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Fluorescence Transients in *Chlorella*: Effects of Supplementary Light, Anaerobiosis, and Methyl Viologen*

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Research in various laboratories has indicated that transitory fluorescence in algae and chloroplasts is emitted by System II chlorophyll a molecules, and that the factor controlling fluorescence is the redox state of Q, the System II electron acceptor [1-9]. With this view in mind, we have studied the effects of supplementary light, anaerobiosis,

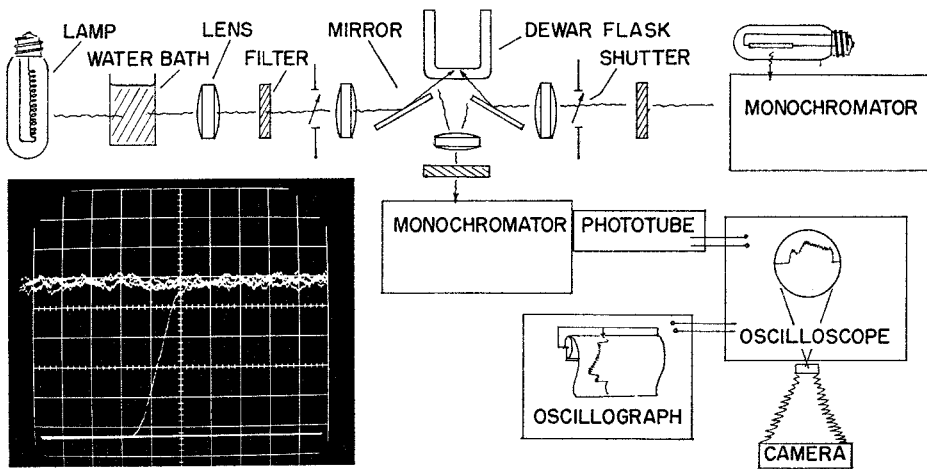


Fig. 1: Diagram of the spectrofluorometer, which includes Bausch and Lomb monochromators, an EMI 9558B phototube, a Tektronix 502A oscilloscope, and a Midwestern Instruments 801B oscillograph. We also used an Eppley thermopile and a Keithley 605A Microvolt-ammeter to measure light intensity, and a B & L Spectronic 505 spectrophotometer equipped with an integrating sphere. The vertically slanting curve in the photograph shows the phototube signal during a shutter opening (horizontal scale: 1 msec/cm).

*) First presented at the 12th Annual Meeting of the Biophysical Society of America, February 1968, Pittsburgh, Pennsylvania; it is published in detail in ref. [14] and [16].

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and methyl viologen, on the fluorescence transient in *Chlorella* within the first second of illumination. We conclude that a dip D preceding the main peak of the transient indicates oxidation of QH and reduced intersystem intermediates (we call these intermediates A) by System I, and that the peak P indicates reduction not only of Q and A but also of X, the electron acceptor for System I. Weak preillumination modifies the transient in several ways, most of which can be explained as due to changes in the redox state of A.

The instrument used to measure the transient is shown in figure 1. *Chlorella pyrenoidosa* was grown in inorganic medium; cells were resuspended for experiments in $\text{NaHCO}_3\text{-K}_2\text{CO}_3$ buffer, $\text{pH} = 9.2$. (These cells have a high quantum yield of O_2 evolution - $0.12 t_0 \times 0.08$.) Samples were studied under cycles of 2-second light exposures and 4-minute dark periods. The exciting light was a broad band centered on 500 nm, with incident intensity of $15,000 \text{ ergs/sec/cm}^2$, above the bend in curves of fluorescence intensity *versus* light intensity. Fluorescence was measured at 685 nm with full bandwidth of 10 nm.

The transient after a 4-minute dark period is shown in figure 2. O, I, P, and S are features labeled by LAVOREL [10] and JOLIOT and LAVOREL [11]. We repeatedly see a dip D between I and P under normal conditions, as evidenced by the sequence of photographs at the top of the figure. A dip has been seen before, in anaerobic cells by KAUTSKY and U.FRANCK [12], and in aerobic cells, by DELOSME [9].

