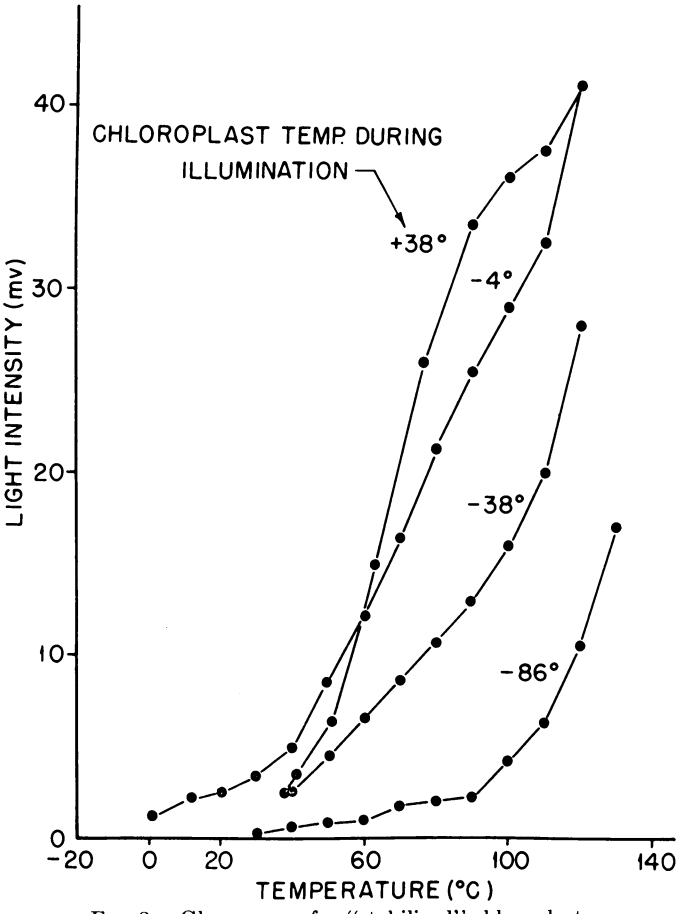

FIG. 3.—Glow curves for "stabilized" chloroplasts.

Figure 3 shows that as the temperature of the sample at the time of illumination is lowered, the amount of energy stored is decreased. After illumination at the temperature of liquid nitrogen, no glow at all is found on heating. At first sight this experiment seems to indicate that since there is a heat of activation for the storage of the light, there must be at least one chemical reaction in the process.

