EMERSON ENHANCEMENT EFFECT AND TWO LIGHT REACTIONS IN PHOTOSYNTHESIS

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Dedicated to the memory of late Professor Robert Emerson

During the 1940's Robert Emerson discovered[1,2] that the quantum efficiency of photosynthesis, plotted as a function of wavelength, drops appreciably long before one reaches the long-wave limit of absorption by chlorophyll a. This decline is referred to as the "red drop." Recently it has become clear that in green cells, this "drop" begins when the form of chlorophyll a, absorbing at the longer wavelength, becomes the prime absorber. Persistence of a certain photosynthetic activity to wavelengths up to 740 mμ could be interpreted either in terms of fractional absorption by the short-wave form of chlorophyll a (Chl a 670) extending into the far red, or by ascribing to the "long-wave form" of chlorophyll a, the capacity of bringing about complete photosynthesis but with a very low yield. Excitation of the "long-wave form" of chlorophyll a alone in green cells certainly is insufficient to bring about all photochemical steps involved in photosynthesis with a high yield. In the case of the red and blue-green algae, the drop occurs at shorter wavelengths[3,4] apparently, when chlorophyll a takes over the role of the main absorber from the phycobilins. It seems that all (or almost all) chlorophyll a in red and blue-green algae has the same photochemical function as do the long-wave forms of chlorophyll a in green plants.

In the photosynthesis laboratory at Urbana, a long series of experiments[4-8] revealed that by exciting one of the "short-wave pigments" (chlorophyll b and Chl a 670 in green algae; phycoerthrins and phycocyanins in red and blue-green algae; fucoxanthol, chlorophyll c and Chl a 670 in diatoms), simultaneously with the long-wave form of chlorophyll a, the "red drop" could be avoided. Simultaneous excitation of two "pigment systems" permits the plants to use efficiently the energy absorbed in the "long-wave" form of chlorophyll a. This synergistic effect is referred to as the "Emerson enhancement effect". Studies

of this effect have given the first evidence for the now widely postulated[8-14] hypothesis of two photochemical systems participating in photosynthesis. The enhancement effect has been studied by manometry[2, 4-8], polarography[11-17], mass spectrometry[18-20] and by the radiocarbon tracer technique.[21] Its finding both in oxygen evolution and in CO_2 uptake shows that it is characteristic of photosynthesis as a whole. Experiments on the Hill reaction[22-24] suggested the involvement of the two pigment systems in this process as well. Further evidence for the existence of two such systems has come from difference spectroscopy[10b, 25-28], electron spin resonance studies [29-31], studies of fluorescence[32-36], and luminescence (afterglow)[37], absorption spectroscopy[38, 39], as well as studies on mutants[40], from flashing light experiments[22,41], and from biochemical investigations.[42]

This paper summarizes some of our recent work on the "red drop" and the enhancement effect in whole cells and in chloroplast preparations.

I. INVESTIGATIONS ON THE "RED DROP" IN PHOTOSYNTHESIS

A. Algae Pioneer studies of Emerson and coworkers showed the occurrence of "red drop" in several algae (Chlorella[1,2], Anacystis[8c], Porphyridium[4,8c], and Navicula[8c,43]). At 20°C, the decline in the quantum yield of photosynthesis in Chlorella begins at 680 mμ, and the yield is halfway down to zero at 696 mμ. This decline is affected by several factors: (a) Presence of appropriate background light ("enhancement effect"). In this case, the decline may disappear altogether, (see figure 1).

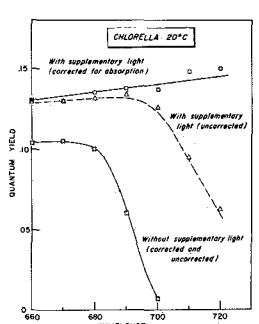

Figure 1. Quantum yield of oxygen evolution as a function of wavelength in thick suspensions of Chlorella (corrected and uncorrected for absorption); (data from Emerson).

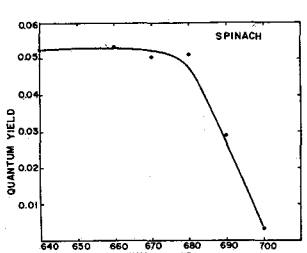

Figure 2. Quantum yield of oxygen evolution in spinach chloroplasts, as a function of wave - length (ferricyanide + catalytic amount of 2,6 DCPIP). (Data from Bakri and Govindjee).