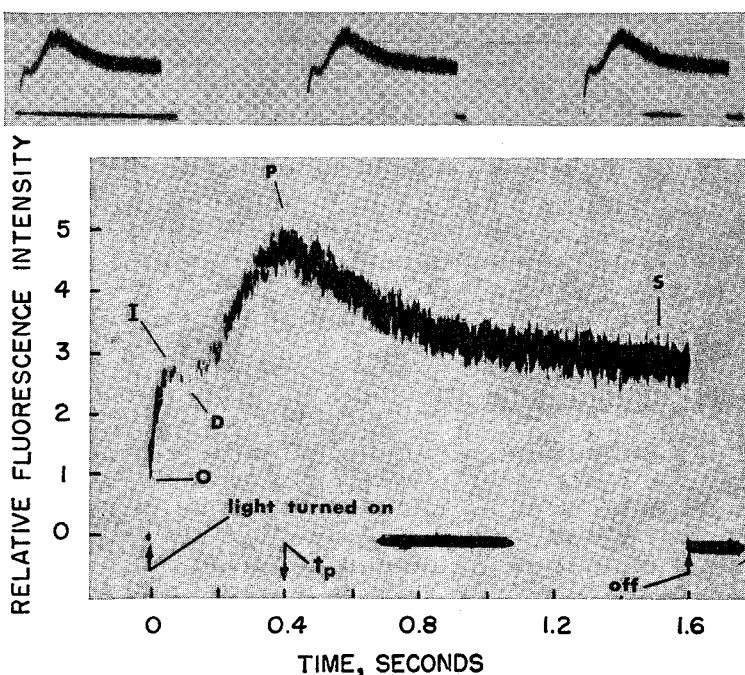


Fig. 2: The fluorescence transient in aerobic *Chlorella pyrenoidosa*. The small photographs were obtained from one sample at 4-minute dark intervals.

Effect of Methyl Viologen

The dip suggests oxidation of AH and QH by System I. Perhaps, then, the peak P represents a temporary accumulation of XH, as well as of AH and QH. Figure 3 shows the transient before and after treating cells with methyl viologen¹⁾, which accepts

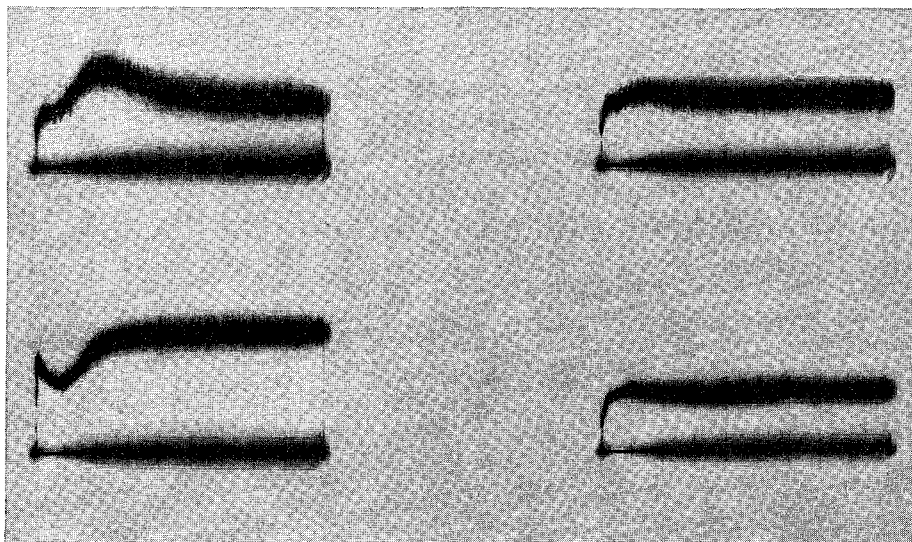


Fig. 3: The effect of methyl viologen on the fluorescence transient in *Chlorella*. Top left, before addition; top right, after addition. Bottom left (new sample), anaerobic cells after addition; bottom right, after admission of air.

electrons only from System I. A concentration of 10^{-4} moles/liter caused the progressive disappearance of P in 15 minutes, with little or no effect on O and I. (The 15 minute development was probably caused by diffusion and cellular penetration, as the cell suspension was not swirled after addition). The figure also shows that treated anaerobic cells displayed a normal anaerobic transient (compare with figure 4), but that admission of air led to an aerobic transient without P. These results are explained by the fact that lack of oxygen allows methyl viologen to accumulate in the reduced form, whence it can no longer oxidize XH. Elimination of P by methyl viologen (under aerobic conditions) suggests that besides Q and A, X is reduced at P.