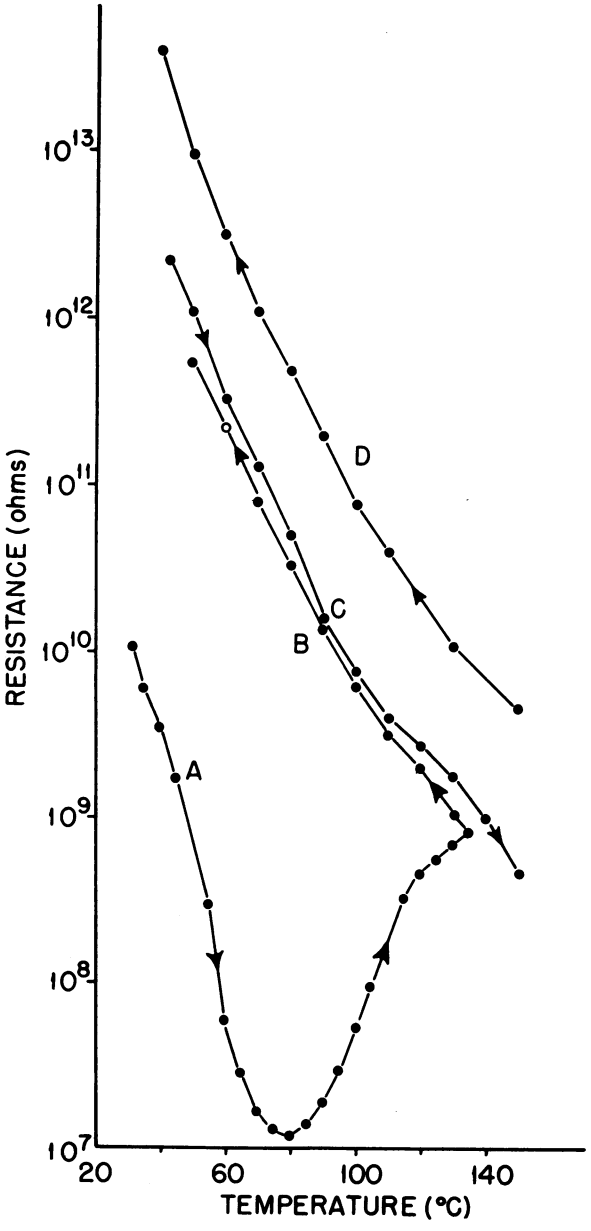

FIG. 4.—Resistance as a function of temperature. Curve *A* was made as temperature of the sample was raised at the rate of 14° C. per minute. Curve *B* was made as the sample was cooled. Curve *C* was made as the sample was heated. Curve *D* was made on cooling the sample down to room temperature, and the points have been raised by one decade in order to avoid congestion on the graph.

However, in solid-state physics, many instances are known where the trapping of an electron involves collision with a phonon, so that, as temperature is lowered, the efficiency of trapping falls. Experiments of the kind represented in Figure 3 cannot help us to decide whether the storage of energy is a chemical reaction or the trapping of electrons.

It now seems certain that the same mechanism that stores energy in the glow curve is also responsible for the delayed light (luminescence) of green plants already

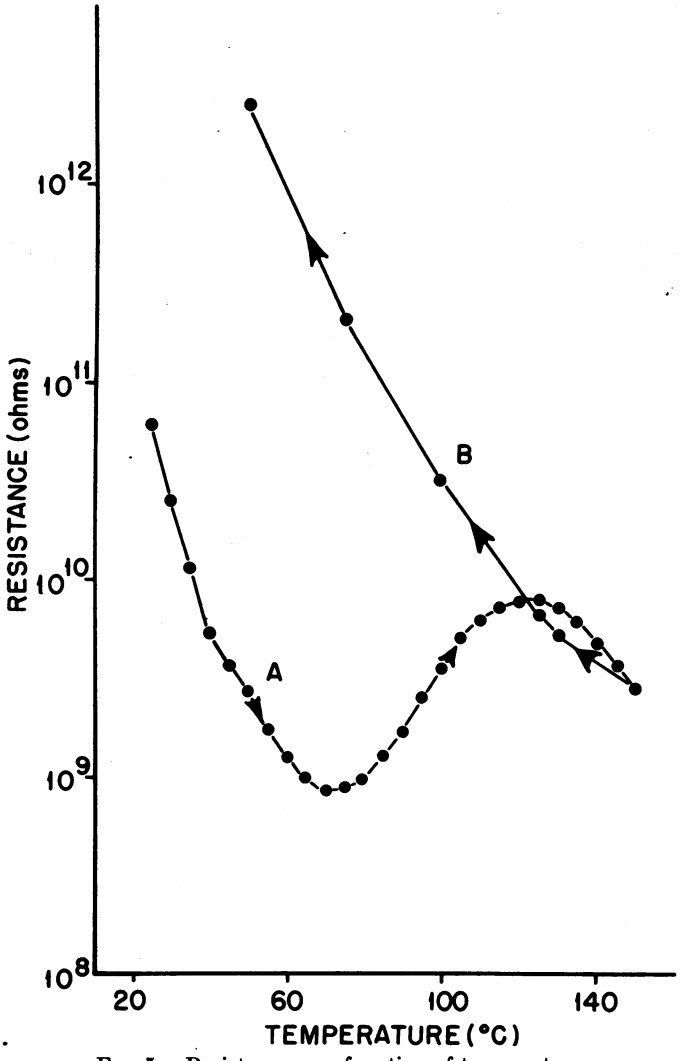

FIG. 5.—Resistance as a function of temperature.

