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5. Since the procedure of Kabat *et al.* (*4*) has been standardized for body fluids and not for extracts from solid tissues, it was necessary to test its applicability to brain extracts. Standard solutions of γG were recovered quantitatively (90 to 110 percent) when added to homogenates of white matter, as well as to plaques from brains of patients that had died of multiple sclerosis.
6. To determine the extent of contribution of residual blood γG in brain, the carboxyhemoglobin method of M. W. Gordon and J. I. Nurnberger [*J. Histochem. Cytochem.* **4**, 84 (1956)] was used. The amount of hemoglobin (Hb) per kilogram of fresh tissue when divided by the amount per milliliter of patient's blood, and multiplied by (1-hematocrit/100), gives the number of milliliters of serum per kilogram of tissue. The average results and standard error of the mean (SEM) obtained were (control value with multiple sclerosis in parentheses): 2.6 ± 0.4 (3.2 ± 1.2), (3.1 ± 1.3) for white matter and plaques, respectively. No real differences as judged by the number of milliliters of serum per kilogram of tissue exist between the control and multiple sclerosis groups in any brain regions tested. The control average values and SEM used for serum γG and albumin were based on serums from 41 normal medical students: 4.5 g per 100 ml (± 0.18) and 1.3 ± 0.04, respectively; and for 97 multiple sclerosis patients: 4.1 ± 0.11 and 1.3 ± 0.04, respectively.
7. Since the plaques of demyelination had, in each case, essentially the same γG concentration as the normal-appearing multiple sclerosis white matter, only the plaque values are shown.
8. A plot of the concentration of γG in cerebrospinal fluid (milligrams per liter) taken from patients 1 year prior to death (based on the data in Table 1) showed a similar correlation.
9. The water content of normal-appearing multiple sclerosis white matter was 716 g per kilogram of tissue; that of plaques was 884 g (Y. Kishimoto, N. S. Radin, W. W. Tourtellotte, J. A. Parker, H. H. Itabashi, *Arch. Neurol.*, in press).
10. Supported by the USPHS (NB 05388). We thank M. J. Ott and E. R. Bryan for technical assistance.

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Light Reaction in Green Plant Photosynthesis: A Method of Study

Abstract. *Frozen green plants* (Chlorella) *carry out a photoreaction which can be assayed by measuring the light produced when the sample is heated. Heating at a rate of several degrees per second gives a measurable signal which shows that green plants can store energy at very low temperatures. The absorption cross section for the unit which carries out the photoreaction is several hundred times larger than that for one chlorophyll molecule, an indication that these are the "photosynthetic units" of photosynthesis.*

Photosynthesis is the process in which green plants reduce carbon dioxide to carbohydrate and oxidize water to oxygen. The energy comes from sunlight absorbed by chlorophyll. Apparently carbon dioxide reduction consists of a series of enzyme reactions, none of which are photochemical (*1*). This series, called the Calvin cycle, is driven by electrons at −0.4 volt and by ATP (adenosine triphosphate). It is not understood how the chlorophyll apparatus uses light energy to lift an electron from the level of water (+0.8 volt) to that of the reductant (−0.4 volt) and makes ATP at the same time.

A procedure which apparently allows us to study the photochemical part of photosynthesis is described here. The experiment consists of four simple steps: (i) a *Chlorella* suspension (or leaf plug) is held in the dark at room temperature (20° to 25°C) for 5 minutes to "relax"; (ii) the sample is frozen while still in the dark, at temperatures from −10 to −200°C; (iii) the sample is illuminated while cold (we believe that the photochemical reaction takes place, but that enzyme reactions do not); and (iv) the sample is rapidly heated in the dark to 100°C.

The light emission (the signal) from the sample as it is heated measures the amount of photoreaction that has taken place. It is the fast heating that makes the signal large compared to the background, which is the light emitted on heating a sample that had zero illumination in step (iii). The background is larger than the signal when the sample is heated slowly, for example 20°C per minute.

