Photochemistry and Photobiology. 1971. Vol. 14, pp. 667–682. Pergamon Press. Printed in Great Britain

FLUORESCENCE INDUCTION IN THE RED ALGA *PORPHYRIDIUM CRUENTUM*

P. MOHANTY, G. PAPAGEORGIOU* and GOVINDJEE
Department of Botany, University of Illinois, Urbana, Illinois 61801, U.S.A.

(*Received* 8 *December* 1969; *accepted* 28 *May* 1971)

Abstract—The intensity dependence and the spectral changes during the fast (sec) and the slow (min) transient of chlorophyll (Chl) *a* fluorescence yield, measured at 685 nm, have been analyzed in the red alga *Porphyridium cruentum*. Both the fast and the slow fluorescence yield changes are affected differently by the inhibitors of electron transport (*e.g.*, DCMU) and by the uncouplers of phosphorylation (atebrin and FCCP). Fixation of *Porphyridium* cells with glutaraldehyde abolishes most of the fluorescence yield changes except for the so-called very fast (*OI*) phase. The same fixed cells, however, reduce DCPIP (a Hill oxidant) but do not evolve O_2 when CO_2 is used as electron acceptor. We interpret these and other results by the hypothesis that fluorescence transients in intact cells are linked to both electron transport and the energy dependent structural changes in the thylakoid membrane.

INTRODUCTION

LIGHT induced changes† in the yield of chlorophyll (Chl) *a* fluorescence have been well documented in the intact green algae both in the fast (sec) and in the slow (min) region (see Refs. [1–3]). Characteristic points in the fast induction are: a low initial level *O*, hump *I*, followed by a dip *D*, a peak *P*, and a temporary steady-state low level *S*. Characteristic points in the slow phase are: the low level *S*, followed by a rise to a maximum *M*, and then a final decline to a terminal steady state level *T*. In the blue-green algae, there is a very slow decline from *M* to *T* [4, 5].

The time course of the rate of oxygen evolution is essentially antiparallel to that of the fast fluorescence changes (*OPS*) except for part of the very fast *OI* phase [6, 7]; both these processes are approximately complementary as their rates add up to a constant [6–8]. At this stage, the fluorescence yield of Chl *a* is mostly determined by the reduction level of the quencher *Q*—the primary electron acceptor of pigment system II [9]. On the other hand, during the *SM* phase (slow induction), the rate of oxygen evolution rises in parallel to fluorescence, and then it reaches a constant level during the *MT* phase [3, 10]. During this *SMT* phase, the reduction level of *Q* does not seem to be the sole determinant of the Chl *a* fluorescence yield [11].

Several hypotheses have been proposed to explain the slow fluorescence change in intact algae. Papageorgiou and Govindjee [3–5] suggested that light induces a spatial alteration of Chl *a* molecules as a result of phosphorylation induced changes in the membrane 'conformation'. In other words, the yield of Chl *a* fluorescence becomes

---

Abbreviations: Chl *a*, chlorophyll a; DCPIP, 2-6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; CMU, 3-(4-chlorophenyl)-1, 1-dimethylurea; FCCP, *p*-trifluoromethoxyphenylhydrazone of ketomalonylnitrile.

*Present address: Department of Biology, Greek Atomic Energy Commission, Democritus, Athens, Greece.

†In this paper, the terms fluorescence induction, transient and change have been used interchangeably.

sensitive to the environment and configuration of the thylakoid membrane besides its sensitivity to the redox level of the primary acceptor Q. Bonaventura and Myers[12], and Murata[13–15] suggested that a change in the rate of the excitation energy transfer from (pigment) system II to I can account for the slow fluorescence change. Murata [13] observed that cations can cause a significant decrease in the rate of this transfer in chloroplasts. Duysens[16] proposed that spatial modifications of the membranes—on which the two photosystems are located—are responsible for the changes in excitation energy transfer (see a recent review[17]). More recently, Vredenberg[18] has shown a correlation of the fluorescence change (*PSMT*) with the depolarization of membrane potential in giant algal cells of *Nitella*; he suggests that the slow fluorescence change is caused by a change in the proton transport across the cellular membranes. It is possible that the ion fluxes across the cellular membranes are associated with changes in the conformation of the thylakoid membranes.

The present study extends the observation on fluorescence transients in algae to *Porphyridium cruentum*—a red alga prototype for photosynthesis research[14, 19–21]; we have analyzed the kinetics of the fast and the slow transient of Chl *a* fluorescence yield in the presence and in the absence of inhibitors of electron transport and uncouplers of phosphorylation. We have also studied fluorescence transients in cells fixed with glutaraldehyde—the latter is known to immobilize the structural changes (see Utsumi and Packer[22], and Deamar *et al.*[23]) but allows Hill reaction to operate (see Park *et al.*[24] and Hallier and Park[25]). Our results show that fixation of intact cells of *Porphyridium* with glutaraldehyde eliminates the slow fluorescence yield change. We suggest that this is a consequence of the elimination of the 'configurational' changes on the thylakoid membranes.

MATERIALS AND METHODS

Porphyridium cruentum was grown in an inorganic medium (see Govindjee and Rabinowitch[26] for details). A stream of 5% CO_2 in air was bubbled to provide CO_2 and to give constant agitation. White 'fluorescent' light was provided from below and the temperature was maintained at $19 \pm 2°C$ by continuous flow of water. Six to eight day old cultures were used in the experiments. Cells were centrifuged at $500 \times g$ for 10 min and suspended in one of the buffers described in the legend of the figures. The final suspension was passed twice through glass wool to eliminate clumped cells.

Glutaraldehyde was purified according to Anderson[27] with Norit EX charcoal. (Glutaraldehyde reacts with free amino and SH groups of amino acids to make intermolecular cross linkages in proteins. Proteins treated in this way still react with antibodies of native proteins and preserve their enzymatic activities[28].) Excess glutaraldehyde was removed by repeated washings with buffer. Glutaraldehyde (3% v/v) fixation was made according to Park *et al.*[24]. Cells were again passed through glass wool to eliminate clumped cells.

Absorption measurements were made with Bauch and Lomb (Spectronic 505) recording spectrophotometer equipped with an integrating sphere. For samples to be used in fluorescence measurements, absorbance was adjusted to 0·03–0·05 at the Chl *a* maximum in the red end of the spectrum.

Fluorescence transient measurements were made as described elsewhere[1]. Fluorescence signal was measured either through a Keithley ammeter or through a

Tektronix oscilloscope. An Esterline Angus or a Midwestern oscillographic recorder was used to record the transient.