(b) Temperature. Lowering the temperature shifts the beginning of the decline to longer wavelengths.[2] At 10°C, the quantum yield[44] starts declining only at 690 mμ and is halfway down at 730 mμ. (c) Growing the algae in "earth extract" (an undefined organic medium). This too, shifts[7] the beginning of the decline to longer wavelengths and causes the enhancement phenomena to disappear. (d) Growing the algae in glucose medium[45] does not shift the beginning of the decline appreciably, although there is a somewhat higher activity at longer wavelengths, as compared to that of cells grown completely in inorganic medium. The persistence of the drop suggests that no significant amount of energy can be supplied to photosynthesis from exogenous respiration. (e) When Chlorella was grown in heavy water, the decline[45] in the yield began at 670 mμ (instead of 680 mμ); no enhancement was observed in these cells. An interpretation of this effect must await studies of the absorption spectra of cells grown in D_2O.

B. Chloroplasts from Higher Plants.

Experiments made on the Hill reaction, using quinone[23], NADP[24] or ferricyanide (in the presence of a catalytic quantity of the dye 2, 6 DCPIP[46a]. (figure 2), revealed a long-wave decline ("red drop") similar to that found in photosynthesis of whole cells. An enhancement effect also has been observed, at least with quinone[23] and NADP[24]. (see section V). This lends support to the previously suggested concept that the Hill reaction and photosynthesis have the same photochemical mechanism.

C. Bacteria.

Uptake of $CO_2 + H_2$ was followed in Rhodospirillum rubrum suspensions at certain selected wavelengths, beyond the major peaks of bacteriochlorophyll, i.e. in a region where one would have expected a decline analogous to the "red drop" in photosynthesis. The quantum yield found at 940, 960 and 980 mμ[46b] were all in the range of 0.10, similar to those found at the shorter wavelengths and suggesting absence of a "red drop". Blinks and van Neil[47] noted the absence of an enhancement phenomenon in bacterial photosynthesis.

D. Discussion.

The question "Does the existence of a "red drop" per se prove the presence of two photochemical systems?" must be answered in the negative. If, for a certain reaction, say - the Hill

reaction with a certain oxidant- the long wave form of chlorophyll a (Chl a 680/Chl a 690) were simply inactive, one would still get a "red drop"!

Conversely, the absence of a "red drop" cannot be taken as proof of the absence of two light reactions, because extensive overlapping of the spectral bands of the two systems could make separation of their effects difficult.

However, the presence of both "red drop" and an enhancement effect clearly suggests the existence of two photochemical systems acting cooperatively in the overall process of photosynthesis.

II. "LIGHT CURVES" OF PHOTOSYNTHESIS IN MONOCHROMATIC LIGHT

Studies of the enhancement phenomena are reliable only if the light curves (the plot of the rate of photosynthesis, R, as a function of light intensity, I) are linear in the intensity range of the experiment, so that by combining the two beams, one does not get into the saturating (or at least less sloping) range, suggesting "negative" Emerson effect. Similarly a "positive" effect can be wrongly inferred when one of the light curves is sigmoid in shape (cf. below). This necessitated a careful investigation of the light curves under different conditions.

A. Light Curve with "Knick"

McCloud[48] (as well as Govindjee[8b,49]) had observed that the saturation rates of photosynthesis in various algae were dependent on the wavelength of light; at the longer wavelengths- beyond 680 mμ - an "early" saturation on a relatively low level was reached. These observations were very difficult to interpret, since saturation was supposed to be imposed by the availability of a limiting enzyme, which should be the same whatever the wavelength of light. Because of the narrow range of light intensities used in my earlier experiments, which had led to the above conclusions, we repeated these measurements on Porphyridium over a wide range of intensities. Two wavelengths were selected -- one absorbed primarily by phycoerythrin (546 mμ) and the other by the "long wavelength" form of chlorophyll a (700 mμ). Advantage was taken of the well-known fact that saturation levels are lower at the lower temperatures. Measurements were made at 3-5°C (see figure 3). The 700 mμ light curve seems to approach the same saturation level as that obtained in

green light, in contradiction to the results of McCloud[48] (and my own earlier conclusions[8b,49]). In an independent study, Pickett and Myers[14e] found the same saturation rate at different wavelengths in Chlorella. The cause of the discrepancy between the newer and the earlier results is that the 700 mμ light curve has a peculiar "break", which was mistaken for "saturation", due to the narrow range of intensities studied.

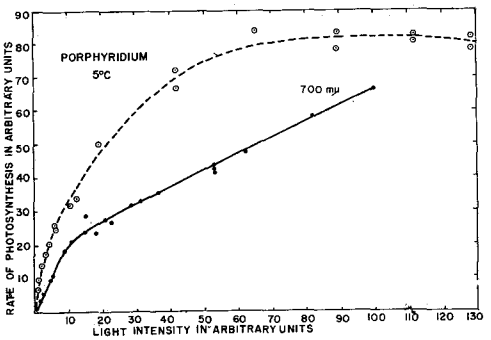

Figure 3. Rate of photosynthesis in Porphyridium as a function of light intensity in two monochromatic beams 545 mμ and 700 mμ measured at 5° C.