I have previously presented an electron-hole picture of photosynthesis (*2*) in an attempt to explain the transfer of electrons from water to the reductant (−0.4 volt). In this picture the electron transfer is carried out by photosynthetic units, each made up of 500 chlorophyll molecules. Each unit has two reaction centers separated in space. A light quantum absorbed by any one of the 500 chlorophyll molecules forms an exciton which runs over the whole unit. An exciton hits reaction center A, breaks up to form a free hole in the chlorophyll, and binds an electron to A. This electron, at −0.4 volt, can go to the Calvin cycle. A second exciton cannot react with A until the electron has moved out of the trap. Similarly, an exciton can react at B to form a bound hole and a free electron in the chlorophyll. Again, a second exciton cannot react with B until the hole has been used in the oxidation of water. The movement of the free electron and hole constitutes the electronic conductor between A and B needed to prevent a reverse reaction between the reducing and oxidizing power. The recombination of the free electron and hole is the mechanism for the production of delayed light (*3*).

If one assumes that, at low temperatures (−100°C), the electron in the reaction center A and the hole in reaction center B were stable, then this explains the fact that at low temperatures green plants emit delayed light after a short flash, but do not emit light after a period of continuous illumination (*4*). This had been observed in Calvin's laboratory during a study of delayed light at low temperatures (*5*). Although the results of Müller and Lumry (*6*) show that the ratio of delayed light to fluorescence in green plants is much less than would be predicted by the electron-hole picture outlined above, this picture will still be used as a matter of convenience to describe the present experiments.

These observations were repeated with a phosphoroscope constructed for the study of delayed light. This instrument is of the Becquerel type and consists of two shutters on a rotating shaft. The first shutter is open for 140 degrees, allowing the exciting light to hit the sample; both shutters are closed for the next 40 degrees, then the second shutter is open for 140 degrees, allowing light from the sample to fall on a photomultiplier, and finally both shutters are again closed for 40 degrees. The signal from the photomultiplier was displayed on an oscilloscope. The sample cavity in this phosphoroscope is 3.2 cm in diameter. This is large enough to allow the cooling of samples by heat conduction down a large aluminum bar, the outer end being cooled with liquid nitrogen. Samples can be held at any temperature between 0° and −130°C.

Samples of *Chlorella* were frozen on the bar and put in the phosphoroscope under the dimmest illumination possible. After the speed of the phosphoroscope was set at 100 cy/sec and the temperature of the bar adjusted to the desired value, the exciting light

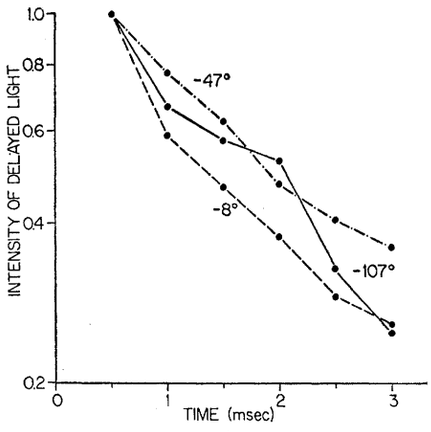

Fig. 1. Log intensity of the delayed light as a function of time. Time is measured from the instant that the second shutter starts to open. The delayed light signals have been normalized to unity at ½ msec.

was turned on by means of an auxiliary shutter. The delayed light diminished during the time that the second shutter was open (Fig. 1). The decrease is approximately exponential, with a time constant (time to reach 37 percent) of 2 to 3 milliseconds. Within the ranges studied, the decrease does not depend upon the temperature.

