TWO PHOTOREACTIONS IN PHOTOSYNTHESIS: EVIDENCE FROM THE DELAYED LIGHT EMISSION OF CHLORELLA

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Following Emerson's\textsuperscript{1,2} finding that the efficiency of photosynthesis drops at long wavelengths and that supplementary illumination at short wavelengths eliminates this long wavelength drop, a number of authors have presented evidence that two photoreactions are involved in photosynthesis.\textsuperscript{3-10} Since 1951, it has been known that for several minutes after illumination photosynthetic organisms emit light from chlorophyll.\textsuperscript{11} Although the physical mechanism of this delayed light production is still uncertain,\textsuperscript{11-13} the emission would appear to allow direct measurement of energy conversion processes within the photosynthetic apparatus. The present experiments on living green plants indicate that the delayed light is emitted by functionally active chlorophyll and that the presence of two pigment systems may be inferred from the wavelength dependence of the decay curves.

\textit{Materials and Methods}.—The time course of delayed light emission was measured by a pumping method similar to that of Strehler and Arnold.\textsuperscript{11} Three hundred cc of Knop's medium containing Chlorella was illuminated by 6,500 Å light (Farrand model No. 300 visible monochromator with 1,000-watt tungsten source, half band width of 350 Å) and by far-red light containing wavelengths longer than 7,000 Å (500-watt tungsten source with Corning filters 2030 and 9836). Illumination of the cells could be performed by the two light sources simultaneously or by each source individually. The cells were pumped with a Maisch metering pump from the illumination chamber, through black rubber tubing, and through glass tubing in front of a DuMont K 1292 photomultiplier, which was cooled to the temperature of dry ice. The signal from the photomultiplier was amplified on an electrometer-amplifier and recorded on a Brown recorder. Black tubing channeled the cells from the photomultiplier to a stainless steel coil in a temperature control bath, then through the pump, and finally back to the illumination chamber. Decay curves for delayed light could be constructed by measuring the intensity of delayed light at various pump speeds, since the time in dark (from illumination chamber to photomultiplier) depended on the speed at which cells were pumped. The volume of unilluminated cells in the pumping system was less than 5 per cent of those in the illumination chamber.

\textit{Chlorella pyrenoidosa} Chick, strain 252, was obtained from the Culture Collection of Algae at Indiana University. The cells were grown in Knop's solution and aerated with 5 per cent CO\textsubscript{2}-95 per cent air at 18°C under continuous red illumination from neon tubes. Before being placed in the delayed light apparatus, exponential phase cultures were diluted to a standard concentration. This was done by first determining the volumetric concentration of cells in a culture by a hematocrit method in which 10 cc of a culture was centrifuged at 500 X g for 5 min. The volume of cells in a culture was read directly from the hematocrit tube, and the culture diluted to a volumetric concentration of 20 mm\textsuperscript{3}/100 cc Knop's solution. This cell concentration absorbed less than 20 per cent of the incident light.
Results.—The delayed light decay curves for saturating intensities of 6,500 Å and for far-red exciting light, given in Figure 1, show that the time course of delayed light emission depended on the wavelength of exciting light. Decay curves produced by the two wavelengths actually crossed each other. The point of intersection depended on temperature, occurring at an earlier time at higher temperatures (i.e., about 4 sec at 18°C and 1.25 sec at 32°C). Simultaneous irradiation with both wavelengths resulted in a decay curve intermediate between the decay curves from the individual wavelengths. Since the decay curves for the individual exciting wavelengths intersect, either wavelength is seen to reduce (or "quench") the delayed light produced by the other, depending on which portion of the decay is observed.

The effect of simultaneous irradiation with both wavelengths has been found to depend on the intensity of exciting light. Table 1 gives a comparison of the delayed light produced by the two wavelengths, individually and simultaneously, at low and high intensities. At extremely low intensities, the effects of the two wavelengths are approximately additive when given simultaneously. At high intensities, which were saturating for this delayed light (but which were on the linear portion of the oxygen evolution dose-response curve), simultaneous excitation resulted in delayed light production intermediate in intensity between the individual wavelengths (as in Fig. 1).

Figure 2 shows the effect of adding various intensities of one wavelength to a saturating intensity of another wavelength. The low intensities of 6,500 Å light had no effect on the delayed light produced by a saturating intensity of far-red, but si-

![Diagram](image_url)

**Table 1**

<table>
<thead>
<tr>
<th>Incident light</th>
<th>Delayed Light</th>
<th>Expected for addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,500 Å, 30 erg/cm²/sec</td>
<td>2.46</td>
<td>—</td>
</tr>
<tr>
<td>Far-red, 80 erg/cm²/sec</td>
<td>2.67</td>
<td>—</td>
</tr>
<tr>
<td>Both wavelengths, at above intensities</td>
<td>5.04</td>
<td>5.13</td>
</tr>
<tr>
<td>6,500 Å, 2,600 erg/cm²/sec</td>
<td>14.3</td>
<td>—</td>
</tr>
<tr>
<td>Far-red, 2,000 erg/cm²/sec</td>
<td>15.6</td>
<td>—</td>
</tr>
<tr>
<td>Both wavelengths, at above intensities</td>
<td>15.0</td>
<td>29.9</td>
</tr>
</tbody>
</table>

*Temperature, 18°C. Delayed light measured at a dark time of 7 sec. Delayed light intensities given in arbitrary units.*
multaneous excitation with higher intensities of 6,500 Å resulted in a reduction of delayed light, compared to far-red alone. This reduction is not merely an intensity effect, since 5,000 erg/cm²/sec of far-red did not result in a reduction compared to the 2,000 erg/cm²/sec of far-red utilized (Fig. 2).

