# Evidences from Action Spectra for a Specific Participation of Chlorophyll *b* in Photosynthesis

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ABSTRACT Rate of oxygen evolution in photosynthesis was measured as the current from a polarized platinum electrode covered by a thin layer of *Chlorella*. The arrangement gave a reproducibly measurable rate of photosynthesis proportional to light intensity at the low levels used and gave rapid response to changes in illumination. Two phenomena have been explored.

The Emerson effect was observed as an enhancement of photosynthesis in long wavelength red light (700 mμ) when shorter wavelengths were added. Two light beams of wavelengths 653 and 700 mμ when presented together gave a photosynthetic rate about 25 per cent higher than the sum of the rates obtained separately.

Large and reproducible transients in rate of oxygen evolution were observed accompanying change in illumination between two wavelengths adjusted in intensity to support equal steady rates of photosynthesis. The transients were found not to be specifically related to long wavelength red light.

Both enhancement and the transients have identical action spectra which are interpreted as demonstrating a specific photochemical participation of chlorophyll *b*.

We report two kinds of evidence for a specific contribution of chlorophyll *b* to photosynthesis. The first is provided by study of the *Emerson effect* (5–7), the increase in photosynthetic effectiveness of long wavelength red light (700 mμ) by added light of shorter wavelength. The second is based upon the chromatic transients, previously reported by Blinks (1–3), which accompany wavelength changes in the incident light beam. Identity of action spectrum establishes relation between the two phenomena studied previously by Emerson and coworkers and by Blinks. The action spectra identify the pigment responsible for both effects as chlorophyll *b*.

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## *Methods*

We have measured photosynthesis with the bare platinum electrode of Haxo and Blinks (11) with the following modification introduced by Haxo (personal communication) to adapt the electrode for use with unicellular algae. A horizontal flat platinum strip about 2 × 15 mm. is inset in black lucite so that it forms the floor of a chamber 0.75 mm. deep. An algal suspension placed in the chamber is confined by a cellophane dialyzing membrane stretched tightly across the top. The electrode chamber is suspended in 650 ml. of aqueous solution contained in a lucite box together with about 200 ml. of gas phase. A tight-fitting lucite cover contains necessary ports and an inset area above the electrode which drops below the solution surface. A large area N/10 KCl–calomel electrode and N/10 KCl–agar–salt bridge complete the return circuit.

In 1957, Dr. Haxo spent several months in this laboratory checking the performance of his recessed platinum electrode and using it to determine action spectra. From his work it was apparent that the electrode, as used, provided a linear measure of oxygen production, gave reproducible measurements over long periods of time, offered the advantage of small sample requirements, gave a crude action spectrum for *Chlorella* photosynthesis quite similar to that here reported, and a corrected action spectrum similar to that obtained by Emerson and Lewis (8), using a manometric method (Haxo, personal communication). We are grateful to Dr. Haxo for a loan of one of the electrodes actually used.

The electrical circuit used is similar to that of Haxo and Blinks (11). An E. M. F. of 0.64 volt was chosen as a value at which there was negligible effect of pH over the range 6.3 to 7.3. Current flow through the electrode was measured on a Varian recorder as a potential drop amplified by a Doelcam model 2HLA-3 amplifier.

Of several solutions tested two have been used in the present work: (*a*) a buffer containing 0.20 M $K^+$, 0.05 M $Cl^-$, and 0.10 M phosphate at pH 6.8 and (*b*) a modified Knops solution containing 0.0025 M $MgSO_4$, 0.0125 M $KNO_3$, and 0.0092 M $KH_2PO_4$ adjusted to pH 6.8. The solutions were saturated with 5 per cent carbon dioxide in air before use and at daily intervals thereafter in the reservoir surrounding the electrode chamber. The solution reservoir was not thermostatted because of a fortuitously steady room temperature of 20 to 21.5°C.

The alga used was *Chlorella pyrenoidosa*, No. 252 of the Indiana collection, grown in a continuous culture apparatus (12). The four separate samples of algae yielding the data presented herein contained cell concentrations of about 6, 9, 18, and 20 c.mm./ml. In the electrode chamber of depth 0.75 mm. these concentrations should have given layers of cells settled on the electrode of thicknesses 4.5, 6.7, 13.5, and 15.3 $\mu$ respectively. These are minimum values probably somewhat exceeded due to settling of cells after deposition of a large drop of suspension and before the dialyzing membrane could be put in place. Once placed in the electrode chamber, separated from a large reservoir of nutrient solution only by a dialyzing membrane, the algae are in a favorable environment. They may be maintained in a virtual resting cell condition with relatively constant light response for periods of weeks. It is a tribute to the method that all the data shown herein were obtained on four samples of total quantity less than 0.5 mg. dry weight used over a period of 6 weeks.