The intensity of the delayed light is

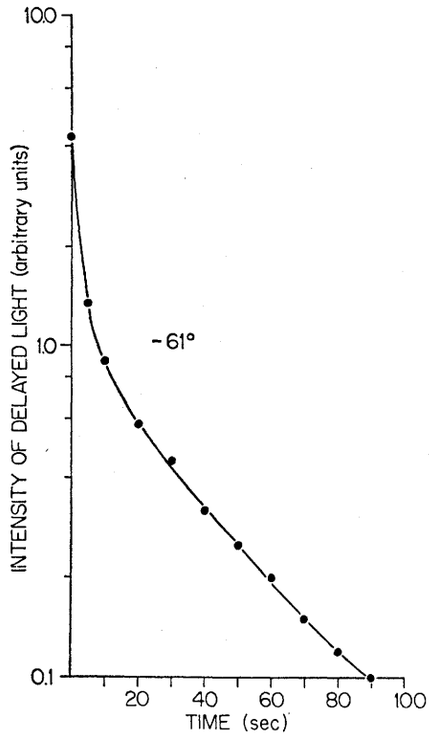

Fig. 2. Log intensity of the delayed light as a function of the time of illumination. The nonexponential nature of this decay may be due to the use of thick (6.5 mm) samples. Use of the aluminum bar for cooling made it necessary to use the thick samples.

a function of the time that the exciting light has been on (Fig. 2). With each revolution of the phosphoroscope, the delayed light is smaller. As can be seen, the frozen plants emit no delayed light after a minute or so. This means that each of the A traps has a bound electron and each of the B traps has a bound hole; therefore the mechanism making delayed light stops.

An electron in a trap at −0.4 volt and a hole in a trap at +0.8 volt together represent the storage of 1.2 ev of energy. This energy is 0.63 ev less than the 1.83 ev energy of excited chlorophyll. If this 0.63 ev can be supplied by thermal fluctuation, then one would expect that heating of the frozen illuminated sample would cause it to emit light. It does.

Initial experiments were made with leaf plugs illuminated while immersed in liquid nitrogen and then transferred to hot water; light emission was measured simultaneously. These experiments established the importance of fast rates of heating in making the signal caused by light large compared to the background. They also showed that the samples must be allowed to relax in the dark at room temperature for at least 2 minutes before being frozen in order to give a constant background. These experiments with leaf plugs were convenient and allowed fast heating of the sample, but as they were not easily reproducible, a simple device was constructed for the use of *Chlorella* suspensions.

The apparatus consists of a type of metal test tube, 15 cm long, 3.8 cm in diameter, with a round bottom having a radius of 5.08 cm. The walls are made of Inconel 0.025 cm thick to allow fast heating. An open metal tube, having a diameter of 2.54 cm, is concentric with the first tube and extends down almost to the bottom. A stream of helium flows slowly down the inner tube and up the annular space between the tubes and out through an exhaust which is sealed against light. The helium stream prevents condensation of oxygen when liquid nitrogen is used as the cooling bath, and it keeps steam out of the center tube, which also serves as light path between sample and photomultiplier during the heating. The apparatus was mounted vertically, directly below the photomultiplier. The EMI 9558B tube was used, operated at 1000 volts. A Corning filter No. 2403 was between sample and light detector. This combination of filter and photocathode limits the

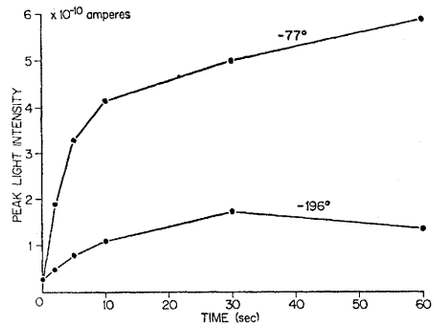

Fig. 3. Peak light intensity as a function of the time of illumination. The shape of the light signal was determined by the electrical circuits, time constant of 9 seconds. The temperature of the sample was raised from −190°C to +95°C by immersing it in a pan of hot water.

light measured to wavelengths between 6400 and 7600 Å. The samples consisted of 2 to 3 mm^3 of *Chlorella* cells in 0.5 ml of Knops solution, with a new sample being used for each point.

The peak intensity of the flash, made by heating the tube with 95°C water, is a function of the time of illumination of the frozen sample (Fig. 3). Red exciting light was used, and the intensity was made low so that the curves took some seconds to reach saturation. The saturation value depends upon the temperature of the sample at the time of illumination. Similar curves can be obtained with blue light and with light from a sodium lamp.