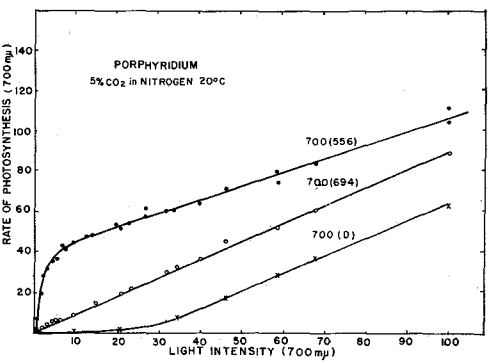

Figure 4. Rates of oxygen evolution, in Porphyridium as a function of light intensity.

My new measurements show a clear "Knick" in the light curve at 700 mμ (figure 3). Kok had observed such broken light curves (see Rabinowitch[50] for a discussion of this "Kok effect"). Hoch and coworkers[20] recently reported light curves of the same type in Anacystis. Jones and Myers[14d], in an independent study, also observed such curves in Anacystis.

B. S-Shaped Light Curves.

Figure 4 (lowest curve) shows a light curve obtained at 700 mμ in Porphyridium cruentum at 20°C under anaerobic conditions. It is clearly S-shaped. The middle curve shows that addition of another beam of the same wavelength, makes the curve linear. With Bannister & Vrooman[17]; I believe that under nitrogen the lower segment of the S-shaped "light curve" is due to immediate consumption of the oxygen evolved in photosynthesis; the oxygen production in photosynthesis, is therefore not registered by the polarograph. At higher intensities, oxygen production increasingly exceeds oxygen consumption (thus destroying the external anaerobic condition). (Reference may be made here to a paper by

Franck et al[51] which suggests several other possible interpretations of S-shaped light curves obtained in white light under anaerobic conditions).

S-shaped light curves are well-known in bacterial photosynthesis[50]; recently they have been found also for the production of ATP by chloroplasts.[52] We observed[46a] an S-shaped light curve for oxygen production by spinach chloroplasts. (The reaction mixture contained excess of ferricyanide and traces of 2,6 DCPIP).

A method to observe enhancement in non-linear light curves is through the experiment shown in figure 4. (1) The rate of reaction as a function of light intensity of the far-red light (A) is first measured (lowest curve, figure 4) (2) The same is done in the presence of another beam (B) of far-red light, of the same wavelength (middle curve, figure 4) (3) The same is done in the presence of supplementary beam (C); the intensity of which is adjusted to give the same rate of reaction as that produced by beam (B). Enhancement is then the difference between the upper curve and the middle curve or

$$E = \frac{R_{1,2} - R_1}{(R_{2,2'} - R_2)} \qquad (1)$$

where E = Emerson enhancement, $R_{1,2}$ = rate in both supplementary and far red beams, R_1 = rate in supplementary beam alone, R_2 = rate in far red beam alone, and $R_{2,2'}$ is the rate in combined far-red beams.

C. Light Curves with Continuously Decreasing Slopes (early saturation effects).

Light curves for various Hill reactions, in general[53] show a decreasing slope with increasing light intensity, (i.e. only a very short linear range is available). This raises the question of the validity of the results[18,21] in which no Emerson enhancement was reported for the Hill reaction. The 650 mμ light curve for NADP reduction shows the above mentioned early bending of the light curve.[24b,54] Due to early "saturation" effects, two beams of 650 mμ light, given together, give a rate smaller than the sum of the two separate rates.[54]. True enhancement can be calculated by taking this fact into account by means of the equation:

$$E = \frac{R_{1,2} - (R_{1,1}' - R_1')}{R_2} \qquad (2)$$

where the symbols have the same meaning as in equation (1), except that R_1', is the rate due to the second (supplementary beam) alone, the intensity of the latter being adjusted so as to give the same rate as that of R_2.

III. ENHANCEMENT EFFECT AND THE TWO PHOTOCHEMICAL SYSTEMS

A. Action Spectra.

The action spectra of the enhancement effect, measured with a background of far-red light, led to the identification of pigments present in one of the two postulated photosystems (system II). Of course, these action spectra do not follow the true absorption curves, but rather the"fractional-absorption" curve of the pigments belonging to system II. Similarly, the action spectra obtained in the presence of background light of shorter wavelength, suggest the fractional absorption curve of pigments in system I. From our previous studies[8], we concluded that system II in diatoms and green algae includes the short-wave form of chlorophyll *a* (Chl *a* 670). (See figure 5 for the absorption spectrum[55] of *Chlorella*, showing bands corresponding to the two forms of chlorophyll *a*). Light energy absorbed by chlorophyll *b* is assumed to be transferred to Chl *a* 670. System I is mainly composed of the long-wave forms of chlorophyll *a* (Chl *a* 680/Chl *a* 690 and "Chl *a* 700"). In figure 6, the solid curve refers to the action spectrum of the enhancement effect in the presence of 700 mμ background light, suggesting the identification of chlorophyll *b* and Chl *a* 670 as components of system II. The dashed curve refers to measurements in the presence of 650 mμ background light. The ratio of the rate due to far-red light to that due to supplementary light is kept constant (1:2). Unlike Myers and Graham[14c], we observed enhancement of 650 mμ action by 670 mμ light. It, thus, appears that Chl *a* 670 occurs in both systems. On the other hand, the long-wave chlorophyll *a* forms belong exclusively to system I; while chlorophyll *b* belongs exclusively to system II.