The electrode assembly without algal cells gave currents of 0.1, 1.8, and 8.4 $\mu$a. when equilibrated with gas mixtures of nominal oxygen content 0, 20, and 95 per cent respectively. In solutions equilibrated with 5 per cent carbon dioxide in air, the current was about 1.8 $\mu$a. without cells. With cells in darkness, the current in various experiments varied between 1.3 and 1.6 $\mu$a. In the experiments to be reported, the current during illumination never exceeded 5 $\mu$a.

Illumination of the electrode chamber was provided by two light beams. The first was presented by a monochromator (Fig. 2 in reference (10)) with slits adjusted to give an emerging beam of 5 m$\mu$ half-band width. An image of the exit slit slightly larger than the electrode was obtained by two lenses and a right angle prism, the latter so arranged that on 90° rotation the slit image was transferred to a Kipp and Zonen compensated linear thermopile. By use of various filters it was established that stray light at 470 and 650 m$\mu$ was effectively less than 4 per cent when the exit beam was filtered only with 6 cm. of water. The intensity of the exit beam was controlled over a wide range by voltage adjustment of the tungsten filament source, by a neutral wedge which could be positioned uniformly on a scale, or by insertion of wire screens.

A second, "reference" light beam presented slightly oblique illumination to the electrode. Its intensity was controlled by lamp voltage and wire screens and its spectral character by either of two interference filters. One filter had peak transmission at 647 m$\mu$ and a half-band width of 11 m$\mu$. The second had a peak transmission at 719 m$\mu$ but was used at an angle of 17° at which its peak transmission was 702 m$\mu$ and its half-band width was 11 m$\mu$. In the discussion below the latter is designated as a 700 m$\mu$ filter. Both interference filters were used with a Corning No. 3480 auxiliary red filter.

The following statements may be made on precision of measurement. Rate of photosynthesis was estimated as the difference current read to 0.5 unit (0.5 per cent full scale or 0.005 $\mu$a.) on the recorder paper. Experience from agreement of duplicates led to the judgment that values obtained had a precision usually better than $\pm 1.0$ units. One test was run at fixed light intensity, using an automatically timed regimen of 2 minutes' light and 2 minutes' dark. Of the fourteen consecutive deflections obtained all were estimated at 34.5 or 35.0 units as the difference current between light and darkness. Perhaps more impressive was the attainable day-to-day stability. A measure of response to a standard light signal at 680 m$\mu$ was used as a daily check upon the algal preparation in use. One of our four preparations gave the following response to the standard light in scale units on each of 8 successive days of use: 44, 44.5, 49.5, 51, 48, 50.5, 51, 48. Variation in the last six values is no greater than that in adjustment of lamp voltage, which was never varied within any one experiment.

## RESULTS

### *Transition Phenomena*

The experiments to be reported were carried out to follow up two observations made in a determination of the action spectrum of photosynthesis in

*Chlorella* for the purpose of checking performance of equipment intended for use with other algae. The first observation was that the time course of rate of oxygen evolution is wavelength-dependent. The effect is shown in Fig. 1 in which the recordings of response to 650 and 700 mμ were superimposed. The character of response to 650 mμ is not always the same but always has one identifiable feature: the initial rise in rate is more rapid. In our early observations, made only at the red end of the spectrum, this feature was dis-