Fluorescence was excited by 540 nm green light (half band width, 20 nm), or with blue–green light (Corning C.S. 4–96 plus 3–73). The intensity of excitation light was varied by inserting calibrated neutral density filters. For transient studies, fluorescence was observed at 685 nm (half-band width, 6·6 nm). A Corning C.S. 2–58 or C.S. 2–64 filter, placed at the entrance slit of the analyzing monochromator, eliminated stray exciting light.

Fluorescence emission spectra were measured as described elsewhere[29]; they were scanned with a 6·6 nm half-band width, and these are presented after correction for the spectral sensitivity of the photomultiplier (EMI 9558 B) and the transmission efficiency of the analyzing monochromator.

Oxygen exchange was monitored with a Haxo–Blinks type of rate electrode[30]. The electrodes were immersed in a buffered electrolyte and gassed with 2% CO_2 in air (temperature, 19° ± 1°C). The samples were illuminated from the top with green light (545 nm interference filter plus Corning C.S. 1–69).

In cells fixed with glutaraldehyde, Hill reaction with DCPIP was measured in saturating ($1{\cdot}5 \times 10^6$ ergs cm^{-2} sec^{-1}) white light, obtained from a 1000-W tungsten lamp, passed through a 11·5 cm water filter. The reaction mixture contained 50 μM DCPIP, 0·05 *M* phosphate buffer, pH 6·8, and 0·1 *M* NaCl.

Chlorophyll *a* was estimated in 80% acetone, assuming an ϵ_{663} of 82 cm^2/mM^{-1}.

All chemical additions to the samples preceded the measurements by at least 10 min to insure penetration into the cell. Dark adaptation lasted 10 min for fluorescence as well as O_2 exchange measurements.

All measurements were carried out at room temperature, 22–25°C.

RESULTS

Time course of Chl fluorescence and the rate of O_2 *evolution*

Figure 1 shows the fast fluorescence transient (*OIDPS*) measured at 685 nm in *Porphyridium cruentum*. Figure 1(A) is an oscillographic record (*I* exc, 16 kergs cm^{-2} sec^{-1}) and Fig. 1(B) is redrawn from a different experiment (*I* exc, 9·2 kergs cm^{-2} sec^{-1}); the latter shows the various characteristic points on the curve. The ratio of *P–O/O* is 1·5 to 1·7 [Fig. 1(A)]; the time of appearance for *P* is about 0·4–0·5 sec, and for *D* is 0·1–0·2 sec.

Figure 2 (top curve) shows the slow fluorescence yield change (*PSMT*) (λ exciting, broad band blue–green light). The extent of *SM* rise and the time to reach *M* varies with the culture condition. Figure 2 (bottom curve) shows the time course of oxygen evolution using saturating green light. The complementary phase of oxygen evolution and fluorescence is noticeable in the *P* to *S* decline phase, but is not seen here in the *DP* phase due to the slow response of the polarograph used. (The initial O_2 spike is less pronounced in *Prophyridium* than in other algae at room temperature[31].) In spite of the difference in the quality of light used for fluorescence (blue–green) and O_2 (green) measurements, the results indicate that the rate of O_2 exchange rises in parallel to the *SM* rise and approaches a constant rate during the *MT* decline phase of fluorescence.

Both the oxygen and the fluorescence transient could be repeated if enough dark

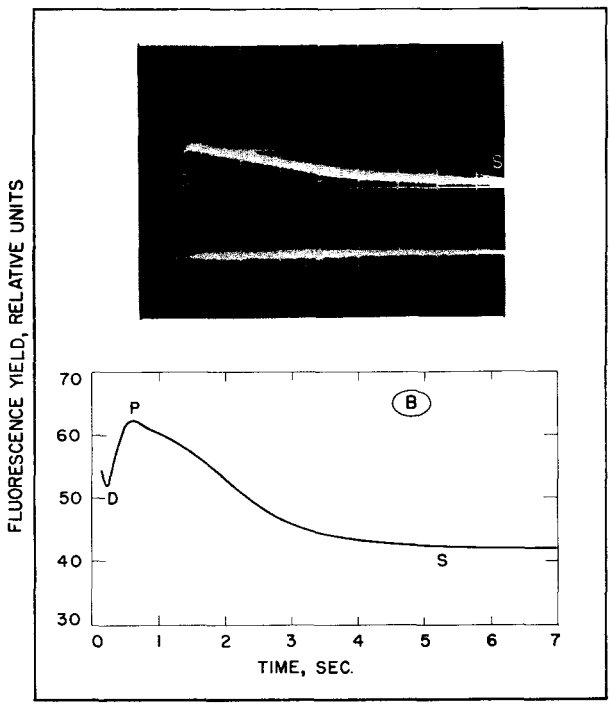

Fig. 1. Time course of the fluorescence yield of Chl *a* in *Porphyridium cruentum*. Top (A): Oscillographic picture of fluorescence transient; 6-day old culture; buffer, carbonate-bicarbonate-sodium chloride, pH 8·5; λ observation, 685 nm (half-band width, 6·6 nm); C.S. 2–58 filter before the photomultiplier; λ excitation, blue-green light (C.S. 4–96 + 3–73) (half-band width, 120 nm); intensity of excitation, $1{\cdot}6 \times 10^4$ ergs cm^{-2} sec^{-1}; ordinate, 0·5 V/division; abscissa, 1 sec/division; note the *OIDPS* points on the transient. Bottom (B): Recorder trace of transient from another culture; 8-days old; buffer, 0·05 *M* Tris-HCl, and 0·25 *M* NaCl, pH 8·5; λ excitation, 540 nm (half-band width, 20 nm); intensity, $9{\cdot}2 \times 10^3$ ergs cm^{-2} sec^{-1}; λ observation, 685 nm (half-band width, 6·6 nm); C.S. 2–64 filter before the photomultiplier; note the pronounced *DPS* transient, *O* is not shown on the trace.

interval was given. In our experiments a 10 min dark interval restored more than 95 per cent of the fluorescence peak at *P*.

Upon the addition of DCMU both fast and slow transients (and O_2 evolution) disappear except the very fast *OI* phase (Fig. not shown; see Ref. [32]).

Intensity of excitation and the fluorescence induction

The time courses of Chl *a* fluorescence yield, recorded at various intensities of illumination, are shown in Figs. 3 and 4. The photochemical nature of the individual processes, that contribute to the induction phenomenon, is evidenced by its dependence on the intensity of excitation—as shown in these figures. Very low intensities fail to induce transients of measureable magnitude. As the excitation intensity is raised, the amplitudes of both the fast and the slow fluorescence changes increase.