The I-D Decline

The I-D decline is prominent in anaerobic cells, as shown in figure 4. The large photograph shows clearly the distinction between O and I, the steep rise and decline from I, and the high and persistent P level. The sequence of small pictures shows the transient

¹⁾ Methyl viologen: 1,1'-dimethyl-4,4'-dipyridilium dichloride. E_0' = - 426 mV [13].

as CO_2 in air was replaced by CO_2 in argon. KAUTSKY and U. FRANCK [12] showed that the I level is high (as in the large photograph) only when the oxygen concentration is below 1%, while P and S are raised by a concentration below 5%. KAUTSKY, APPEL, and AMANN [1] noticed that the transient of anaerobic cells excited by flashing rather than continuous light is prolonged (as confirmed in figure 5), and that the prolongation is proportional to the dark time between flashes. Thus, the I-D decline requires light and does not proceed in darkness.

We tested which pigment system sensitizes the decline by measuring the hastening of D by different background lights. 705 nm was chosen to excite System I, and 650 nm for System II (some 650 nm light reaches System I); half-maximum bandwidths were 10 nm, incident intensity at 705 nm was 1500 ergs/sec/cm², and intensity at 650 nm was adjusted with neutral density filters to give absorbed intensity equal to that at 705 nm. Effects

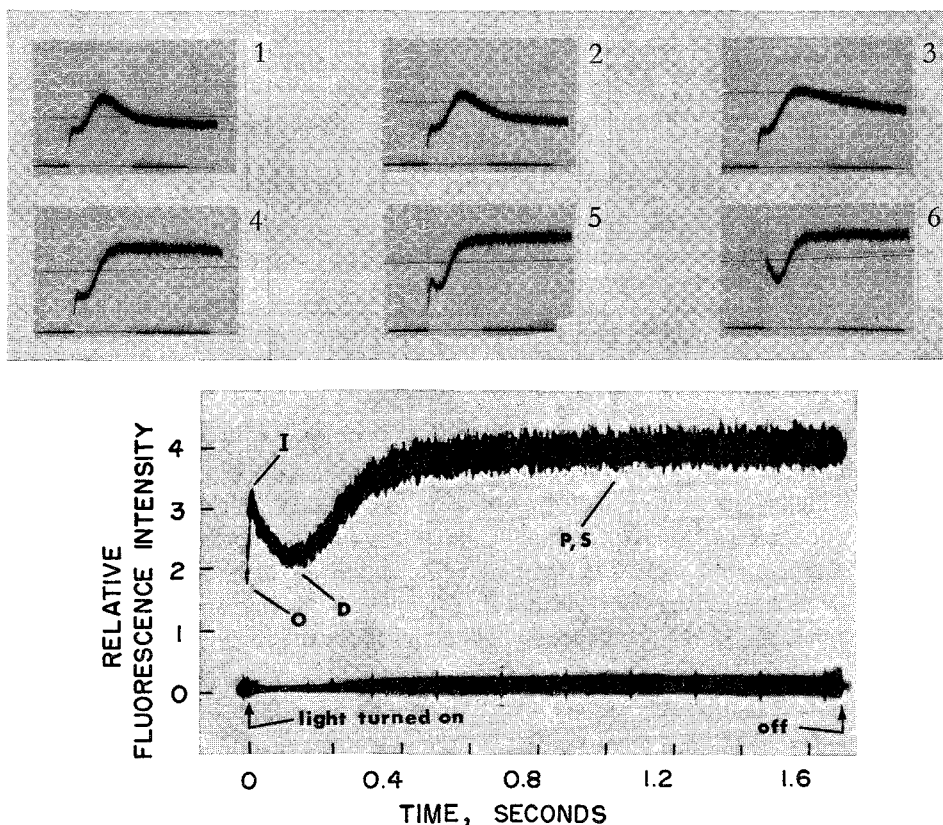


Fig. 4: Change of the fluorescence transient in *Chlorella* during oxygen removal. The small photographs were taken at 4 minute intervals as 2.0% CO_2 in air was replaced by 2.6% CO_2 in argon. Replacement began a few seconds after picture 1. The large photograph, from a different anaerobic experiment, shows the distinction between O and I.

were tested in several experiments, each involving at least 5 trials at each wavelength, alternated to eliminate influence of long-term fluorescence changes. Shutters for the background and flashing exciting lights were opened simultaneously.