Soret bands. Fork[11] has shown that, in red algae, in the action spectrum of enhancement with a background of green light, system I exhibited a Soret band. This was confirmed by Blinks.[15] The demonstration of this band in green algae and diatoms has not met with success in our hands, perhaps due to a strong overlap of ab-

sorption bands of systems I and II in the blue end of the spectrum.

The action spectrum of the enhancement effect with a 700 mμ background light in Chlorella[8c], showed, however, a hump around 440 mμ, in addition to several other bands, due to chlorophyll b and Chl a 670. This 440 mμ band may be due to the Soret band of Chl a 670.

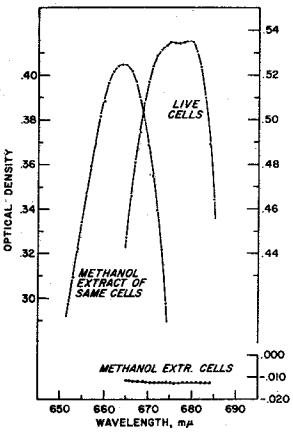

Figure 5. Absorption spectrum of Chlorella and pigments from Chlorella, (measuring band width 1 mμ, after Cederstrand).

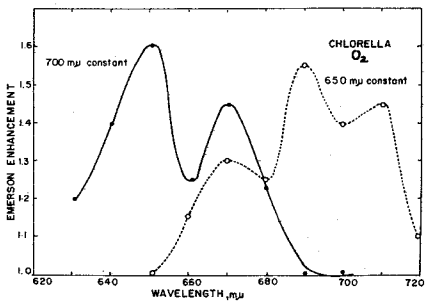

Figure 6. Action spectra of enhancement effect, in Chlorella, as a function of wavelength.

B. LIGHT INTENSITY AND THE ENHANCEMENT EFFECT

Enhancement was measured, both as a function of the intensity

of the supplementary light when 700 mμ light is kept constant, and as a function of increasing intensity of far-red light, when the supplementary light is kept constant. Figures 7 and 8 show that: (1) With an increase in the intensity of supplementary light, the enhancement reaches a plateau and then declines (perhaps we reach the region where the slope of the light curve decreases). (2) With an increase in the intensity of 700 mμ light, the enhancement decreases. Similar results were obtained with 650 and 700 mμ light, and with 670 and 700 mμ light. No enhancement was seen between 690 and 700 mμ at any of the intensities used. Due to the use of manometry in the above measurements, effects of light on the oxygen uptake could not be avoided. With this in mind, we checked part of our results in Chlorella with a mass spectrometer[19]. Enhancements by factors up to 8-10 could be obtained by selecting the appropriate intensity and wavelengths, (650 mμ and 720 mμ). Enhancement increases with an increase in the intensity of 650 mμ light, and decreases with an increase in the intensity of far-red light (at 720 mμ).

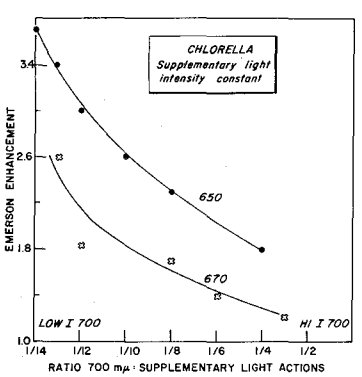

Figure 7. Enhancement effect, in Chlorella, as a function of far-red light intensity.

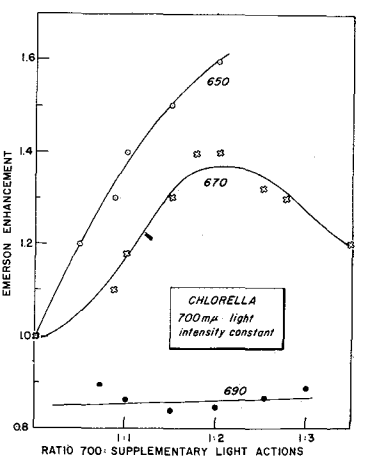

Figure 8. Enhancement effect, in Chlorella, as a function of supplementary light intensity.

