The "Second Wave" of Fluorescence Induction in Chlorella pyrenoidosa*

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When dark adapted photosynthetic organisms are subjected to continuous exciting illumination, one observes a complex kinetic response of the capacity of chlorophyll a (Chl a) *in vivo* to fluoresce. The whole process is composed of two parts: a fast fluorescence transient lasting for a few seconds [1–8], followed by a slower change which may require several minutes for completion [7, 9–16]. The research on the fluorescence induction was initiated, in 1931, by KAUTSKY [17], and was followed by an extensive body of experimental evidence. Attempts to synthesize this information into a consistent hypothesis met with success only recently, and only in respect to the fast fluorescent transient [3–5].

The study of the slow fluorescence induction has been reopened recently in the light of the currently accepted photosynthetic theory of two consecutive photoreactions, linked by a sequence of spontaneous dark oxido-reduction steps. Results obtained in our laboratory [11–14] and in that of BANNISTER's [15, 16] suggest a photoactivation of a photochemically *inert* and non-fluorescent fraction of Chl a as the cause of this induction. Furthermore, we favor the hypothesis of a relation between the slow fluorescence induction and the light-induced structural changes that accompany phosphorylating electron transport.

The Time Course of the Slow Fluorescence Induction

The experiments to be described were performed with 3–6 days old *Chlorella pyrenoidosa* cells, grown in inorganic medium over weak white light. The cells were resuspended in Tris – (hydroxymethyl) – aminomethane, 0.02 M, NaCl 0.04 M medium, buffered at pH 8.0. A dark adaptation time of 15 min preceded all measurements. A fluorometer, described earlier [19], was used in all measurements. Other experimental details are given in the legends of the figures.

Curve W in fig. 1 depicts the time course of the fluorescence yield with continuous, broad blue band, excitation. The fluorescence rises, within a minute, from the level S at the end of the fast transient to a maximum M, and then declines slowly to a terminal level T. The latter is always lower than the S-level.

If at the end of the induction the excitation is turned off, a gradual increase of the fluorescence yield toward the initial S-level can be monitored with the help of short (2–3 sec) and weak excitation flashes. If, however, the excitation is turned off during the rising or the fast decaying portion of the induction, the fluorescence yield goes through a minimum before it attains its initial S-level (fig. 1, curves A and B). Since curves A
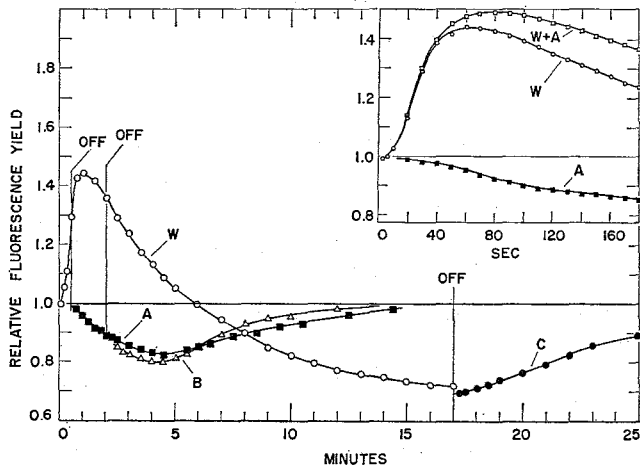

Fig. 1: Time course of the relative fluorescence yield ($f = F_t/F_3''$) in continuous and flashing light in *Chlorella*. In continuous light (W); in the dark, measured with light flashes (A, B, and C). Excitation: Blue band, Corning filter, C.S. 5–56; half-band width, 130 nm; incident intensity, 2.5 kergs. cm^{-2}. sec.$^{-1}$. Observation: $\lambda = 685$ nm, half-band width, 16.5 nm; Corning filter, C.S. 2–60. Insert: Data up to 3 min. drawn on an expanded time scale (W and A); fluorescence induction minus the dark changes (W + A).

and B are almost superimposed, even though they correspond to different times of removal of the excitation, we can assume that the dark decay of the fluorescence yield is triggered by a minimal light dose. Very brief light exposures, interrupting a long dark adaptation period, do not elicit dark induction phenomena.