Effects were expressed as fractional changes in the time of D, then averaged and divided by absorbed intensity to give relative quantum yield. The relative quantum

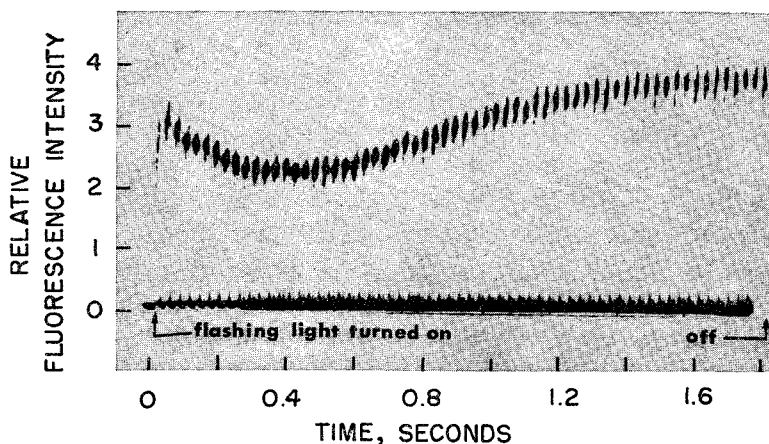


Fig. 5: The anaerobic transient excited by flashing light (40 msec on and off). D is delayed in flashing light (compared to continuous light) because the I-D decline progresses only during the light periods [1]. Compare D in figure 4.

yields in one experiment were, for 650 nm, 7.0, and for 705 nm, 8.2. The computed standard deviations were about 30% of the average effects. Several experiments gave similar results. The results rule out the possibility of a long-wavelength "red drop" in quantum yield.

Since System I light effectively sensitizes the I-D decline, and methyl viologen eliminates P, we believe that D represents oxidation of AH and QH by System I, and that P represents accumulation of XH, AH, and QH [14].

Effects of Preillumination

Expanding the work briefly reported at Brookhaven [15], we have studied the effects of weak preillumination at various wavelengths as a function of preillumination intensity, in both aerobic and anaerobic conditions. The procedure involved replacement of the 4-minute dark period in our light-dark cycles by preillumination. Full bandwidth was 10 nm, and incident intensities were 1-750 ergs/sec/cm². In general, these intensities were 100 to 1000 times weaker than the exciting light intensity. Preillumination with $\lambda \geq 690$ nm is called preillumination-1, and with $\lambda \leq 680$ nm, preillumination-2.

Figure 6 shows the results in aerobic cells of preillumination at 710 nm, expressed as percentage changes *versus* absorbed preillumination intensity. P is decreased in nonlinear

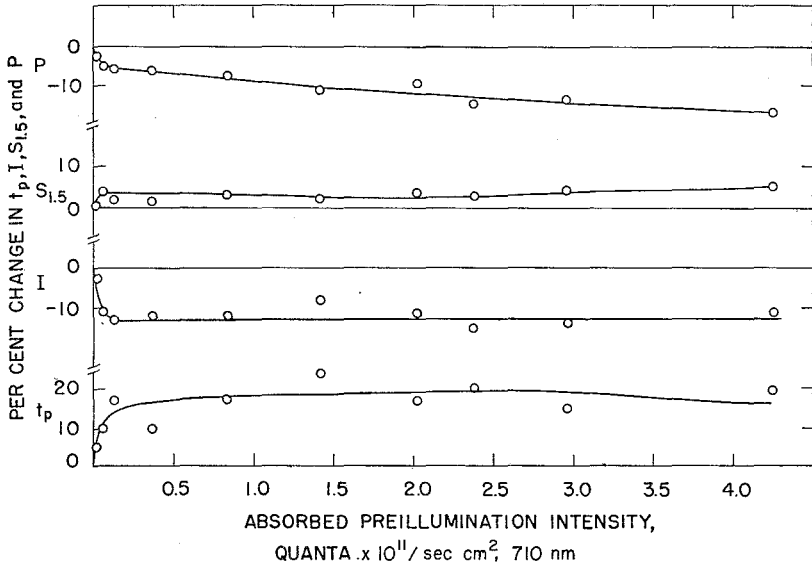


Fig. 6: The effects on points of the transient caused by different intensities of 710 nm preillumination replacing the usual 4-minute dark period.