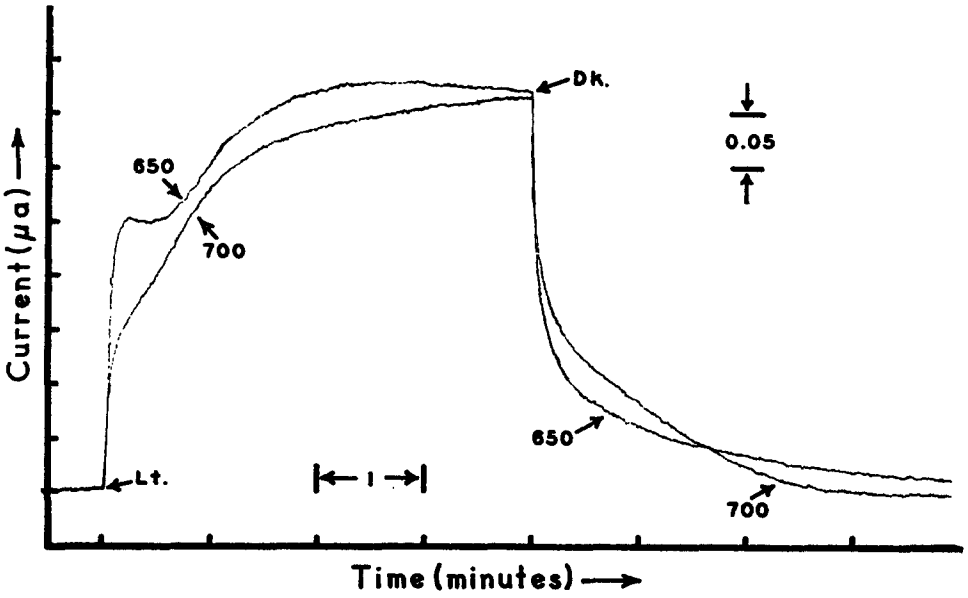


FIGURE 1. The time course of photosynthesis in *Chlorella* under low intensity monochromatic illumination. A photograph of an original recording in which the records at 650 and 700 mμ were superimposed.

tinguished at 650 and to a lesser extent at 640 and 660 mμ as compared to adjacent wavelengths.

The feature shown in Fig. 1 was readily identified with the phenomena reported by Blinks (2) for wavelengths 560 *vs.* 675 mμ in the red alga *Porphyra.* We therefore proceeded to look for chromatic transients similar to those also reported by Blinks. Our monochromator and reference beams were adjusted in intensity to give equal steady state rates of photosynthesis and the two beams were then presented alternately without any dark transition. Alternation of such matched beams of 700 and 650 mμ gave record A of Fig. 2. For comparison record B shows the result of alternating matched beams of 700 and 690 mμ. We interpret the minor fluctuations in record B as a result of lack of perfect shutter synchronization and less than desired resolution of the 700 mμ beam by the interference filter used.

The transients shown in Fig. 2A are significant in two respects. First, they are observed at very low light intensities where the steady rate of photosynthesis is demonstrably light-limited. Second, they are large in magnitude. Fig. 2 was recorded in the same experiment and with the same current sensitivity used for Fig. 1. The current upswing attending the 700 → 650 mμ transition is about 25 per cent of the current difference maintained by steady state photosynthesis. The reverse transition 650 → 700 mμ is accom-

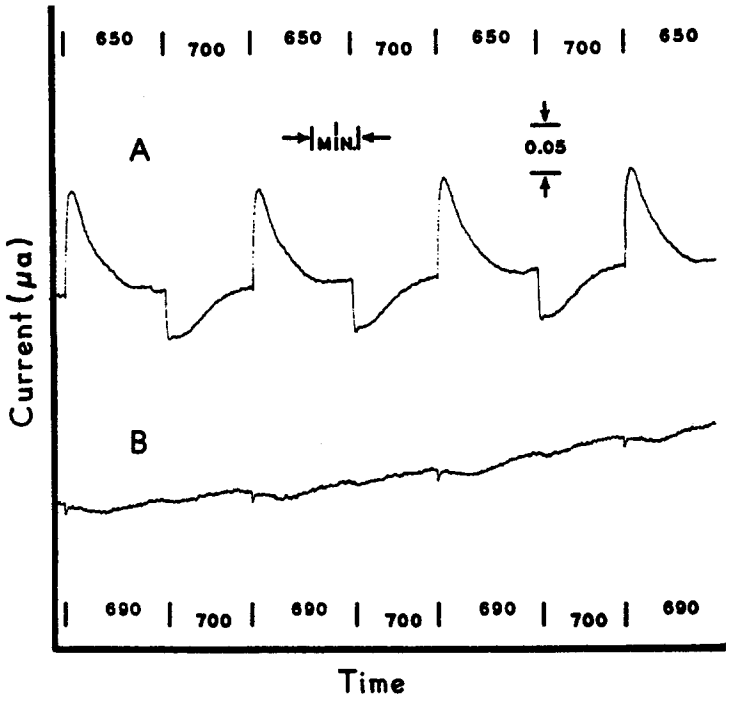


FIGURE 2. Chromatic transients observed by alternating two light beams of intensities adjusted to sustain equal steady rates of photosynthesis. Record A, alternating beams of 650 and 700 mμ. Record B, alternating beams of 690 and 700 mμ.

panied by a downswing in current which is smaller and somewhat less regular in shape. Both transients are consistently reproducible phenomena. They show minor variations in time course apparently due to second order effects which we disregard for the present.