These results show that continuous illumination of *Chlorella* induces not only a light-dependent change but a light triggered one as well. The light triggered decay, however, cannot account for the total decay of the fluorescence yield. This is shown in the insert of fig. 1, where the curve W + A corresponds to a hypothetical time course obtained in the absence of dark induction. The dark decay also illustrates the feasibility of the induction phenomena in the absence of sustained photosynthetic electron flow.

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We may now ask two questions: (a) Is the fluorescence induction *completely* independent of the photosynthetic electron flow? (b) Is the fluorescence induction the manifestation of a photochemical change?

To answer the first question we followed the fluorescence time courses in samples poisoned with 3 - (3′,4′ - dichlorophenyl) - 1, 1 - dimethylurea (DCMU) and o-phenanthroline. We note here that because we normalize all time courses at 3 sec of illumination, both poisoned and non-poisoned samples have time courses starting from the same point of the ordinate. In the actual recorded time courses, however, poisoned samples exhibited the usual 2 to 3 times stronger fluorescence relative to that of the normal samples. The results shown in fig. 2 suggest an elimination of the second wave in the poisoned samples. Only a very slow and weak fluorescence change persists.
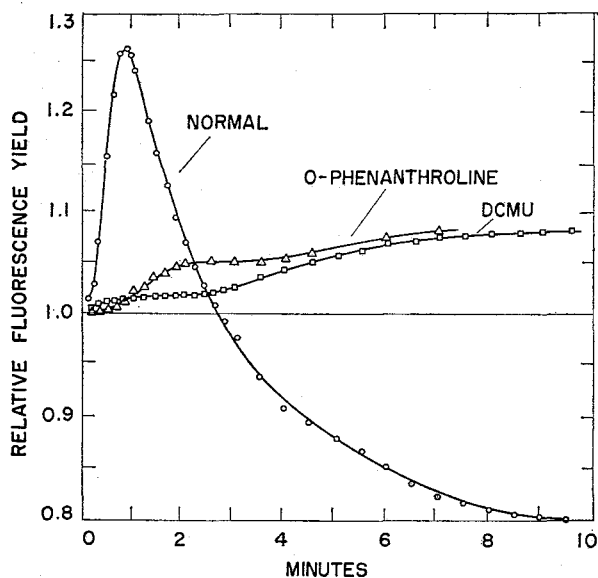

Fig. 2: Time course of the relative fluorescence yield ($f = F_t/F_3''$) in *Chlorella*. Control; with DCMU, 5×10^{-5} M; with o-phenanthroline, 5×10^{-4} M. Excitation: $\lambda = 436$ nm; half-band width, 16.5 nm; incident intensity, 2.1 kergs. cm.$^{-2}$ sec.$^{-1}$. Observation: $\lambda = 689$ nm; half-band width, 16.5 nm; Corning filter, C.S. 2–60.

The photochemical character of the processes, which underly the fluorescence induction phenomena, is illustrated by their dependence on the intensity of excitation (fig. 3). We observe that both the amplitude and the duration of the induction wave increase with the exciting light intensity, being hardly discernible for weak excitation. We made several attempts to describe the fluorescence time course in terms of a superposition of elementary kinetic functions but we had no success. Our failure can be traced to our inexact knowledge of the individual processes which contribute to the induction phenomena.
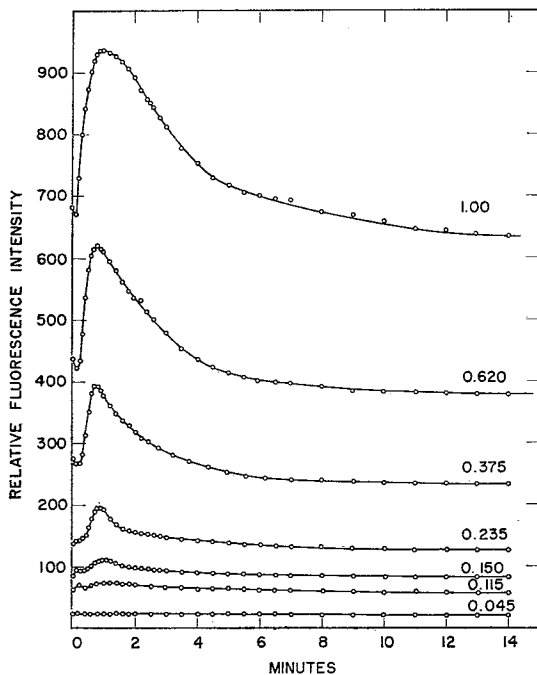
Fig. 3: Time course of fluorescence intensity in *Chlorella* for different excitation intensities. Intensity (indicated on the graph), 1.00 = 6.6 kergs. cm.$^{-2}$ sec.$^{-1}$. Excitation: Blue band, Corning filter C.S. 5–60; half-band width, 100 nm. Observation: λ = 685 nm; half-band width, 16.5 nm; Corning filter, C.S. 2–60.
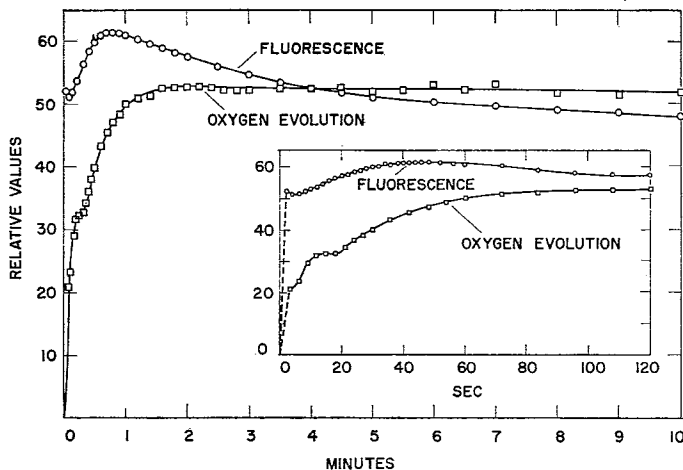