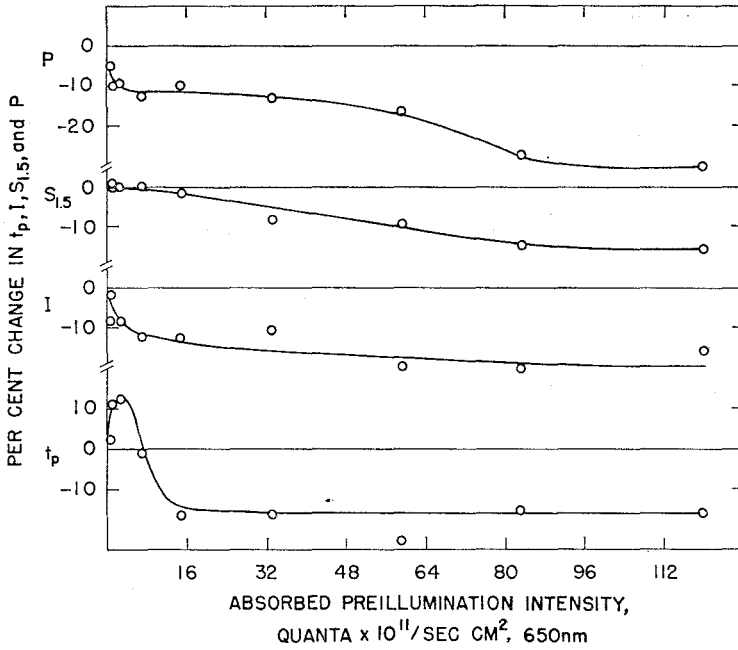


Fig. 7: The effects of 650 nm preillumination; see legend of fig. 6.

fashion, decreasing steeply at low intensity, and more gently at higher intensity. S is increased, and the effect saturates. I is decreased up to about 10%, and the effect saturates. In ref. [16], we show that O is not decreased by preillumination-1 of these intensities; thus, in the dark-period oxidation of QH is complete, without the aid of preillumination-1. However, this result, and the fact that I is decreased, signify that dark-period oxidation of AH is not complete; also I is affected by the concentration ratio A/AH and that the O-I rise represents reduction of both Q and A. At much higher exciting light intensities, only Q is reduced during O-I. (See MORIN [8] and DELOSME [9].) The last curve shows that preillumination-1 increases t_P , the time of P, i.e., that P has been delayed. The effect saturates at the same intensity as the other effects. Experiments for 725 and 700 nm gave results similar to the above.

Results at 650 nm are shown in figure 7. Those at 660 and 670 nm were similar. Effects on P are the same as at 710 nm, but the absorbed intensity is 30 times higher. S is decreased now rather than increased. These S changes are opposite to those reported by GOVINDJEE et al. [17], BUTLER [2], BUTLER and BISHOP [18], and DUYSSENS and SWEERS [3]. However, the conditions in our experiments are different, especially the use of preillumination of long duration. In the literature, immediate effects were studied. We believe the S changes are long-time effects, perhaps involving conformational changes (as proposed for long-term fluorescence induction by PAPAGEORGIOU [19], and PAPAGEORGIOU and GOVINDJEE [20, 21]), rather than short-time changes in the redox state of Q. (See the discussion in ref. [16].) The effect on I is the same as at 710 nm, except for the higher preillumination intensity. The t_P is increased at low intensity, but decreased at high intensity.