We interpret the transients of Fig. 2A as actual transients in rate of oxygen evolution. Explanation in terms of carbon dioxide is eliminated. Reducing the carbon dioxide from 5 per cent to equilibration with air with attendant change of about 0.4 pH unit has negligible effect on the electrode current.

The transients of Fig. 2A are wavelength-dependent in magnitude but relatively constant in shape. The action spectrum for the transient effect was

obtained by the following procedure. The shutter system was arranged to alternate the reference and monochromator beams. The reference beam was defined by an interference and auxiliary red filter to give a band pass peaked at 700 mμ. The monochromator beam at a chosen wavelength, λ, was adjusted by the neutral wedge to give an intensity such that, after switching light beams and completion of the transients (*ca.* 2 minutes), equal rates of photosynthesis were sustained by the two beams. The neutral wedge scale was noted and several successive transients recorded. This procedure was repeated through the spectrum. After completion of the recordings the monochromator beam was diverted to the thermopile and energy measured at each wavelength and corresponding wedge scale.

The procedure used actually yields two action spectra. The action spectrum

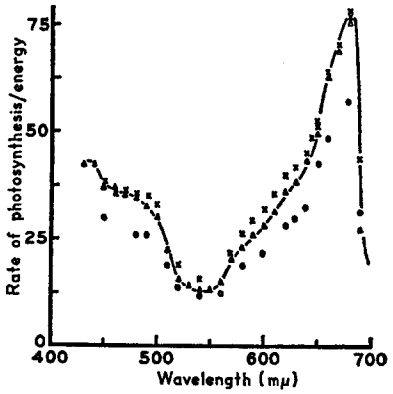


FIGURE 3. Action spectrum of photosynthesis of *Chlorella*. Three separate experiments are presented without adjustment. The curve is drawn through the best set of data.

of photosynthesis, obtained as reciprocal of energy for constant rate of photosynthesis, is presented in Fig. 3. The three sets of data come from three experiments on two different samples of *Chlorella* but are not arbitrarily normalized in any way. Except for the low height of the blue peak the curve appears as expected. At this point we wish to make no issue of the precise shape of Fig. 3 since we hope to improve resolution by use of a narrower electrode and to simplify the procedure by automatic recording. The intent of this presentation is only to provide comparison with the other spectra which follow.

Fig. 4A presents the action spectrum of the 700 → λ transient. Probably the best measure of magnitude of the transient would be the area encompassed. However, as a simpler and reasonably effective measure we have chosen the initial peak height. Further, because it is larger and more uniform, we have chosen the 700 → λ upswing. The spectrum is not materially shifted on the wavelength axis if the λ → 700 downswing or the total envelope is taken as an alternative measure. Again the data of three experiments are plotted together without any arbitrary normalization. Before

examining the meaning of the spectrum obtained we digress to a second phenomenon with an identical action spectrum.

### *Enhancement*

A second early observation in our work was that the action spectrum for photosynthesis can be shifted by a small procedural variation. In preliminary work we obtained action spectra by the procedure of Haxo and Blinks (11). At each wavelength the rate of photosynthesis was estimated as the difference in currents observed in darkness and in light after a 3 to 4 minute exposure.

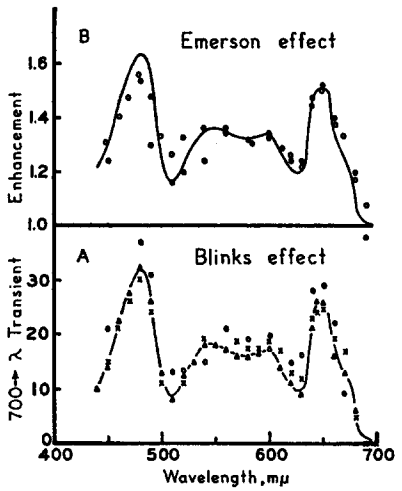


FIGURE 4. Action spectra. A, the 700 → λ transient as per cent of the steady rate of photosynthesis. B, the 700 + λ enhancement reckoned in terms of 700 mμ alone ($rate_{700\ with\ background}$) / ($rate_{700\ without\ background}$). Curve B is drawn to duplicate curve A in order to facilitate comparison.