Figure 5 shows a plot of *tp*, the time in sec to reach the peak *P*, as a function of intensity of exciting light. It is clear that *P* is delayed as the exciting intensity is lowered; at low intensities, the Bunsen–Roscoe law is obeyed. However, from Fig. 4, it is clear

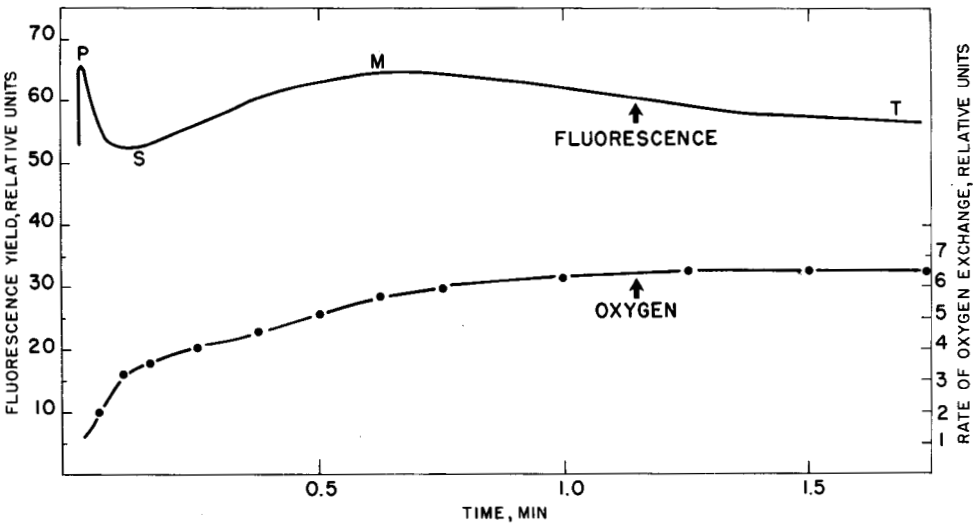

Fig. 2. Time course of the fluorescence yield of Chl *a* and the rate of O_2 exchange in a 6-day old culture. Top curve: Recorder trace of fluorescence transient—experimental conditions as described in the legend of Fig. 1(A); the fast *OI* phase is not shown. Bottom curve: Oxygen exchange as measured with a Haxo–Blinks type electrode, and recorded on a Heathkit recorder; λ excitation, 545 nm light; intensity, $1{\cdot}2 \times 10^4$ ergs cm^{-2} sec^{-1}; buffer, 0·05 *M* potassium-sodium phosphate, plus 0·25 *M* NaCl, pH 7·0; other details as in materials and methods.

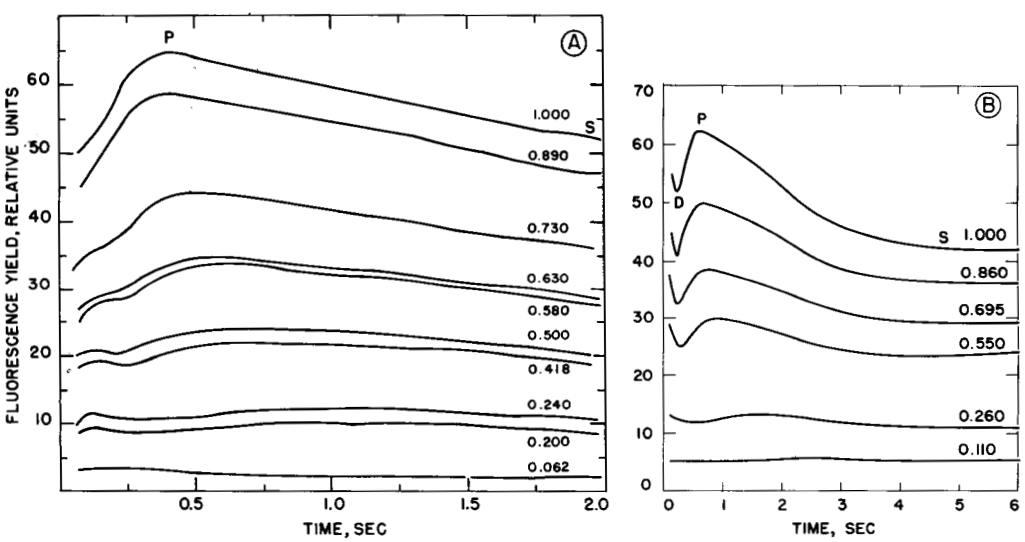

Fig. 3. Fast transient of the fluorescence yield of Chl *a* at different intensities of excitation. Left (A): Recorder traces; numbers on the curves are fractions of the maximum intensity used; intensity 1·00 = $1{\cdot}8 \times 10^4$ ergs cm^{-2} sec^{-1}; other details as in Fig. 1(A). Right (B); Intensity 1·000 = $9{\cdot}2 \times 10^3$ ergs cm^{-2} sec^{-1}; other details as in Fig. 1(B).

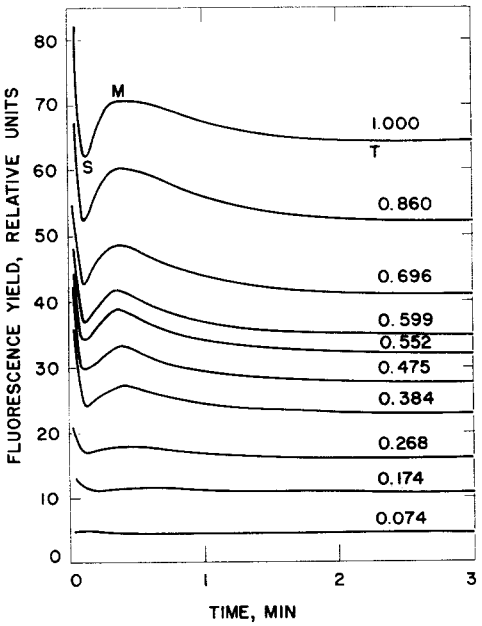

Fig. 4. Slow transient of the fluorescence yield of Chl *a* at different intensities of excitation. Intensity 1·000 = $9{\cdot}2 \times 10^3$ ergs cm^{-2} sec^{-1}; other conditions as in Fig. 1(B).