With sodium light it is possible to estimate the absorption cross section for the unit that is being charged in these experiments. Kohn (7) showed that in photosynthesis the unit that absorbs the light has an absorption cross section some 360 times that of a single chlorophyll molecule. Applying Kohn's calculations to the curve obtained with sodium light, I estimate that the unit is 110 times the cross section for one chlorophyll molecule. This estimate is too low because the frozen sample reflects considerable light and thus reduces the intensity at the position of the cells. The approximate agreement between my estimate and that by Kohn strongly suggests that the photosynthetic units which carry on photosynthesis at normal temperatures are being charged.

The saturation value of the charging curve depends upon the temperature. One would expect from Kohn's equation, however, that the saturation value would depend only on the num-

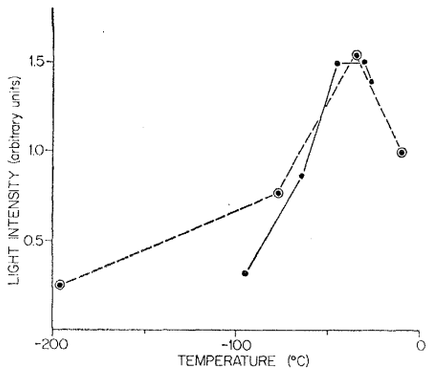

Fig. 4. The saturation value of curves like those shown in Fig. 3 is given by the dotted line as a function of the temperature of the sample at the time of the illumination. The intensity of the delayed light as a function of the temperature, at the instant the exciting light is turned on, is shown by the solid line.

ber of cells in the sample. In Fig. 4 the saturation value is given as a function of the temperature at the time of illumination, as is the maximum intensity of the delayed light studied in the phosphoroscope. The similarity between the two curves suggests that delayed light production and the charging of the units are closely related.

The signal discussed above is dependent on the temperature of the sample at the time of illumination. If the samples are held in the dark at higher temperatures after having been illuminated at a low temperature, then the signal is smaller and depends upon the time it is exposed at the higher temperature.

Figure 5 gives the results of an experiment in which all samples were frozen at −77°C, exposed to light for 1 minute, and then held in the dark at the temperature indicated on the

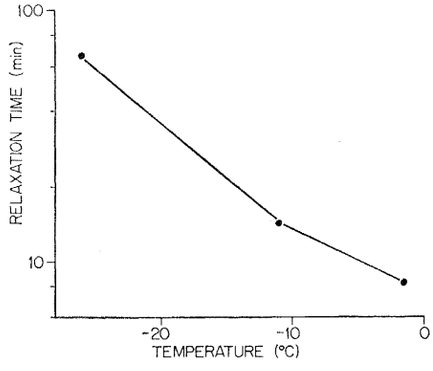

Fig. 5. The relaxation time (time to decrease to 37 percent) is plotted as a function of the temperature. The points were calculated on the assumption that the decay was exponential.

curve for a time long enough to reduce the signal to approximately one-half or one-quarter of the original. The samples were then cooled again to −77°C, and the signal was observed on heating to +95°C. Relaxation times were calculated on the assumption of a simple exponential decrease.

Glow curves were made from −70° to 100°C by heating at the rate of 20°C per minute. The results varied from one run to the next (Fig. 6). The background (that is, the glow curves with no illumination) consisted of three peaks at 20°, 55°, and 95°C. Samples that had been illuminated for 1 minute at −70°C in addition to the background, gave peaks at 0° and 30°C. The signal due to illumination was smaller than the background.

A detailed comparison between these results and those given by the fast heating (Fig. 3) shows that the maximum intensity of the background is approximately the same in the two experiments. The signal due to the illumination behaves quite differently. Here the integral of the intensity for the fast heating is equal to, or somewhat larger than, the integrated light output of the light signal when the heating rate is slow. This explains why the signals are much larger than the background in Fig. 3.

In the apparatus used to make Fig. 3, the sample is not heated at a uniform temperature. This can be seen by watching the sample melt. The ice takes several seconds to disappear. This makes no difference in the charging curve, since one can use a long time constant (9 seconds) in the light-measuring circuit. But for a glow curve one wants to know the intensity of the emitted light at each temperature.