IV. ENHANCEMENT EFFECTS IN CHLOROPLAST REACTIONS

We have consistently observed the enhancement phenomenon in the Hill reaction with isolated chloroplasts, using quinone[23] and NADP[24] as oxidants. These experiments clearly show the existence of an enhancement when a far-red beam is supplemented by light of a shorter wavelength. The action spectrum of this effect has peaks at 650 mμ (due to chlorophyll b) and 675 mμ (due to chlorophyll a). (See figures 9 and 10). Enhancement of NADP reduction was accompanied by enhancement of oxygen evolution. The situation may be different with other Hill oxidants, such as certain dyes, or ferricyanide.

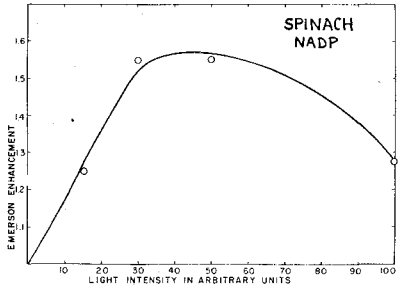

Figure 9. Enhancement in NADP reduction as a function of 650 mμ light intensity in spinach chloroplasts. (after R. Govindjee and Govindjee and Hoch).

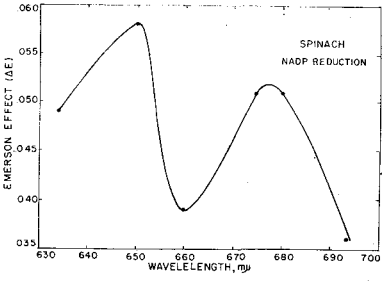

Figure 10. Action spectrum of Emerson enhancement effect in NADP reduction for spinach chloroplasts. (after Yang and Govindjee).

V. FLUORESCENCE STUDIES

We reported[32] that the 685 mμ fluorescence excited by 436 mμ light is quenched by the addition of 700 mμ light. This effect can be interpreted in terms of two photochemical systems. Butler[33], Duysens and Sweers[35] obtained similar results. To clarify the mode of action of different oxidants by observing

their effect on the fluorescence yield of "P 700" and Chl a 670, we first measured the action spectra of fluorescence at room, and at liquid nitrogen temperatures.

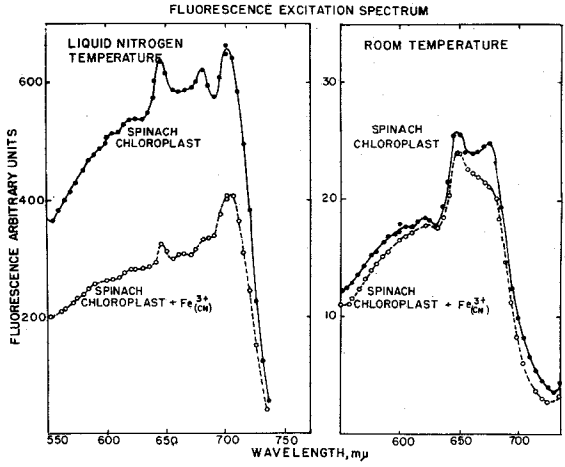

Figure 11. Fluorescence excitation spectra of isolated spinach chloroplasts, measured at 22° C (right) and at -196° C (left); note a twentyfold increase in the fluorescence yield. The appearance of a new band at 700 mμ in the cooled sample is clearly demonstrated; tentatively, it can be assigned to P 700—a pigment (or pigments) absorbing at 700 mμ. Ferricyanide (10^{-3}M) quenches fluorescence yield (see open circles) at all the wavelengths—due to the formation of non-fluorescent oxidation products of the pigments (after Louisa Yang and Govindjee).

First, we show (see figure 11) the action spectrum of fluorescence of a chloroplast suspension, measured at 758 mμ and at -196° C. A peak at 705 mμ appears (which is not present at room temperature). This provides independent confirmation of Butler's[56] prior findings. The peak is believed to be due to P 700.

In Anacystis, fluorescence spectra (see figure 12) (excited by 436 and 605 mμ light, absorbed primarily by chlorophyll a and phycocyanin respectively) show (1) that chlorophyll a fluorescence, when excited via energy transfer from phycocyanin, is much stronger than when it is excited directly; (2) that excitation in the Soret band of chlorophyll a is not transferred to phycocyanin; (3) that energy transfer yield from phycocyanin to chlorophyll a is much less than 100%, as some energy is lost as phycocyanin fluorescence (peak at 650 mμ); (4) upon cooling to -196° C, new peaks appear at 696 mμ, 718 mμ, and a shoulder at 760 mμ.

The ratio of intensities of the 718 and the 685 mμ fluorescence bands at -196° C is higher when chlorophyll a is excited, than when phycocyanin is excited. This suggests two separate chlorophyll a systems: (1) one system in which the energy preferentially goes to the part of chlorophyll a that fluoresces at 718 mμ; and (2) another system in which the energy preferentially goes to the chlorophyll a that fluoresces at 685 mμ.