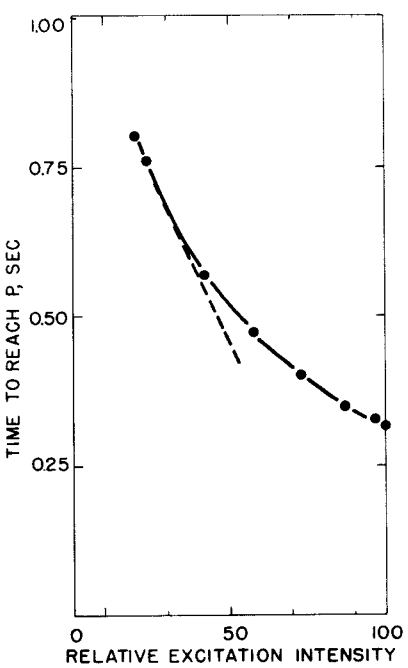

Fig. 5. Time (*tp*) for the appearance of peak *P* as a function of the intensity of exciting light. 100 on abscissa = $1{\cdot}8 \times 10^4$ ergs cm^{-2} sec^{-1}; this plot was obtained from experiments similar to those in Fig. 3(A) except that oscillographic pictures were taken as in Fig. 1(A).

that the time to reach M did not change significantly with light intensity except, perhaps, at very low light intensity.

Figure 6 (A and B) shows our attempts to separate the different processes that are operative in both the fast and slow induction phases. In these figures, an average rate function defined as $F_{t_1} - F_{t_2}/t_2 - t_1$ is plotted against the intensity of excitation. (Rates defined in terms of half-times could be misleading due to superposition of a number of processes, which, in general, may not be first order.) It is evident that SM and MT phases saturate at lower intensity than the DP phase.

Figure 7 illustrates the constancy of the relative yield (ϕF_T) at the terminal steady state T in the intensity range this experiment was made. On the other hand, fluorescence yield quotients f_M and f_T (which measure the ratio of fluorescence intensity at M and T with respect to that at S normalized as unity) show characteristic dependence on the same intensity of excitation. Upon increasing the intensity (I), the amplitude of S increases proportionately more than at T; this is inferred from a comparison of the ϕ_{FT} and f_T curves. But, in the same intensity range, the amplitude of M increases much more and, thus, f_M increases with I. Upon further increase in intensity, neither M and S increase as much as T; but, both S and M increase in the same proportion leading to a

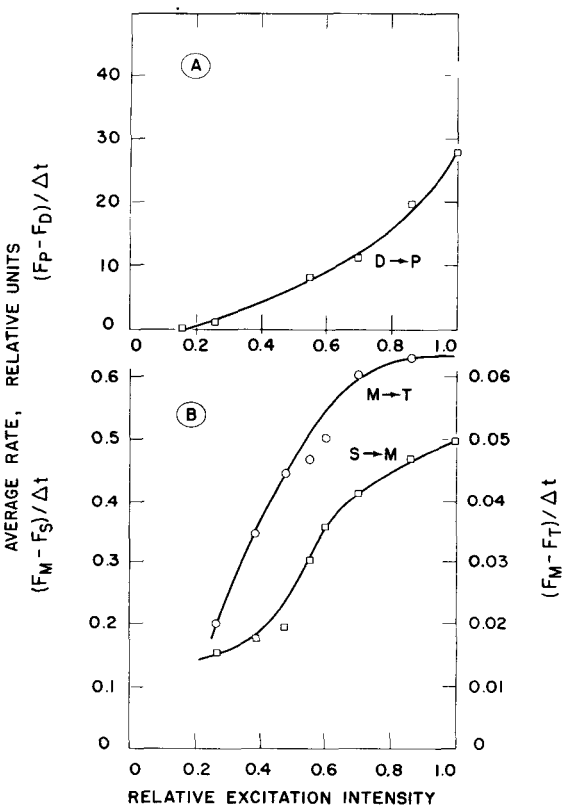

Fig. 6. Average rate of change in the fluorescence yield as a function of the intensity of exciting light. (A): For DP rise; (B) for SM rise, and MT decline; intensity $1{\cdot}0 = 9{\cdot}2 \times 10^3$ ergs cm^{-2} sec^{-1}; all other conditions same as in Fig. 1(B).

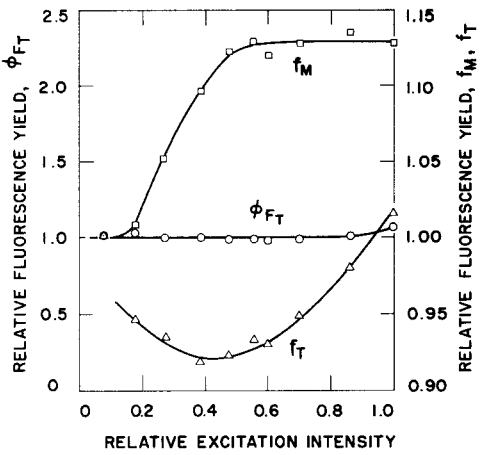

Fig. 7. Relative fluorescence yield at the stationary state T (ϕF_T) and the quotients $f_M (= F_M/F_S$ and $f_T (= F_T/F_S)$ as functions of the intensity of excitation. Intensity $1{\cdot}0 = 9{\cdot}2 \times 10^3$ ergs cm^{-2} sec^{-1}; all other conditions as in Fig. 1(B).

constancy in f_M. From all these data, we suggest that the underlying processes for the fluorescence for *SM* rise and *MT* decline are different.

Spectral changes during the fluorescence induction

We obtained the fluorescence spectra of *Porphyridium cruentum* at different stages of the induction by observing the fluorescence time course at various wavelengths. Dark periods of suitable length (10 min) were interposed between the recordings to assure the reproducibility of the results. Figure 8 shows the spectra at *D*, *P*, and *S*, as well as the difference spectra *P–D* and *P–S*. The difference bands are peaked at 687 nm for the *P–D*, and at 684 nm, for the *P–S* spectra while their half-band widths are about 25 nm. In Fig. 9 the fluorescence spectra at *S* and *M* and the difference spectrum *M–S* are given. The maximum of the difference band is at 684 nm and its half-band width is 23 nm. (Samples from different cultures were used for the spectra presented in Figs. 8 and 9.)