We repeated the procedure with a continuous background illumination from the reference beam in place of darkness. The spectral character of the background was found to modify significantly the resulting action spectrum. In subsequent analysis we came to a simplified method which reveals that at least some part of the phenomenon observed is identical with that reported by Emerson and coworkers (5–7).

If we varied intensity of a monochromatic beam by screens calibrated *in situ*, the rate of steady photosynthesis observed was always linear with light intensity. However, if in addition to the monochromatic beam we imposed a constant background illumination of proper wavelength, the rate of photosynthesis deviated from linearity. The effect is shown in Fig. 5 for a variation in intensity at 650 mμ superimposed on darkness or on a constant background of 700 mμ.

The effect is demonstrated more convincingly by an inverted procedure which gives rise to Fig. 6. One of the two light beams, designated the signal beam, was held at constant intensity of a desired wavelength (as 700 mμ).

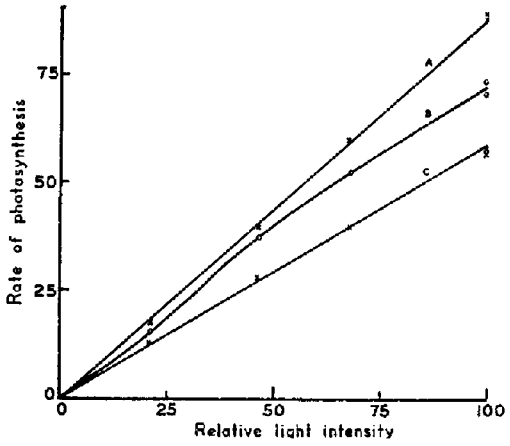


FIGURE 5. Rate of steady photosynthesis *vs.* relative light intensity at 653 mμ. Curves A and C, no background illumination. Curve B, with a constant background of about 25 rate units of photosynthesis at 700 mμ. Values of intensity for curves B and C were identical. Curve A is a repetition of curve C with the light intensity about 50 per cent greater.

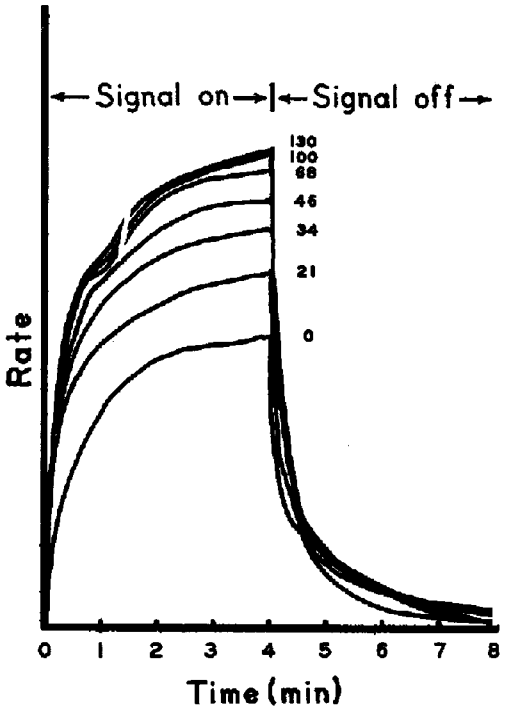


FIGURE 6. Tracings of records showing response to a standard light signal of 700 mμ superimposed on a background of 653 mμ of the relative intensities noted on the curves.

We observed the rate of photosynthesis sustained by the signal beam. Then we observed independently the rate of photosynthesis sustained by the second or reference beam of chosen wavelength (as 653 mμ). Third, leaving the reference beam on as background and electrically biasing out the recorder deflection corresponding to its rate of photosynthesis, we again observed the rate of photosynthesis produced by the signal beam. The procedure, repeated at different intensities of the reference beam, gives rate of photo-

TABLE I

PROTOCOL OF EXPERIMENTAL DATA OF FIGS. 7 AND 8

Rate units of photosynthesis observed at each of three intensities of a signal beam when superimposed on rate units of photosynthesis corresponding to varied intensity in a reference beam. A rate unit is a difference current of 0.01 μa. The 700 mμ beam was defined by an interference filter plus a RG5 red filter. The 653 mμ beam was presented from the monochromator.