described.[7] When examined with the high-speed phosphoroscope,[8] the dried chloroplast films show the same light emission at short times as do *Chlorella* and leaves. Furthermore, *Chlorella* suspensions and leaf plugs also give glow curves when they are heated after being illuminated. When *Chlorella* and leaves are heated, steam condenses on the optical parts and interferes with the light measure-

ment. The technical problem of making glow curves under pressure to eliminate the fogging has not yet been solved. But enough experiments have been made to say that the glow curves are much the same as with the dried material, except that the peak of intensity seems to be at a lower temperature. The idea that there are a number of light-emitting elements with different activation energies nicely explains the decay curves found for the delayed light.[7, 8]

Crystals that store energy by electron traps show, during the time that the exciting light is on, an increased electrical conductivity. The phenomenon is known as "photoconductivity." If it could be shown that dried chloroplasts are photoconducting, the idea of electron traps being responsible for the glow curves and luminescence would be more believable. A number of experiments have been

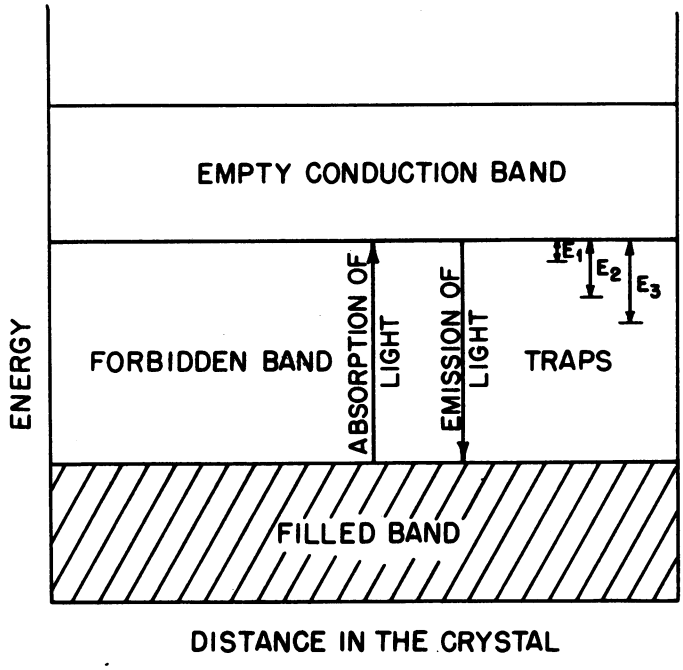

FIG. 6.—A schematic representation of the energy levels in a crystal.

made, but so far we have no unequivocal evidence that red light can increase the conductivity. The experiments do show that infrared light caused a momentary increase in the conductivity. Two explanations of the effect can be given. First, it is well known that infrared light can untrap electrons (transfer them from the trap to the conduction band). Second, as will be shown, the resistance of the dried chloroplasts is decreased by higher temperature. The infrared light would raise the temperature of the sample.

It has been found in Dr. Calvin's laboratory in Berkeley that dried films of chlorophyll are photoconducting (private communication). Since chlorophyll has a high concentration in the grana, it might be argued that the grana must be photoconductive. It should be remembered that the grana are small, discrete bodies imbedded in the stroma of the chloroplasts. Electrical connections made to the

dried material must measure predominantly the electrical properties of the continuous phase.

Since the experiments designed to show that grana are photoconducting were inconclusive, Mr. J. B. Davidson, of this laboratory, suggested that the resistance of the dried chloroplast material be measured under conditions similar to those used for the glow curves.

Figure 4 gives the resistance as a function of the temperature as the temperature of the sample was raised at the rate of 14° C. per minute. The large spike at 80° C., curve A, is a sort of glow curve in conduction. In these dried samples it would seem that this increased conduction must be caused by the freeing of electrons from traps. After the first heating, the spike did not appear again unless the sample had been illuminated as in Figure 5. Experiments with filters showed only blue light to be effective.