These results indicate that throughout the fluorescence time course the fluorescence induction is restricted to changes in Chl *a* (684–687 nm). The constancy of the allophycocyanin fluorescence yield (660 nm) rules out light-induced changes in the efficiency of excitation energy transfer from allophycocyanin to Chl *a*. However, an increase in the yield of fluorescence in system II may be accompanied by a decrease in the fluorescence of system I because of decrease in the efficiency of energy transfer from Chl *a* of system II to that of system I. Examination of Fig. 8, and Table 1 shows that the ratio of fluorescence intensity at the peak of Chl *a* (mostly system II) to that at 710 nm (mostly system I) is higher at the *P* (2·68) than at the *S* level (2·46), and is highest (4·31) for the *P–S* spectrum. This result could be interpreted to mean that at *P*, as compared to *S*, there is much more Chl a_{II} than Chl a_I fluorescence; it appears that as the fluorescence yield changes from *P* to *S* an increase in the efficiency of transfer from system II to system I occurs. In the language of Duysens[16], and Bonaventura and Myers[12], the light 'state' 1 is converted to light 'state' 2. Figure 9 also shows that the

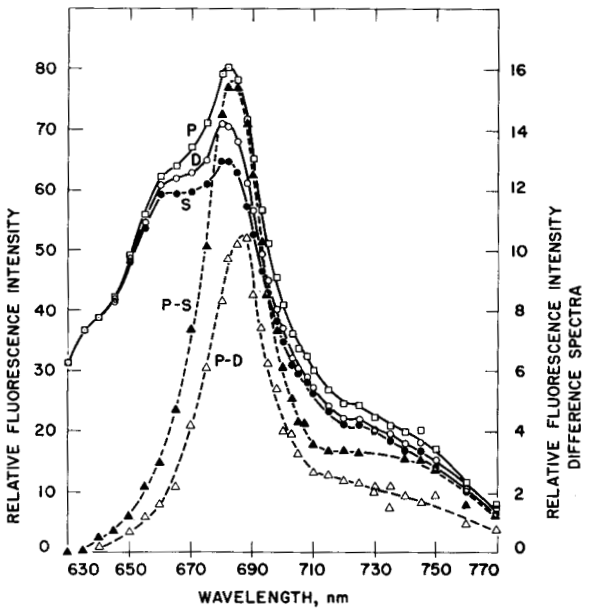

Fig. 8. Fluorescence emission spectra at the stage *D* (open circles), *P* (open squares), and *S* (solid circles). Difference spectra *P–D* (open triangles) and *P–S* (solid triangles) on an expanded (5X) ordinate scale. Details as in Fig. 1(B) with the exception that C.S. 2–58 was used instead of C.S. 2–64 filter.

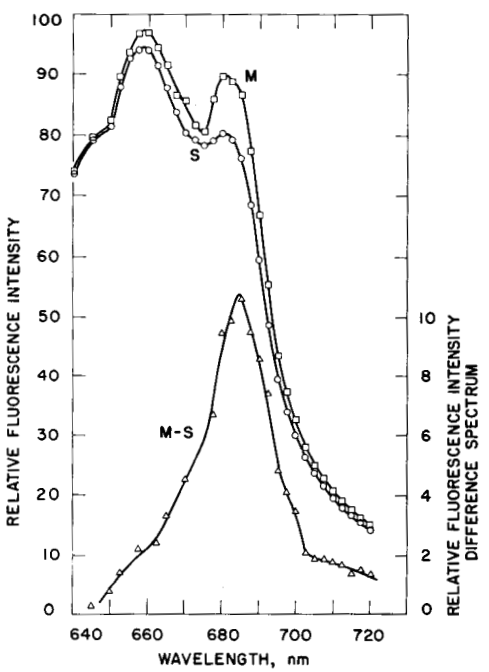

Fig. 9. Fluorescence emission spectra at the state *S* (open circles) and *M* (open squares) of the slow fluorescence transient. Difference emission spectrum *M–S* (open triangles) on an expanded (5X) ordinate scale. A different culture—grown at much higher intensity—was used in this experiment than in Fig. 8; other details as in Fig. 1(B).

Table 1. Ratios of fluorescence intensity at the peak (mainly system II) to that at 710 nm (system I) at different portions of fluorescence transient

Experiment No. 1*		Experiment No. 2†		Adjusted to experiment No. 1
D	2·63	*S*	4·12	2·46
P	2·68	*M*	4·39	2·62
S	2·46	*M–S*	6·12	3·66
P–S	4·31	—	—	—

*Culture number 1, incident intensity, 1.
†Culture number 2, incident intensity, 2.

ratio of the intensity at the fluorescence peak to the fluorescence intensity at 710 nm increases when the yield changes from *S* to *M*. The exact value of this ratio at *S* was very different from that in the previous experiment partly because of the different culture used here. In this culture (Fig. 9), as compared to the previous one (Fig. 8), the efficiency of energy transfer from allophycocyanin to Chl *a* was lower as is evidenced by the higher allophycocyanin to Chl *a* fluorescence. Thus, one can suggest that the observed fluorescence yield changes from *S* to *M* have again been brought about by a change from state 2 to state 1.

Effect of inhibitors of electron transport and uncouplers of photophosphorylation on slow fluorescence changes

The requirements of photosynthetic electron flow for fluorescence transient can be demonstrated by the use of potent inhibitors like CMU and DCMU. In the presence of CMU and DCMU, Chl *a* fluorescence yield in *Porphyridium* rises to a maximum within 2–3 sec and then remains constant for an extended period of illumination (Fig. 10). Upon DCMU poisoning the maximum yield was higher than that at *P*; this is so because the intensity of exciting light used here was not high enough to get true *P* as defined by Lavorel[33]. With weak illumination, the fluorescence rises slowly to a maximum in DCMU poisoned *Porphyridium*. In the dark the reduced Q is reoxidized due to recombination of the oxidized donor $Z(Z^+)$ and the reduced acceptor $Q(Q^-)$ of system II[32, 34]. (The effects of hydroxylamine (another inhibitor of O_2 evolution) on *Porphyridium*, and their interpretations are described in another paper by P. Mohanty *et al.*[32].)

At low concentration (2–5 μM) of uncoupler FCCP (Fig. 10B), the *P* to *S* decline rate becomes slower, but the oxygen evolution was not suppressed to any great extent at the steady state (3 min of illumination). However, at higher concentrations (10 μM or more) of FCCP, a suppression of O_2 exchange is seen. Atebrin, at a concentration usually used to uncouple photophosphorylation in chloroplast reaction (10 μM), inhibited fluorescence changes to the same extent as 10 μM FCCP. (No oxygen exchange experiments could be carried out with Atebrin, as this compound reacted with our electrode system.) Phloridzin, the energy transfer inhibitor of ATPase[35] did not suppress significantly the slow *SM* rise phase.