| | Signal beam 700 mμ | | | | Signal beam 653 mμ | | |
|---|---|---|---|---|---|---|---|
| Reference beam 653 mμ | Int. 100 | Int. 65 | Int. 44 | Reference beam 700 mμ | Int. 100 | Int. 65 | Int. 34 |
| 0 | 49.5 | 32 | 21.5 | 0 | 99 | 62 | 32 |
| 13.5 | 57 | 38.5 | 27.5 | 14 | 107 | 72 | 39 |
| 29 | 63 | 44.5* | 33 | 30.5 | 123‡ | 85 | 43.5 |
| 64 | 76 | 54.5 | 39 | 45 | 131 | 91 | 46.5 |
| 94 | 83 | 57.5 | 40.5 | 67 | 137 | 92 | 47.5 |
| 138 | 84 | 57 | 40.5 | | | | |

* This figure gives the circled points of Fig. 8A and 8B by the following calculations:

A, enhancement (for 700 mμ) = 44.5/32 = 1.39

ratio 653/700 = 29/32 = 0.91

B, enhancement, $(\text{rate}_{700+653})/(\text{rate}_{700} + \text{rate}_{653}), = \frac{44.5 + 29}{32 + 29} = 1.21$

λ653, per cent of (λ653 + λ700), = 100 × 29/(29 + 32) = 47.5

‡ This figure gives the triangle points of Fig. 8A and 8B by the following calculations:

A, enhancement (for 700 mμ) $= \frac{123 - 99 + 30.5}{30.5} = 1.79$

ratio 653/700 = 99/30.5 = 3.24

B, enhancement, $(\text{rate}_{700+653})/(\text{rate}_{700} + \text{rate}_{653}), = \frac{123 + 30.5}{99 + 30.5} = 1.18$

λ653, per cent of (λ653 + λ700), = 100 × 99/(30.5+99) = 76.5

synthesis in response to a standard signal of one wavelength superimposed on measured rate units of photosynthesis of the same or any other wavelength.

When the wavelengths of the signal and reference beams are the same, the response to a standard signal is constant within the range of intensities which we have used for the reference beam. When the two beams are chosen as 653 and 700 mμ, then response to a standard signal is not constant. A typical experiment is illustrated by Fig. 6 as the superimposed tracings of a

family of curves. The only variable between curves is the intensity of the reference or background light which is indicated in relative units for each curve.

The results of a more complete experiment are presented as a protocol in Table I since various kinds of analysis can be made. The saturation curves obtained directly from the data are plotted in Fig. 7. A number of other similar but less complete experiments yielded similar results except that some plots, as in Fig. 7, had a slightly S-shaped character instead of being initially linear.

The magnitude of enhancement obtained by mixing 653 and 700 mμ

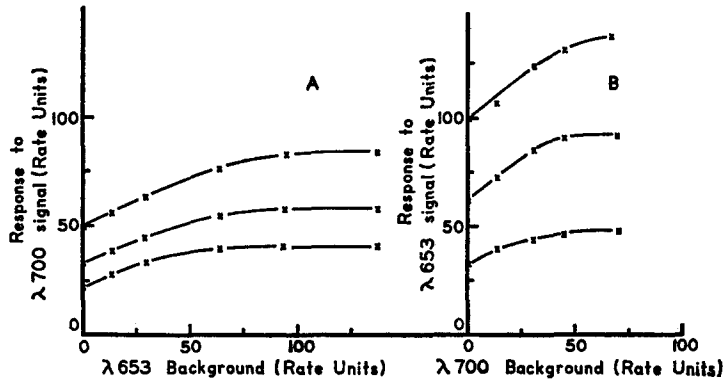


FIGURE 7. Steady rate of photosynthesis in response to each of three intensities of a constant signal superimposed upon varied intensity of background. Both response to signal and intensity of background are measured in rate units of photosynthesis. Curve A, 700 mμ signal, 653 mμ background. Curve B, 653 mμ signal, 700 mμ background.

may be reckoned in several ways. If the effect is considered to be unidirectional, that is, the effect of 653 mμ light on the photosynthetic utilization of 700 mμ light, then enhancement should be reckoned in terms of rate of photosynthesis due to 700 mμ light alone. This has been done in Fig. 8A in which all data may be fitted to a single curve by plotting enhancement of 700 mμ photosynthesis against the ratio of rate units of photosynthesis for 653/700 mμ. The maximum value of enhancement reckoned in this way is about 1.8 and it is half-saturated by about equal rate units of 653 and 700 mμ.