Results at 680 and 690 nm were intermediate compared to those at shorter and longer wavelengths. Comparing all results, we found that absorbed intensities for the saturation regions in the curves increased as the wavelength decreased. In some earlier experiments,

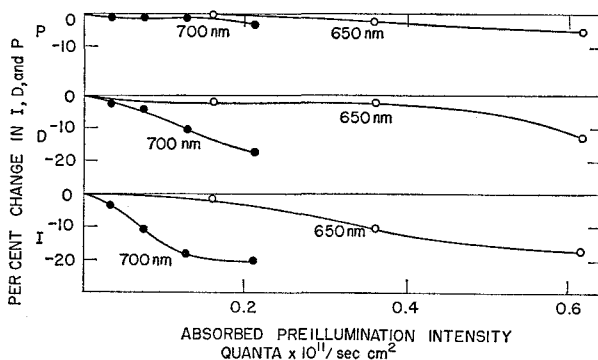


Fig. 8: The effects of 700 nm and 650 nm preillumination in anaerobic cells. Plots for t_P and S at 1.5 seconds are omitted; they are unmeasurable because P does not decline for several seconds in anaerobic cells.

we employed 30 second rather than 2 second exciting light exposures [15]. Then, the preillumination effects on P included an increase at lowest intensities [16]. We do not explain these results here.

Effects of preillumination of anaerobic cells are shown in figure 8. P, I, and D are decreased by 700 nm light (black circles) at much lower intensities than for 650 nm (open circles). The decreases are thus sensitized by System I.

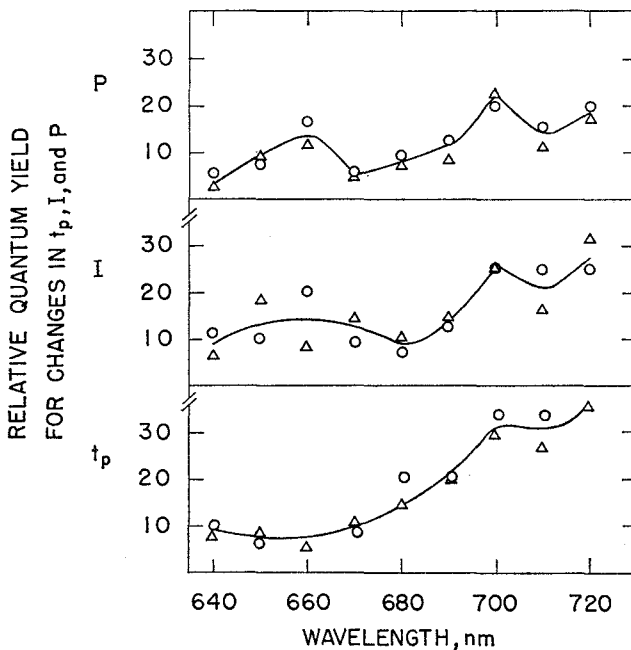


Fig. 9: The relative quantum yields for the increase of t_p and the decreases of I and P, versus preillumination wavelength. Preillumination intensities were below the saturation regions of figures 6 and 7. (For discussion see ref. [16]).

Figure 9 shows the relative quantum effectiveness of different preillumination wavelengths in aerobic cells. The spectra were obtained from single samples, and the intensities chosen to be in the linear and steeply sloping portion of the curves of figures 6 and 7. Spectra are shown for P, I, and t_p ; an S spectrum was omitted because S effects were small at the low intensities used. All three spectra show clear sensitization at long wavelengths, with peaks at 700 nm, and perhaps at 720 nm if absorption data at 720 nm are regarded as sufficiently accurate.

Results in figures 6 through 9 are consistent in indicating that the preillumination effects on P, I, and t_p at low intensity are caused by light reaching System I. These effects are explainable as involving an increase in the concentration ratio A/AH . However, the gentle decrease in P at higher preillumination intensity, and the t_p decrease for high

intensity preillumination-2, do not involve further changes in A/AH, because at intensities where these effects appear, the I decrease caused by changes in A/AH is already saturated.

Since the P, I, and t_p effects of low intensity preillumination-2 are the same as those of preillumination-1, and absorbed intensities of preillumination-2 are higher, we suggest that dim light reaching System II is wasted. Such a suggestion has previously been made by MURATA et al. [22] and JOLIOT [4], and discussed recently by MALKIN [23].

Conclusion

In our experimental conditions, the O-I rise represents reduction of Q and A; D represents oxidation of AH and QH by System I; and P represents reduction of X, A, and Q. The redox changes of X and A influence fluorescence yield by affecting the redox state of Q.