To make a glow curve which shows some structure, a copper plug was made with a round bottom to fit the Inconel tube. The flat upper face of this plug had a well 0.8 mm deep and 12.7 mm in diameter. The copper plug weighed 73 g and was chromium plated. The *Chlorella* suspension in the well was covered with a glass cover slip. Experiments were made to show that the glass cover slip did not emit any appreciable light upon heating. The time constant in the light-measuring circuit was 0.5 second.

The large mass of the plug slowed the rate of heating to about 2°C per second in the neighborhood of 0°C. But the fact that no part of the suspension was further than 0.8 mm from the copper helped to keep all parts at

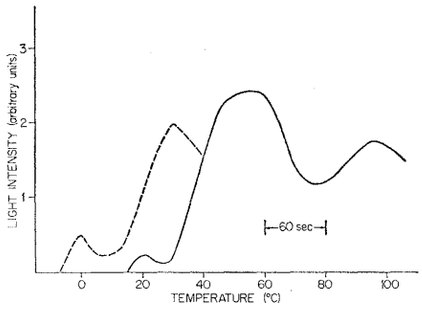

Fig. 6. This schematic glow curve gives the intensity of the emitted light as a function of the temperature as the sample is heated at the rate of 20°C per minute. The solid line is the background. The dotted line gives the signal due to illumination at −70°C.

the same temperature. When the ice was heated from −196° to 95°C, it melted 46 seconds after the hot water was added. Once the ice started to melt, it all disappeared in ½ second.

Glow curves were made with the copper plug by keeping the samples in the dark for 5 minutes at 20°C, then freezing them at −196°C (Fig. 7). The samples were then warmed to the temperatures indicated on the graph, exposed to white light for 1 minute, and

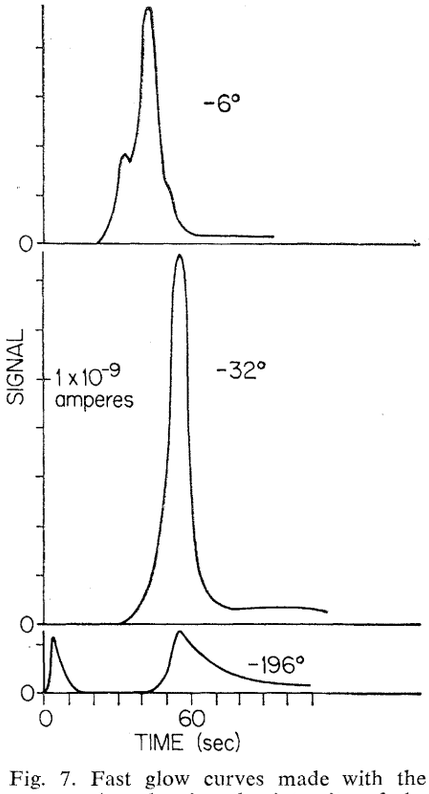

Fig. 7. Fast glow curves made with the copper plug showing the intensity of the emitted light as a function of the time of heating. The temperature of the sample at the time of illumination is shown.

cooled again to −196°C. The glow curves were made by heating with a water bath at 95°C.

The glow curve for the illumination at −196°C consists of two spikes, one at about 4 seconds and one at 53 seconds after starting the heating. The 4-second spike is not due to light absorbed by chlorophyll, since it is not seen after illumination with red light. Furthermore, the charging curve for this spike is quite different from that shown in Fig. 3. For illumination at temperatures higher than −32°C, the spike at 53 seconds becomes smaller and a new spike appears at about 43 seconds. At still higher temperatures, as shown by the curve for −6°C, the spike at 53 seconds has almost disappeared. The maximum light intensity now is at 43 seconds, with a new spike showing at about 30 seconds.

These results show that there is structure to the glow curves; more than one activation energy must be involved. It would be interesting to use the activation energy found from the glow curve to calculate the level of trap A and compare it with various redox potentials. Unfortunately, the fast glow curves are not yet good enough.