Fig. 4: Time course of the fluorescence intensity and the rate of oxygen evolution in *Chlorella*. Excitation: λ = 480 nm; half-band width, 16.5 nm; incident intensity, 3.1 kergs. cm.$^{-2}$ sec.$^{-1}$. Observation: λ = 685 nm; half-band width, 16.5 nm; Corning filter, C.S. 2–60. Insert: Data up to 2 min on an expanded time scale.

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Fluorescence Induction and the Rate of the Photosynthetic Electron Flow

In spite of the existence of dark components in the slow fluorescence induction, the inhibition of the change with DCMU and o-phenanthroline clearly indicates a correlation with the non-cyclic electron flow. The question therefore arises whether there is a complementary change in the rate of oxygen evolution. The latter remains constant during the decay phase of the fluorescence induction wave (fig. 4). The absence of an apparent competition between photochemistry and fluorescence during the slow induction was ascertained previously by McAlister and Myers [18].

A parallel increase in the rate of photosynthesis and the yield of Chl a fluorescence has been always interpreted as an "activation" process. For the slow induction, Bannister and Rice [16] picture this activation as involving whole System II photosynthetic units, whose inactive form is converted to a photosynthetically active and fluorescent one. This interpretation cannot account for the decay portion (M–T) of the wave, nor for the light triggered induction in dark.

Our interpretation [13, 14] is less phenomenological and more mechanistic. Since the activation must occur on the expense of the rate of internal conversion, we have to consider processes which can achieve that. Assuming that the radiationless dissipation of the excitation is due to regions of *aggregated* Chl a, we have to accept that processes which tend to disperse the pigment molecules will suppress the internal conversion.

Light induced structural changes have been documented extensively both in chloroplasts and in algae (for reviews see references [20–22]). These changes, which occur at rates comparable to those of the fluorescence induction, have been shown to be associated with the conservation of a fraction of Chl a excitation as adenosine triphosphate (ATP). In view of this information, we proceeded to assess the effects of phosphorylation uncouplers on the fluorescence kinetics.

Fluorescence Induction and Photophosphorylation

A correlation between photophosphorylation and fluorescence induction was first proposed by Strehler [23] in 1953, on account of their similar kinetics in *Chlorella*.
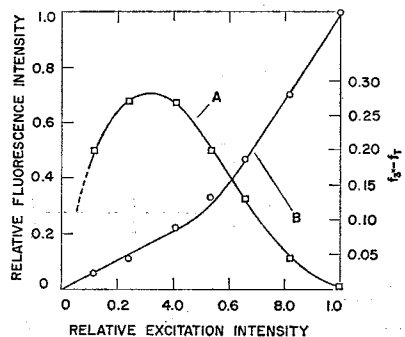
Fig. 5: The fluorescence change $f_3'' - f_T$ (A) and the fluorescence intensity at T (B) as a function of the incident light intensity in *Chlorella*. Intensity 1.0 = 25.5 kergs. cm.$^{-2}$ sec.$^{-1}$. Excitation: Blue band, Corning filter, C.S. 4–72; half-band width, 150 nm. Observation: $\lambda = 685$ nm; half-band width, 16.5 nm; Corning filter, C.S. 2–60.