Fixation of photosynthetic membranes by glutaraldehyde and fluorescence induction

Intact cells of *Porphyridium* fixed with glutaraldehyde did not show any change in

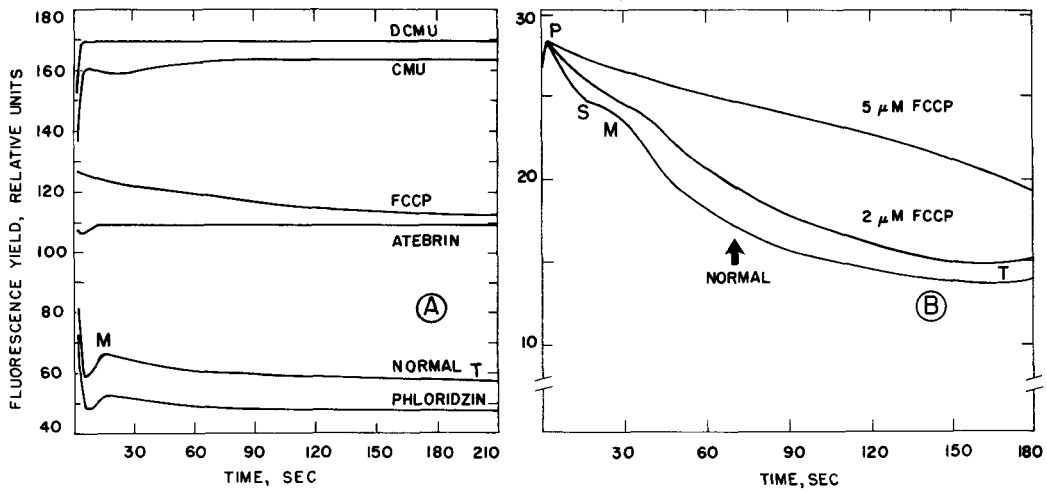

Fig. 10. Effects of oxygen evolution inhibitors and of photophosphorylation uncouplers on the slow change of Chl *a* fluorescence. Left (A): Normal cells; with 10 μM DCMU; with 10 μM CMU; with 10 μM FCCP; with 10 μM atebrin; with 2 mM phloridzin. All curves are plotted on the same scale; details as in the legend of Fig. 1(B). Right (B): Transients with and without FCCP (2 μM or 5 μM) – normalized at peak P; details as in Fig. 1(A).

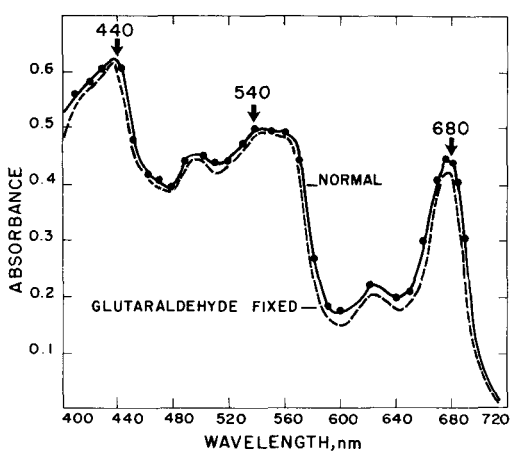

Fig. 11. Absorption spectra for normal (solid) and fixed (dashed) cells adjusted at the red peak. Fixation was in 3% (v/v) glutaraldehyde (see text for details); buffer, 0·05 M potassium phosphate plus 0·25 M NaCl.

the absorption spectrum of the pigments *in vivo* (Fig. 11). However, Cohen-Bazire and Lefort-Tran[36] have reported a depression in the concentration of phycoerythrin although no change in energy transfer from phycocyanin to Chl *a* was observed upon fixation[37].

The DCPIP reduction in the fixed cells, suspended in a phosphate buffer at pH 6·8, was found to be about 30–80 μmoles of dye reduced/mg-Chl/hr in saturating white light.

Figure 12 shows measurements of oxygen evolution with CO_2 as oxidant in both

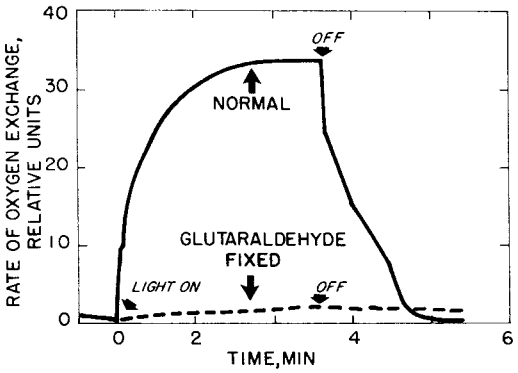

Fig. 12. Time course of oxygen exchange in the normal (solid) and glutaraldehyde fixed (dashed) cells with CO_2 as electron acceptor. Details as in Fig. 2.

normal and fixed cells upon illumination with 540 nm light. No measureable oxygen evolution was observed in the fixed cells.

Glutaraldehyde fixed *Porphyridium* cells are devoid of DPSMT fluorescence change (Fig. 13 A and B); the very fast *OI* phase is, however, present. We do not know if these fixed cells make ATP or not, although fixed chloroplasts are known not to phosphorylate[38]. We, however, suggest that the loss of ability to fix CO_2 and thus evolve O_2 with CO_2 as oxidant, noted above, may be related to the possible unavailability of ATP needed for the Calvin–Benson cycle. The suppression of slow fluorescence change in the fixed algal cells observed here and of light induced scattering and volume changes in the isolated chloroplasts[38, 39] lends support to the hypothesis that all these phenomena may originate from the changes in the lamellar organization that is associated with the energy coupling during photosynthesis.

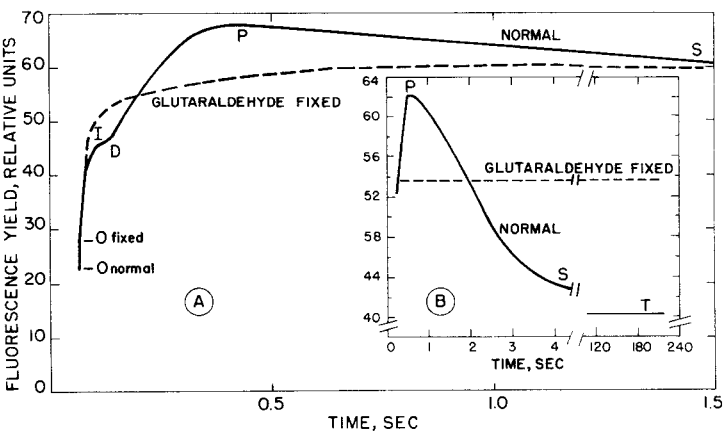

Fig. 13. Transient of Chl *a* fluorescence yield in normal (solid) and glutaraldehyde fixed (dashed) cells. Samples were adjusted to equal absorbance. Left (A); *OIDPS* phase; intensity of excitation, $1{\cdot}8 \times 10^4$ ergs cm^{-2} sec^{-1}; buffer, same as in Fig. 2; the estimation of *O* was made in a separate experiment on the same samples; other details as in Fig. 1(A). Right (B): *DPS*, and a portion of *T* phase; excitation and observation as in Fig. 1(B).