The 750 mμ peak in the excitation spectrum (see figure 13) is observed consistently, and is perhaps due to P 750 N. The 760 mμ shoulder in the fluorescence spectrum is due to the fluorescence of this pigment. The 696 mμ band may be due to the "unknown trap" of photosystem II (cf. also 57 and 58).

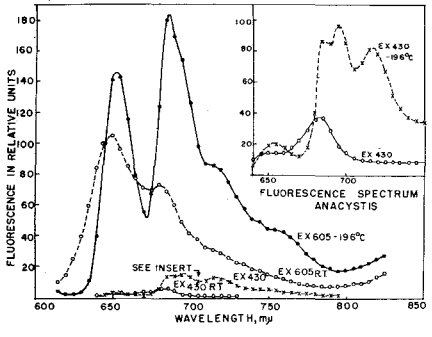

Figure 12. Fluorescence emission spectrum of Anacystis. (after Spencer and Govindjee).

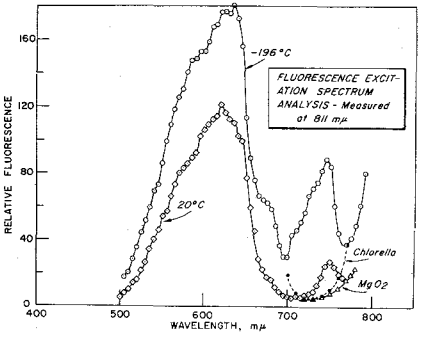

Figure 13. Fluorescence excitation spectrum of Anacystis at two temperatures (after Spencer and Govindjee).

VI. CONCLUDING REMARKS AND SUMMARY

1. The quantum yield of photosynthesis in Chlorella begins to decrease at 690 mμ and declines to about 50% at 730 mμ (at 10° C). No analogous decline was noticed in bacterial photosynthesis. This may mean that a photochemical system of type II is absent in bacteria.

2. A prior knowledge of the shape of the "light curve" (the plot of the rate of the O_2 evolution as a function of light intensity) is necessary to calculate the enhancement factor. A study of this relationship shows the existence of: (a) S-shaped curves, e.g. in photosynthesis under anaerobic conditions, and in O_2 evolution in chloroplasts in the presence of ferricyanide with catalytic amounts of DCPIP; (b) curves with a discontinuity or "knick" (700 mμ light curve for oxygen evolution in Porphyridium,) (c) "light curves" with a rapidly decreasing slope with increasing light intensity.

3. The Emerson enhancement effect first increases linearly with an increase in supplementary light intensity, then saturates and finally declines. It decreases with an increase in the intensity of far-red light.

4. The data presented substantiate the hypothesis of two light reactions involved in both photosynthesis and the Hill reaction. From the two action spectra of the enhancement effect, measured with constant far-red and with constant supplementary beam, respectively, and from fluorescence studies, there emerges a picture of two photochemical systems: System I. This is composed of Chl a 680/690 and its "trap," P 700. The P 700 is nonfluorescent at room temperature, and fluorescent at liquid nitrogen temperature. Its fluorescence peak is at 718 mμ. Some Chl a 670 also belongs to this system. System II. This is composed of Chl a 670, and the "accessory pigments." Chl a is fluorescent at room temperature.

5. In Anacystis, measurements, at -196° C, of fluorescence spectra, excited at 436 mμ (chlorophyll a) and 605 mμ (phycocyanin), show that the relative heights of fluorescence bands at 687 mμ and at 718 mμ are very different. The ratio of the maxima of the 718 mμ fluorescence band to that of the 687 mμ band is greater when Anacystis is excited by 436 mμ light, than when it is excited by 605 mμ light. This suggests the existence of two kinds of chlorophyll a: one that receives its energy primarily from the phycobilins, and another that receives its energy directly from chlorophyll a.

VII. ACKNOWLEDGEMENTS

Continued support from the National Science Foundation (G 19437 and GB 1610) is acknowledged. A grant from the Charles F. Kettering Foundation to Professor Rabinowitch, provided some of the instruments used in the present study. Thanks are due to Maarib Bakri, Carl Cederstrand, Rajni Govindjee, Mary Osbakken, Jobie Spencer and Louisa Yang for their valuable help in this investigation. Part of the work presented here was done at RIAS, Baltimore, and at the Carnegie Institution of Washington, Stanford. My thanks are due to Drs. Eugene Rabinowitch, Bessel Kok, George Hoch, Olga Owens, C. Stacey French, David Fork and N. R. Murty for their interest.