A very rough calculation of the activation energies can be made. We use the differential equation for a glow curve given by Randall and Wilkins (*9*), and the frequency factor of 2.5×10^9 found for dried chloroplasts (*8*). We know that 46 seconds after the start of the heating the copper plug is at 0°C, and, if we assume that the heat flow into the plug is proportional to the difference in temperature between the plug and the water at 95°C and that the heat capacity is constant, then we can calculate the temperature of the sample at any time. The activation energies of the three spikes shown in Fig. 7 are 0.57, 0.52, and 0.47 ev. These values are in rough agreement with the 0.63 ev that we expected.

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Antimicrobial Specificity of Leukocyte Lysosomal Cationic Proteins

Abstract. *Differences in antimicrobial specificities against* Staphylococcus aureus, Streptococcus faecalis *(group D), and* Proteus vulgaris *exist among the electrophoretically separable components of lysosomal cationic proteins of polymorphonuclear leukocytes.*

In previous reports we have described the isolation from polymorphonuclear leukocytes of a group of cationic proteins that possessed antimicrobial activity (*1*). The proteins were distinct from and more basic than lysozyme and ribonuclease. Fractions of such proteins have also been shown to account for a considerable part of the tissue-damaging activity (*2*) and pyrogenicity (*3*) of polymorphonuclear lysosomes.

The lysosomal cationic proteins exhibited electrophoretic heterogeneity that varied from species to species (*4*). This heterogeneity might be attributed to: (i) various degrees of aggregation of single protein species (in this case it could be expected that the biological activities of the electrophoretic components might be essentially similar); or (ii) true chemical heterogeneity corresponding to a mixture of different molecular species of proteins (in this case it would be expected that on resolution these protein species would differ substantially in biological activities).

By means of sucrose density gradient electrophoresis we have resolved the constituents of lysosomal protein to a degree of homogeneity that has made possible comparative studies of the antibacterial specificities of the resolvable components.

This report presents evidence that the components of the polymorphonuclear lysosomal cationic proteins thus resolved manifest a form of specificity in their antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis* (group D), and *Proteus vulgaris*, suggesting the existence of a true chemical heterogeneity of the cationic components.

Polymorphonuclear leukocytes were obtained from aseptic peritoneal exudates of rabbits. The lysosomal fraction was prepared by homogenization and differential centrifugation of polymorphonuclear cells in 0.25*M* sucrose (*5*). The lysosomal fraction was extracted with 0.01*N* HCl, dialyzed against distilled water, and lyophilized. Fifty milligrams of lysosomal proteins were subjected to ascending electrophoresis in a sucrose density gradient column (LKB Instruments, Washington, D.C.) at 25°C for 10 hours (acetate buffer *p*H 4, ionic strength 0.01, 700 volts, 16 ma) according to the procedure described elsewhere (*6*). At the end of the run, 5-ml fractions were collected. Protein in the fractions was estimated by Lowry's method (*7*). Lysozyme was determined according to a standard method (*8*). For antibacterial assay the photometric method of Muschel (*9*) was modified. Cultures of *Streptococcus faecalis* (group D), *Staphylococcus aureus*, and *P. vulgaris* were grown overnight in 40 ml of trypticase soy broth. On the day of the test the bacteria were washed twice with distilled water and resuspended in 5 ml of distilled water. A portion (0.25 ml) of the suspension was transferred into 10 ml of trypticase soy broth and cells were grown in a shaker bath at 37°C for 4 hours. At the end of the period a 1 : 10 dilution of the culture was prepared in fresh trypticase soy broth and was further incubated for 1 hour. This culture was finally adjusted with appropriate dilutions in trypticase soy broth to give an optical density of 0.15 ± 0.01 at 650 mμ. The cells were then centrifuged to remove trypticase soy broth and resuspended in citrate-phosphate buffer (*p*H 5.6) to the original volume. The standardized bacterial suspension (0.3 ml) was added to 1.7 ml of citrate-phosphate buffer (*p*H 5.6) containing known quantities of sample from each lysosomal fraction. After the tubes were incubated for 1 hour for maximum inhibitory effect, 5 ml of trypticase soy broth was added to the incubation mixture and the tubes were returned to the water bath at 37°C. Incubation was carried out until the optical density in the control tubes reached the range of 0.4 to 0.45. A measure of the antibacterial activity was obtained by dividing the optical density of each tube by the optical density of the control and multiplying by 100.