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STREHLER, moreover, found that the steady-state ATP content exhibits a light intensity "optimum". A similar optimum appears also in the plot of the fluorescence yield at 3 sec. illumination minus that at the terminal state T ($f_{3''}$–f_T) *versus* the excitation intensity (fig. 5, curve A). The quantity $f_{3''}$–f_T measures the light-induced reduction of the fluorescence yield, after the completion of the process. In the same figure (curve B) a light curve
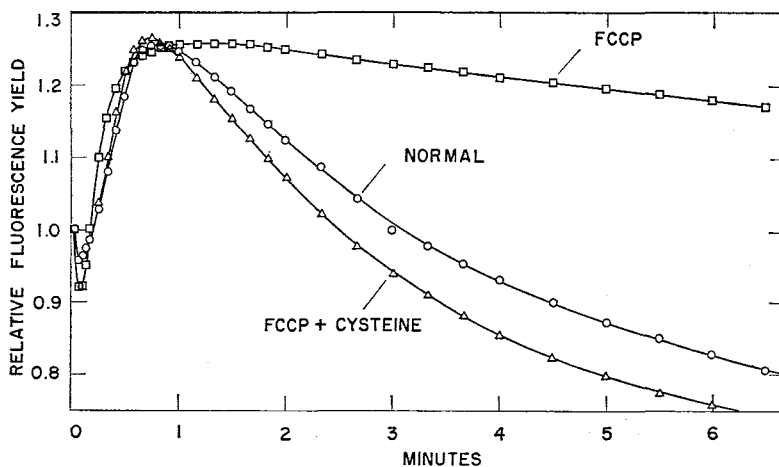

Fig. 6: Time course of the relative fluorescence yield ($f = F_t/F_{3''}$) in *Chlorella*. Control; with 10^{-5} M FCCP; with 3×10^{-5} M FCCP and 10^{-3} M cysteine. Excitation: $\lambda = 480$ nm; half band width, 10 nm; incident intensity 1.4 kergs. cm.$^{-2}$ sec.$^{-1}$. Observation: $\lambda = 685$ nm; half-band width, 11.6 nm; Corning filter, C.S. 2–60 (Additions were made at least 30 min prior to illumination).
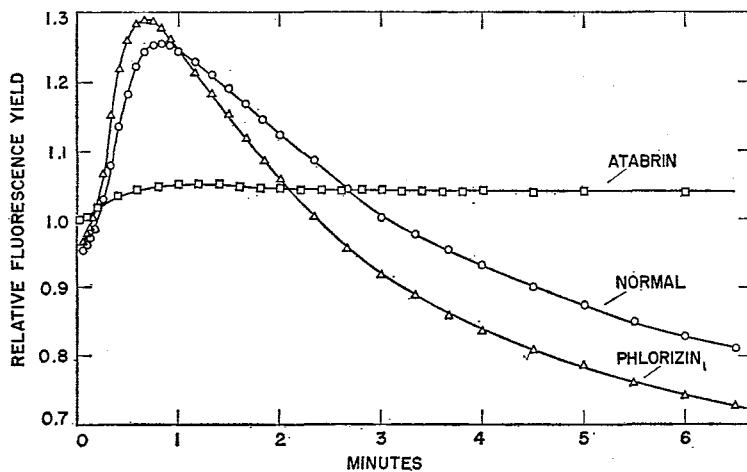

Fig. 7: Time course of the relative fluorescence yield ($f = F_t/F_{3''}$) in *Chlorella*. Control: with 3×10^{-5} M atabrin; with 2×10^{-2} M phlorizin. Excitation and observation as in fig. 6.

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$(F = f(I_{exc}))$ of the steady-state fluorescence intensity is given. It appears that the light intensity optimum of $f_{3.}$–f_T coincides with the break in the $F = f(I_{exc})$ curve and thus also with beginning light saturation of photosynthesis.