REFERENCES

1. Emerson,R. & Lewis,C.M., Amer. J. Bot. 30, 165, 1943.
2. Emerson,R., Chalmers,R., & Cederstrand,C., Proc. Nat. Acad. Sci. 43, 133, 1957.
3. Haxo,F.T. & Blinks,L.R., Jour. Gen. Physiol. 33, 389, 1950.
4. Brody,M. & Emerson,R., Jour. Gen. Physiol. 43, 251, 1959.
5. a. Emerson,R., Science, 125, 746, 1957; b. Science, 127, 1059, 1958; c. Annl. Rev. Plant Physiol. 9, 1, 1958.
6. Emerson,R. & Chalmers,R., Phycol. Soc. Amer. News Bull. 11, 51, 1958.
7. Emerson,R., & Rabinowitch,E., Plant Physiol., 35, 477, 1960.
8. a. Govindjee & Rabinowitch,E., Science 132, 355, 1960; b. Biophysic. J., 1, 73, 1960; c. Govindjee, Ph.D.thesis, University of Illinois, 1960; d. Thomas,J.B. & Govindjee, Biophysic. J. 1, 63, 1960; e. In "Light & Life" (edt. McElroy, W.D. & Glass,B.), The Johns Hopkins Press, p. 475, 1961; f. Rabinowitch,E. & Govindjee in "Light & Life" (edt. McElroy, W.D. & Glass,B.), The Johns Hopkins Press, p. 378, 1961; g. Govindjee, Paper presented at the annual meeting of Biophysics Society, 1962.
9. a. Franck,J., Proc. Nat. Acad. Sci., U.S., 44, 941, 1958; b. Hill,R., & Bendell,F., Nature 186, 136, 1960.
10. a. Duysens,L.N.M., & Amesz,J., Biochim et Biophys. Acta, 64, 243, 1963; b. Witt,H.T., Müller,A., & Rumberg,B. Nature, 197, 987, 1963; c. Kok,B., Cooper,B., & Yang,L. in "Studies on Microalgae & Photosynthetic Bacteria" (edt. Jap.Soc.Plant Physiol.), The University of Tokyo Press, p. 373, 1963; d. Clayton,R.K., Annl. Rev. Plant Physiol., 14, 159, 1963; e. Rabinowitch,E., Paper presented at the Paris conference, 1963; f. Calvin,M., & Androes,G.M., Science, 138, 867, 1962; g. Gaffron,H., Presidential address at the first symposium on

Bacterial Photosynthesis, Yellow Springs, Ohio, 1963; h. Franck,J., & Rosenberg,J.L. (unpublished).
11. Fork,D.C., Ph.D. thesis, University of California, 1961.
12. McCloud,G.C., Science, 133, 192, 1961.
13. a. French,C.S. & Fork,D.C., Biophysic. J., 1, 669, 1961; b. "Mechanism of Photosynthesis (edt. Tamiya,H.), Pergamon Press, London, p. 122, 1963; c. Smith,J.H., & French,C.S., Annl. Rev. Plant Physiol., 141, 81, 1963.
14. a. Myers,J. & French,C.S., Plant Physiol., 35, 963, 1960; b. Jour. Gen. Physiol. 43, 723, 1960; c. Myers, J. & Graham, Jo-Ruth, Plant Physiol. 38, 105, 1963; d. Jones,L.W., & Myers,J. Paper presented at A.I.B.S., Amherst, Massachusetts, 1963; e. Pickett,J.M. & Myers,J., Paper presented at A.I.B.S.; Amherst Massachusetts, 1963.
15. Blinks, L.R., Proc. Nat. Acad. Sci., 46, 327, 1960.
16. Haxo,F.T. in Comparative Biochemistry of Photoreactive Systems (M.B. Allen, edt.), Academic Press, p. 339, 1960.
17. Bannister,T.T. & Vrooman,M.J., (in manuscript).
18. Mayne,B.C. & Brown, A.H. in "Studies on Microalgae & Photosynthetic Bacteria," (edt. Jap.Soc.Plant Physiol.), The University of Tokyo Press, p. 347, 1963.
19. Govindjee, Owens,v.H. & Hoch,G., Biochim et Biophys Acta, 75, 281, 1963.
20. a. Hoch,G., Owens,O.v.H. & Kok,B., Arch. Biochem. Biophys., 101, 171, 1963; b. Owen,O. & Hoch,G., Biochim et Biophys Acta 75, 183, 1963.
21. Gibbs,M., Fewson,C.A. & Schulman,M.D., Carnegie Institution Yearbook, 1963 (in press).
22. a. Bishop, P.M. & Whittingham,C.P., Nature, 197, 1225, 1963; b. in "Studies on Microalgae & Photosynthetic Bacteria," (edt. Jap.Soc.Plant Physiol.), The University of Tokyo Press, p. 291, 1963.
23. a. Govindjee,R., Ph.D. thesis, University of Illinois, 1961; b. Govindjee,R., Thomas,J.B., & Rabinowitch,E., Science, 132, 421, 1960; c. Govindjee, R., & Rabinowitch,E., 1, 377, 1961.
24. a. Govindjee,R., Govindjee & Hoch,G., Biochem. Biophys. Res. Comm., 9, 222, 1962; b. Govindjee,R., Govindjee & Hoch,G., Plant Physiol., 1963 (in press); c. Yang,L., & Govindjee (in preparation).
25. a. Müller,A., Rumberg,B. & Witt, H.T., Proc. Roy. Soc. (London), Series B 157, 313, 1963; b. Müller,A., Fork,D.C., & Witt,H.T.Z. Naturforschung, 18b, 142, 1963.
26. Duysens,L.N.M., & Amesz,J., Biochim et Biophys. Acta, 64, 243, 1962.
27. Kok,B. & Gott,W., Plant Physiol, 35, 802, 1960.
28. Rabinowitch,E., J. Phys. Chem., 66, 2537, 1963.