Preillumination effects on P, I, and t_p at low intensity are caused by light reaching System I, and apparently involve an increase in the concentration ratio A/AH. Dim light reaching System II appears to be wasted. The changes in S we believe to be long-term induction effects, perhaps involving conformational changes.

References

- [1] KAUTSKY, H., W. APPEL, and H. AMANN: *Biochem. Z.* **332**, 277 (1960).
- [2] BUTLER, W.L.: *Biochim. Biophys. Acta* **64**, 309 (1962); see also *Biochim. Biophys. Acta* **66**, 275 (1963).
- [3] DUYSSENS, L.N.M., and H.E. SWEERS in: "Studies on Microalgae and Photosynthetic Bacteria", Japanese Society of Plant Physiologists, (Ed.) p. 353, Univ. of Tokyo Press (Tokyo 1963).
- [4] JOLIOT, P.: *Biochim. Biophys. Acta* **102**, 135 (1965).
- [5] KREY, A., and GOVINDJEE, *Biochim. Biophys. Acta* **120**, 1 (1966).
- [6] MALKIN, S., and B. KOK: *Biochim. Biophys. Acta* **126**, 413 (1966).
- [7] MALKIN, S.: *Biochim. Biophys. Acta* **126**, 433 (1966).
- [8] MORIN, P.: *J. Chim. Phys.* **61**, 674 (1964).
- [9] DELOSME, R.: *Biochim. Biophys. Acta* **143**, 108 (1967).
- [10] LAVOREL, J.: *Plant Physiol.* **34**, 204 (1959).
- [11] JOLIOT, P., and J. LAVOREL: *Bull. Soc. Chim. Biol.* **46**, 1607 (1964).
- [12] KAUTSKY, H., and U. FRANCK: *Biochem. Z.* **315**, 139 (1943).
- [13] BLACK, C.: *Biochim. Biophys. Acta* **120**, 332 (1966).
- [14] MUNDAY, J. C., JR., and GOVINDJEE: *Bioph. J.* **9**, 1 (1969).
- [15] GOVINDJEE, J. C. MUNDAY JR., and G. PAPAGEORGIOU in: J. M. OLSON (Ed.) "Energy Conversion by the Photosynthetic Apparatus", p. 71, Brookhaven Symposia in Biology, No. 19 (Upton, New York 1966).

*) *Note added in proof*: At the time we received the proof of this paper (February 1969) a detailed paper by BANNISTER and RICE [24] on fluorescence induction was in print. They have compared some of our data presented here and elsewhere [14-16, 19-21, 25] with those obtained in their laboratory and in DUYSSENS' laboratory [26]. (See ref. [24] for similarities and differences in results and interpretations.)

- [16] MUNDAY, J. C., JR., and GOVINDJEE: *Bioph. J.* **9**, 22 (1969).
- [17] GOVINDJEE, S. ICHIMURA, C. CEDERSTRAND, and E. RABINOWITZ: *Arch. Biochem. Biophys.* **89**, 322 (1960).
- [18] BUTLER, W. L., and N. I. BISHOP in: "Photosynthetic Mechanisms of Green Plants", p. 91, National Academy of Sciences, National Research Council, Publ. No. 1145 (Washington, D.C., 1963).
- [19] PAPAGEORGIOU, G.: Doctoral Thesis, University of Illinois, 1968.
- [20] PAPAGEORGIOU, G., and GOVINDJEE: *Biophys. J.* **7**, 375 (1967).
- [21] PAPAGEORGIOU, G., and GOVINDJEE: *Biophys. J.* **8**, 1299, 1316 (1968).
- [22] MURATA, N., M. NISHIMURA, and A. TAKAMIYA: *Biochim. Biophys. Acta* **120**, 23 (1966).
- [23] MALKIN, S.: *Biochim. Biophys. Acta* **153**, 188 (1968).
- [24] BANNISTER, T. T., and G. RICE: *Biochim. Biophys. Acta* **162**, 555 (1968).
- [25] PAPAGEORGIOU, G., and GOVINDJEE: in H. METZNER (Ed.): "Progress in Photosynthesis Research", Vol. II, 905-912, Tübingen 1969.
- [26] DUYSSENS, L. N. M., and A. TALENS: in H. METZNER (Ed.): "Progress in Photosynthesis Research", Vol. II, 1073-1081, Tübingen 1969.