Presence of the uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) causes a delay of the decay portion of the induction wave (fig. 6). Since FCCP is also known to suppress the light-induced formation of ion gradients and the associated absorbancy changes in chloroplasts [20], a correlation of these processes with fluorescence induction can be assumed. The suppression of the decay rate of the induction wave is more severe at low light intensities. At light intensity of 5 kergs cm^{-2} sec^{-1}, half-maximal inhibition of the decay was obtained at 4.5×10^{-6} M FCCP. As shown in fig. 6, addition of cysteine hydrochloride (at a 100-fold concentration) removes the FCCP inhibition.

The effects of the uncouplers atabrin and phlorizin are shown in fig. 7. Atabrin, which is known to stimulate the light-induced absorbancy change in chloroplasts, although it suppresses photophosphorylation [24], has a severe effect on the fluorescence induction. On the other hand, phlorizin, which functions as a phosphorylase inhibitor [25], exerts no profound influence on the induction wave, except for an acceleration of the rise and decay phases.

We interpret our results as indicative of a relation between the light-induced structural changes, which accompany phosphorylating electron transport, and the process of the slow fluorescence induction. Direct and parallel measurements of the time course of structural changes, the ATP levels, and the rates of electron transport and fluorescence induction are now required to fully substantiate our hypothesis.

Acknowledgment

References

[1] LAVOREL, J.: Plant Physiol. **32**, 204 (1959).
[2] KAUTSKY, H., W.APPEL, and H.AMANN: Biochem. Z. **332**, 277 (1960).
[3] DUYSENS, L.N.M., and H.E.SWEERS in: "Studies on Microalgae and Photosynthetic Bacteria." Edited by the Japanese Society of Plant Physiologists, p. 353, University of Tokyo Press, Tokyo 1963.
[4] JOLIOT, P.: Biochim. Biophys. Acta **102**, 135 (1966).
[5] JOLIOT, P.: Brookhaven Symp. in Biology **19**, 418 (1966).
[6] DELOSME, R.: Biochim. Biophys. Acta **143**, 108 (1967).
[7] GOVINDJEE, J.C., MUNDAY JR., and G.PAPAGEORGIOU: Brookhaven Symp. in Biology **19**, 434 (1966).
[8] MUNDAY, J.C., JR.: Ph. D. Thesis, University of Illinois (1968).

[9] WASSINK, E.C.: Advances in Enzymology **11**, 91 (1951).

[10] RABINOWITCH, E.: "Photosynthesis and Related Processes", Vol. II, part 2, p. 1375, Interscience, New York (1956).

[11] PAPAGEORGIOU, G., and GOVINDJEE: Biophys. J. **7**, 375 (1967).

[12] PAPAGEORGIOU, G.: Ph. D. Thesis, University of Illinois (1968).

[13] PAPAGEORGIOU, G., and GOVINDJEE: Biophys. J. **8**, 1299 (1968).

[14] PAPAGEORGIOU, G., and GOVINDJEE: Biophys. J. **8**, 1316 (1968).

[15] BANNISTER, T.T.: Biochim. Biophys. Acta **143**, 275 (1967).

[16] BANNISTER, T.T., and G.RICE: personal communication.

[17] KAUTSKY, H.: Naturwiss. **19**, 964 (1931).

[18] McALISTER, E.D., and J.MYERS: Smithsonian Inst. Publs. Misc. Collections **99**, 1 (1940).

[19] GOVINDJEE in: J.B.THOMAS and J.H.C.GOEDHEER (Eds.), "Currents in Photosynthesis", p. 93, Ad. Donker, Rotterdam 1966.

[20] GOOD, N.E., S.IZAWA, and G.HIND in: D.R.SANADI (Ed.), "Current Topics in Bioenergetics", Vol. 1, p. 75, Academic Press, New York 1966.

[21] JAGENDORF, A.T., and E.URIBE: Brookhaven Symp. in Biology **19**, 215 (1966).

[22] PACKER, L., D.W.DEAMER, and A.R.CROFTS: Brookhaven Symp. in Biology **19**, 281 (1966).

[23] STREHLER, B.L.: Arch. Biochem. Biophys. **43**, 67 (1953).

[24] DILLEY, R.A., and L.P.VERNON: Biochemistry **3**, 318 (1963).

[25] CORI, G.F., G.T.CORI, and A.A.GREEN: J. Biol. Chem. **151**, 39 (1943).