29. Heise,J.J., Ph.D. thesis, Washington University, St. Louis, Mo., 1962.
30. a. Allen,M.B., Piette,L.H. & Murchio,J.C. Biochem. Biophys. Res. Comm., 4, 271, 1961; b. Biochim et Biophys Acta, 60, 539, 1962.
31. a. Beinert,H., Kok,B., & Hoch,G., Biochem. Biophys. Res. Comm., 7, 209, 1962; b. Kok,B. & Beinert,H., Biochem. Biophys. Res. Comm. 9, 349, 1962.
32. Govindjee, Ichimura,S., Cederstrand,C., & Rabinowitch,E., Arch. Biochem. Biophys. 89, 322, 1960.
33. Butler,W.L., Biochim et Biophys Acta, 64, 309, 1962.
34. Brody,S.S., & Brody,M., Archiv. Biochem. Biophys. 95, 521, 1961.
35. Duysens,L.N.M., & Sweers,H.E., in "Studies on Microalgae & Photosynthetic Bacteria," (edt. Jap.Soc. Plant Physiol.), The University of Tokyo Press, p. 353, 1963.
36. Lavorel,J., Biochim et Biophys Acta, 60, 510, 1962.
37. Goedheer,J.H.C., Biochim et Biophys Acta, 64,294, 1962.
38. French, C.S. in Light & Life (edt. McElroy,W.D. & Glass,B.) Johns Hopkins Press, p. 477, 1961.
39. Allen, M.B., Murchio,J.C., Jeffrey, S.W. & Bendix, S.A. in "Studies on Microalgae & Photosynthetic Bacteria," (edt. Jap. Soc. Plant Physiol.), The University of Tokyo Press, p. 407, 1963.
40. a. Bishop, N.I., Nature, 195, 55, 1962; b. Bishop, N.I. & Gaffron, H., Biochem. Biophys. Res. Comm., 8, 471, 1962.
41. French, C.S. in Carnegie Institution Yearbook, 1963 (in press); b. Govindjee,R., Govindjee & C.S. French (unpublished).
42. Losada,M., Whatley,F.R. & Arnon,D.I., Nature, 190, 601, 1961.
43. Tanada, T., Am. J. Bot. 38, 276, 1951.
44. Govindjee (unpublished).
45. Emerson, R. (unpublished).
46. a. Bakri, M. & Govindjee (unpublished); b. Govindjee, R., & Govindjee (unpublished).
47. Blink, L.R. & van Niel, C.B. in "Studies on Microalgae & Photosynthetic Bacteria," (edt. Jap. Soc. Plant Physiol.), The University of Tokyo Press, p. 297, 1963.
48. McCloud, G. C., Plant Physiol. 36, 114, 1961.
49. Rabinowitch, E. in Mechanism of Photosynthesis, (edt. Tamiya, H.), The Pergamon Press, London, p. 15, 1963.
50. Rabinowitch, E., Photosynthesis & Related Processes, Interscience Publishers, Inc., New York, 1956.
51. Franck, J., Pringsheim, P., & Ladd, D.T., Arch. Biochem., 7, 103, 1945.
52. a. Turner, F.F., Black, C.C. & Gibbs, M., J.B.C., 237, 577, 1962; b. Shen, Y.K., & Shen, G.M., Scientia Sinica, 11, 1097, 1962.

53. Ehrmentraut, H., & Rabinowitch, E., Arch. Biochem., 38, 67, 1952.
54. Yang, L. & Govindjee, (unpublished).
55. Cederstrand, C., (unpublished).
56. French, C.S. in "Studies on Microalgae & Photosynthetic Bacteria" (edt. Jap. Soc. Plant Physiol.). The University of Tokyo Press, p. 271, 1963.
57. Duysens, L.N.M., Ph.D. thesis, University of Utrecht, 1952.
58. a. Bergeron, J.A. & Sybesma, C., abstract in Fed. Proc., 1963; b. Kok, B. (Personal Communication to L. Yang).