DISCUSSION

The time course of Chl *a* fluorescence yield in *Porphyridium cruentum* (Figs. 1 and 2), after a period of darkness, is similar to that of green algae and higher plants, but not blue green algae* – both in the sec and the min range [1–4, 10, 18].

These changes are restricted to the fluorescence yield of Chl *a*, while the phycocyanin yield remains constant throughout the induction period (cf. with Refs. [4, 40]). (Also, see Refs. [41–44] for changes in emission spectra after excitation or prolonged adaptation with light of various colors.) Consequently the emission spectrum of *Porphyridium* during the induction period is variable (Figs. 8 and 9). The room temperature (25°C) emission spectrum shows a higher F685 at *M* than at *S*. We also confirm the earlier observation of Lavorel [33] that *P* has more system II emission. These observations are in agreement with the suggestion of Murata [14] that alterations in the efficiency of energy transfer from Chl *a* of system II to Chl *a* of system I occur without any change in the efficiency of energy transfer from phycocyanin to Chl *a* [see Results]. Chlorophyll *a* seems to behave like an environmentally sensitive probe (cf. with Ref. [45]).

During the slow *MT* decay phase, the oxygen evolution remains almost constant (Fig. 2). This observation emphasizes that these slow fluorescence changes are not directly linked to the oxidation reduction level of electron carriers. Furthermore, the time to reach *P* varies inversely with exciting light [1], while the time to reach *M* remains largely independent of exciting light intensity except at very low intensity (Figs. 4 and 5). This observation – *in Porphyridium* [this paper] and in *Chlorella* [3] – is consistent with the hypothesis that the fast *OIDP* phase reflects largely a photochemical utilization [1, 2] of excitation energy at the system II reaction center, while the slow *SMT* change is a light induced change in the yield not related to the level of *Q* [11]. The involvement of different underlying processes for the fluorescence yield changes during *S* to *M* and *M* to *T* phases are deduced from an analysis of Fig. 7 [see Results]. Additional evidence to support this contention is derived from the early light saturation of *SM* rise phase than of the *DP* phase in *Porphyridium* (Fig. 6) and in other algae [3, 4].

Poisons of electron transport like CMU and DCMU eliminate most of the slow changes in Chlorella [3] and in *Porphyridium* [this paper]. In some cultures of *Porphyridium*, DCMU-insensitive slow changes of fluorescence have occasionally been seen. But in most cases, DCMU poisoned cells retain only the fast *O–I* phase [32]. However, Bannister and Rice [10] have reported a DCMU resistant fluorescence change in the green alga *Chlamydomonas* at high intensity of excitation. Similar slow fluorescence changes were observed in a mutant strain of *Chlamydomonas* lacking in an electron carrier on the reducing side of system II [11]. Also, blue green algae *Anacystis* [4], *Schizothrix* [46], and *Phormidium* [unpublished observations] show prolonged induction of fluorescence even in the presence of DCMU and orthophenanthroline. This 'process' may be related to the operation of 'cyclic' electron transport of system I *in vivo* and, perhaps, also to the DCMU-resistant photodepression of O_2 uptake [47].

Apparently, it seems that the slow fluorescence changes in the case of *Porphyridium*

*In very old cultures of *Porphyridium*, however, Mohanty [unpublished] has observed transients that are similar to those in blue-green algae in the sense that there is an extremely slow *MT* decline phase.

and *Chlorella* need both the cyclic and noncyclic electron flow. It may be possible that in the case of *Porphyridium* (as we grow it in our laboratory)—unlike blue green algae [4]—there is probably a limited extent of cylic electron flow. Only if the extent of the cyclic flow increases upon blocking the noncyclic electron flow with DCMU, would a higher magnitude of slow fluorescence change be expected, otherwise not. Preliminary experiments show that poisoned cells and chloroplasts could be made to exhibit slow fluorescence yield changes upon the addition of cofactors that are known to accelerate system *I*-mediated cyclic electron flow (Mohanty, unpublished). These results, together with the effect of uncouplers (see below), favor the hypothesis that the slow fluorescence change is related in some way to energy coupling during photosynthesis.

Uncouplers of photophosphorylation such as FCCP and atebrin abolish portions of the slow fluorescence change in *Chlorella* [3], *Anacystis* [4], and *Porphyridium* [this paper]. The FCCP, even at low concentrations, affects the fluorescence kinetics of *Porphyridium* (Fig. 10); it slows down the *P* to *S* decline phase and suppresses the slow *S* to *M*. The striking effect of these uncouplers on the fluorescence yield, without significant change in the rate of oxygen evolution, suggests that the slow fluorescence change and the phosphorylation are somehow interrelated. Higher concentrations of FCCP ($10\ \mu M$) and atebrin abolish completely the slow fluorescence change.

If uncouplers, indeed, uncouple phosphorylation *in vivo*, as they do in isolated chloroplasts, the simplest interpretation of the slow Chl *a* fluorescence change is that it is linked to energy coupling mechanism. Since in isolated chloroplasts, structural changes have been shown to be linked to energy coupling ability (see Ref. [39]), the observed fluorescence change is, perhaps, related to such structural changes.

Fixation of cells and chloroplasts with glutaraldehyde (or formaldehyde) immobilizes the structural changes as shown by the absence of scattering and volume changes [38, 39]. Unfortunately, CO_2 reduction is also abolished, perhaps due to the lack of ATP formation. Fixed chloroplasts do not have the ability for transmembrane ion transport [38, 39]. Fixed cells have, however, normal electron transport ability in that they can reduce idophenol as well as viologen dyes [25, this paper]. Fixed cells do not exhibit slow fluorescence changes [Fig. 13(B)]. These observations strongly suggest that electron transport *per se* contributes very little to the slow fluorescence changes in intact cells; the latter seem to be more intimately related to the energy coupling process than electron flow during photosynthesis.

It is not possible from these studies to evaluate if the slow fluorescence changes are related to proton transport across cellular membranes as experiments with giant alga *Nitella* seem to indicate [18]. However, we believe that fluorescence changes are directly related to the energy dependent structural changes of the thylakoid membranes. Other phenomena like the proton movement and phosphorylation affect fluorescence via these structural changes. However, it must be added that Packer and his associates [39] have shown that, upon illumination, glutaraldehyde fixed chloroplasts can take up proton—although at a reduced rate. (Movements of other ions into and out of chloroplasts are, however, largely eliminated.) If we assume that fixed cells behave as fixed chloroplasts, then it follows that proton uptake alone is not solely responsible for *PSMT* fluorescence yield change in whole cells.