The resistance of the samples after the spike has been "annealed out" by the heating follows the equation for an intrinsic semiconductor,[9]

$$\ln R = C + \frac{w}{2kT},$$

where w is the difference in energy between the bottom of the conduction band and the top of the filled band. In Figure 7 curves C and D of Figure 4 have been plotted as $\ln R$ versus $1/T$. From the slope of the line, w has a value of approximately 2.1 ev. This is somewhat smaller than the 4.6 ev. calculated by Gergely and Evans for proteins.[10] That the resistance of dried protein films follows the equation for an intrinsic semiconductor has been known from the work of Baxter[11] and Eley *et al.*[12]

The freeing of electrons shown by the spike in Figures 4 and 5 cannot be closely connected with the glow curves. The spikes in conduction appear at much lower temperature than the peak of intensity in the glow curve, and the glow curve can be made after illumination by red light, whereas blue light is needed for the spike.

The experiments described in this paper do not, unfortunately, give a definite answer to the question "Are chloroplasts semiconductors?" Perhaps experiments on photoconductivity or on the Hall effect may. But, owing to the high electrical resistance shown by the chloroplast samples, such experiments are difficult. However, the similarity between the glow curves made with chloroplasts and those made with crystals, the variation in the resistance of dried chloroplasts as a function of the temperature in the manner of a semiconductor, and the spike in conduction produced by heating all can be understood by assuming that electrons do move in a conduction band in the chloroplast.

If it is established that chloroplasts are semiconductors, then our ideas on the first step in photosynthesis may need some revision.

SUMMARY

1. Glow curves analogous to those made with inorganic crystals can be made with *Chlorella* suspensions, leaves, and dried chloroplasts.

2. On heating, the electrical resistance of dried chloroplasts shows changes that can be interpreted as the freeing of trapped electrons.

3. Although the experiments reported here do not prove that grana act as

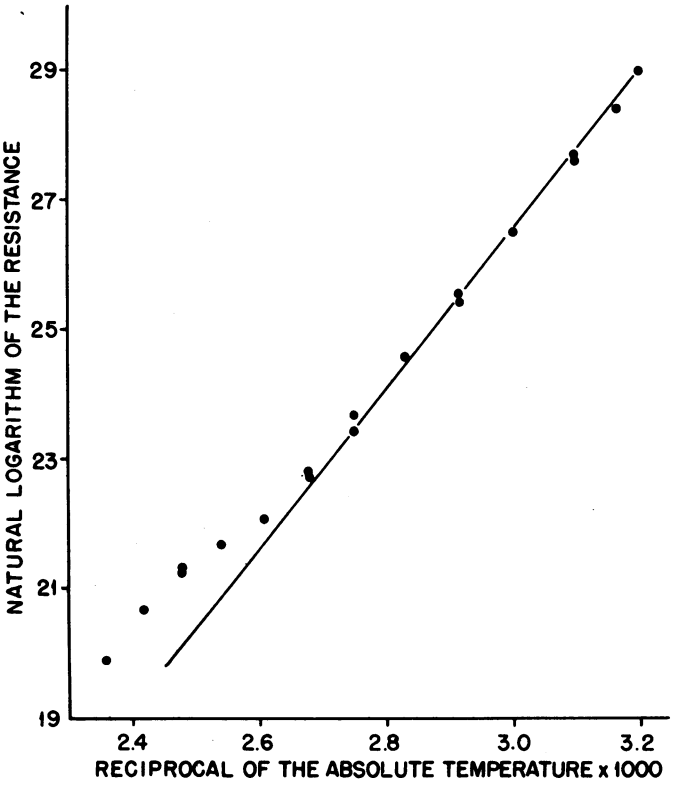

FIG. 7.—The natural logarithm of the resistance as a function of the absolute temperature. The points plotted are those from curves *C* and *D* of Figure 4. If *k* is taken as 8.6×10^{-5} ev. per degree, the calculated value of *w* is 2.1 ev.

semiconductors, they do suggest that the first act in photosynthesis may have at least as much in common with solid-state physics as with the chemistry of solutions.

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