If the two different decay curves were somehow produced by a single pigment system, then when both exciting wavelengths result in the same amount of delayed light there should be no effect of shifting from one wavelength to the other. Such an experiment was carried out by measuring the delayed light at the crossover point of the decay curves from 6,500 Å and far-red light. Under these conditions, a shift from one exciting wavelength to the other caused changes in the delayed light emission, which then gradually returned to the steady-state (Fig. 3). A shift from 6,500 Å to far-red resulted in a negative transient (or temporary reduction in delayed light production) while a shift from far-red to 6,500 Å resulted in a positive transient. These delayed light chromatic transients are very similar to the chromatic transients which have been reported for oxygen production,14-16 and the transients are of the same sign in both cases.

Discussion.—Chromatic transients of oxygen evolution imply the presence of two photosynthetic pigment systems.14-16 Since there are similar chromatic transients in the delayed light emission of living plants (Fig. 3) and because the delayed light is emitted from the first excited singlet state of chlorophyll,17, 18 it therefore appears that the delayed light comes from chlorophyll that is functionally involved in photosynthesis.

The finding that a saturating dose of 6,500 Å light produced a different decay curve from a saturating dose of far-red (Fig. 1) implies that the two exciting wavelengths are not absorbed by the same pigment system. This is a direct physical demonstration of the presence of two photosynthetic pigment systems. It might therefore be expected that the two decay curves would be additive after simultaneous excitation with both wavelengths. On the contrary, simultaneous excitation of the two pigment systems at saturation resulted in a decay which was more or less intermediate between the decay from the individual wavelengths (Figs. 1, 2). Had a single pigment system been present, the amount of delayed light at saturation would have been equal for all exciting wavelengths or wavelength combinations. The lack of additivity at saturating intensities of exciting light implies that the two photoreactions are linked, presumably through a chain of enzymatic reactions.

The older evidence concerning involvement of enzymes in delayed light emission has been summarized by Strehler.19 Recently Sweetser, Todd, and Hersh20 found that chemical inhibitors of photosynthesis could change the shape of the delayed light decay curve. A good deal of data support Katz's hypothesis that a chloro-
phyll semiconducting system is involved in the first steps of photosynthesis.\textsuperscript{22-30} The extremely long time of decay,\textsuperscript{31, 32} as well as the very low intensity,\textsuperscript{17} could be explained by trapping or diffusion of electrons (or holes) in such a semiconductor system. The extreme complexity of the delayed light decay would then be explained by the presence of enzymatic systems which utilize high-energy electrons from the chlorophyll semiconducting system.

Delayed light excited by the two wavelengths is additive at very low exciting intensities (Table 1) for which no measurable photosynthesis takes place. This result may reflect a requirement for more than one type of absorption act within an individual photosynthetic unit (group of cooperating chlorophyll molecules) for efficient photosynthesis. The existence of photosynthetic units has long been suggested by Kohn's observation\textsuperscript{33} that the absorption cross section for photosynthetic oxygen evolution is $9.7 \times 10^{-14}$ cm$^2$ (for the 6,200–7,000 Å region), which is the size of 360 chlorophyll molecules. In the present experiments, the additive effect of delayed light produced by the two pigment systems at very low intensities of exciting light is interpreted as suggesting the presence of the two pigment systems in every photosynthetic unit. The additivity at low exciting intensities would then be explained, since it would be unlikely for both pigment systems in an individual unit to undergo an absorption act within the turnover time of the presumed enzymatic link. This explanation is consistent with the calculation that exciting intensities (given in Table 1) which were additive in delayed light production resulted in the order of 1 quantum/sec falling on a photosynthetic unit. Since Emerson and Arnold\textsuperscript{34, 35} found the turnover time for a photosynthetic unit to be $10^{-2}$ sec, it is clear that at these intensities two absorption acts would have little probability of occurring within the turnover time of an individual photosynthetic unit.

Summary.—The time course of delayed light emission from living Chlorella depends on the wavelength of exciting light. Two distinct decay curves result when wavelengths are used that selectively excite the two photosynthetic pigment systems. The decay curves intersect, and the time of intersection depends on temperature.

At extremely low intensity, effects of the two exciting wavelengths are additive. At high intensity, simultaneous irradiation results in a decay curve intermediate between that obtained at each wavelength.

Chromatic transients of delayed light are observed upon shifting from one wavelength to the other. These transients are of the same sign and similar in shape to oxygen chromatic transients.
These results imply that delayed light is emitted by functionally active chlorophyll, and provide direct evidence that different photosynthetic pigment systems are absorbing the two wavelengths. The additivity results are interpreted as evidence that the individual photosynthetic units contain two pigment systems linked by an enzyme chain.

The author is sincerely grateful to William Arnold for assistance in building the delayed light apparatus, for generous encouragement, and for many helpful discussions.

Note: During the preparation of this manuscript, summaries of two forthcoming papers by Goedheer (Biochim. et Biophys. Acta Previews, 2, Nos. 3 and 4, 1962) indicated that he also ascribes certain aspects of delayed light emission to the presence of two pigment systems.

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