Light triggered changes in the lamellar conformation and configuration alter the probability of trapping of excitation at the reaction centers. It has been suggested [12, 13, 15, 16; also see 3,4] that spatial configuration or the 'state' of the two photosystems

controls the spill over of excitation from system II to system I. In chloroplasts as well as in intact algal cells, the ionic environment seems to regulate the final level of fluorescence yield (Mohanty, unpublished). Immobilization of structural changes by glutaraldehyde wipes out this dynamic distribution of excitations although redox reactions occur at a measureable rate. Under this condition, the slow fluorescence transients disappear. We hope that future research will show how the thyalkoid conformation regulates the yield of fluorescence and how the 'energy coupling' is linked to conformational and configurational changes.

Acknowledgements—We are grateful to the National Science Foundation for support. We thank American Norit Company for charcoal, and Dr. P. G. Heytler for a generous gift of FCCP.

REFERENCES

1. J. C. Munday, Jr. and Govindjee, *Biophys. J.* **9**, 1 (1969).
2. J. C. Munday, Jr. and Govindjee, *Biophys. J.* **9**, 22 (1969).
3. G. Papageorgiou and Govindjee, *Biophys. J.* **8**, 1316 (1968).
4. G. Papageorgiou and Govindjee, *Biophys. J.* **8**, 1299 (1968).
5. G. Papageorgiou and Govindjee, *Biophys. J.* **7**, 375 (1967).
6. P. Joliot, *Biochim. Biophys. Acta* **102**, 135 (1965).
7. P. Joliot, *Photochem. Photobiol.* **8**, 451 (1968).
8. R. Delosme, P. Joliot and J. Lavorel, *Compt. Rend. Acad. Sci.* **249**, 1409 (1959).
9. L. N. M. Duysens and H. E. Sweers. In *Studies in Microalgae and Photosynthetic Bacteria, Japanese Society of Plant Physiology*, p. 353, University of Tokyo Press, Tokyo (1963).
10. T. T. Bannister and G. Rice, *Biochim. Biophys. Acta* **162**, 555 (1968).
11. P. Mohanty, J. C. Munday, Jr. and Govindjee, *Biochim. Biophys. Acta* **223**, 198 (1970).
12. C. Bonaventura and J. Myers, *Biochim. Biophys. Acta* **189**, 366 (1969).
13. N. Murata, *Biochim. Biophys. Acta* **189**, 171 (1969).
14. N. Murata, *Biochim. Biophys. Acta* **172**, 242 (1969).
15. N. Murata, *Biochim. Biophys. Acta* **205**, 379 (1970).
16. L. N. M. Duysens, Paper presented at the International Conference on Photosynthetic Unit, Gatlinburg, Tennessee, U.S.A. (1970).
17. Govindjee and G. Papageorgiou, In *Photophysiology* (Edited by A. C. Giese), Vol. 6, pp. 1–46, Academic Press, New York (1971).
18. W. P. Vredenberg, *Biochim. Biophys. Acta* **223**, 230 (1970).
19. M. Brody and R. Emerson, *J. Gen. Physiol.* **43**, 251 (1959).
20. A. Krey and Govindjee, *Biochim. Biophys. Acta* **120**, 1 (1966).
21. E. Gantt and S. F. Conti, *J. Cell. Biol.* **26**, 365 (1965).
22. K. Utsumi and L. Packer, *Arch. Biochem. Biophys.* **121**, 633 (1967).
23. D. W. Deamer, K. Utsumi and L. Packer, *Arch. Biochem. Biophys.* **121**, 641 (1967).
24. R. B. Park, J. Kelly, S. Drury and K. Sauer, *Proc. Natl. Acad. Sci., U.S.* **55**, 1056 (1966).
25. U. W. Hallier and R. B. Park, *Plant Physiol.* **44**, 535 (1969).
26. Govindjee and E. Rabinowitch, *Biophys. J.* **1**, 73 (1960).
27. P. J. Anderson, *J. Histochem. Cytochem.* **15**, 652 (1967).
28. A. F. S. A. Habeeb and R. Hiramoto, *Arch. Biochem. Biophys.* **126**, 16 (1968).
29. Govindjee, In *Currents in Photosynthesis* (Edited by J. B. Thomas and J. H. C. Goedheer) p. 93, Ad Donker, Rotterdam (1966).
30. J. Myers and J. Graham, *Plant Physiol.* **38**, 1 (1963).
31. Govindjee and R. Govindjee, *Carnegie Inst. Wash. Yearbook* **63**, 468 (1964).
32. P. Mohanty, T. Mar and Govindjee, *Biochim. Biophys. Acta*, in press (1971).
33. J. Lavorel, *Biochim. Biophys. Acta* **60**, 510 (1962).
34. P. Bennoun, *Biochim. Biophys. Acta* **216**, 357 (1970).
35. G. D. Winget, S. Izawa and N. E. Good, *Biochemistry* **8**, 2067 (1969).
36. G. Cohen-Bazire and M. Lefort-Tran, *Arch. Mikrobiol.* **71**, 245 (1970).
37. J. D. Clement-Metral and M. Lefort-Tran, *FEBS Letters* **12**, 225 (1971).
38. J. West and L. Packer, *J. Bioenergetics* **1**, 405 (1970).
39. L. Packer, J. M. Allen and M. Stark, *Arch. Biochem. Biophys.* **128**, 142 (1968).
40. J. L. Rosenberg, T. Bigat and S. Dejaegere, *Biochim. Biophys. Acta* **79**, 9 (1964).
41. H. I. Virgin, *Physiol. Plantarum* **7**, 560 (1964).

42. C. S. French and V. K. Young, *J. Gen. Physiol.* **35**, 873 (1952).
43. A. K. Ghosh and Govindjee, *Biophys. J.* **6**, 611 (1966).
44. S. S. Brody and M. Brody, *Arch. Biochem. Biophys.* **82**, 161 (1959).
45. B. Chance, *Proc. Nat'l. Acad. Sci., U.S.* **67**, 560 (1970).
46. L. N. M. Duysens and A. Talens, In *Progress in Photosynthesis Research*, (Edited by H. Metzner), Vol. 2, p. 905, International Union of Biological Sciences, Tübingen (1969).
47. F. P. Healey and J. Myers, *Plant Physiol.* **47**, 373 (1971).