Enhancement may be reckoned also in terms of total light presented, namely as

$$\frac{\text{Rate}_{653+700}}{(\text{Rate}_{653}) + (\text{Rate}_{700})} \quad \begin{matrix}\text{(presented together)} \\ \text{(presented separately)}\end{matrix}$$

Enhancement reckoned in this way is plotted in Fig. 8B and shows a maximum value of about 1.25 when the 653/700 ratio is about 2/1 in terms of rate units of photosynthesis.

With the above information at hand it became possible to determine an action spectrum for the Emerson effect. For this purpose we chose a 700 mμ reference beam. The monochromator beam at a selected wavelength, λ, was adjusted by the neutral wedge to an intensity such that, after switching light beams and completion of transients, equal rates of photosynthesis were sustained by the two beams. The neutral wedge scale was noted and one set of transients recorded. The monochromatic beam was then added to the reference beam for a period of time necessary to obtain a new steady rate of photosynthesis. The procedure gives action spectra for steady photosynthesis, for the chromatic transient, and for enhancement. The action spectrum for

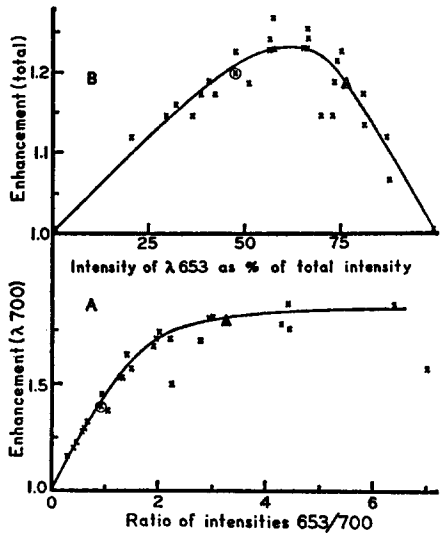


FIGURE 8. Characteristics of the 653 + 700 mμ enhancement from the data of Table I and Fig. 7. A, enhancement, reckoned as $rate_{700}$ with background divided by $rate_{700}$ without background, *vs.* ratio of intensities 653/700 measured in rate units of photosynthesis. B, Enhancement, reckoned as $(rate_{700+653})/(rate_{700} + rate_{653})$ *vs.* per cent of total units of photosynthesis in the 653 mμ beam.

enhancement is presented in Fig. 4B. The identity of action spectra for the 700 → λ transient and the 700 + λ enhancement establishes a basic relation between the two phenomena.

We have also inverted the above procedure by using 647 mμ for the reference beam in order to observe the λ → 647 transient and the λ + 647 enhancement. The results can be simply described. The λ → 647 transient gives an action spectrum almost completely inverted from that for 700 → λ transient shown in Fig. 4A. However, the λ + 647 enhancement was observed only at values of λ of 690 mμ or greater and showed no further detail in the action spectrum.

## DISCUSSION

We take the action spectra of the $700 \rightarrow \lambda$ transient and the $700 + \lambda$ enhancement as evidence for a specific contribution of chlorophyll *b* to both phenomena. The evidence is not completely firm since we have no reliable data from which the ratio of chlorophylls *a*/*b* absorption *in vivo* may be determined. However, estimates may be made from curves for the extracted pigments (13) by making reasonable corrections for known spectral shifts observed from those *in vivo* to those in organic solvent extracts. Further, we have available an estimate of a chlorophyll *b*/*a* ratio of 0.3 (kindly provided by J. R. Graham from multiple analyses on extracted pigments of *Chlorella ellipsoidea*). From these data the 483 and 653 mμ maxima and the 633 mμ minimum are explainable; explanation of the 513 mμ minimum is uncertain because of very low absorption by the chlorophylls in this region. We attribute the 480 peak to chlorophyll *b*. While carotenoids also absorb in the 480 mμ region, the single sharp peak of the action spectrum looks more like the blue chlorophyll *b* peak than like the broad carotenoid absorption with several humps.

The data on enhancement confirm and extend the observations of Emerson *et al.* (6). With the later data of Emerson (5), cited for seven wavelengths within our range, our action spectra are in rough agreement even as to approximate magnitude. The only discrepancy is for the relative height in the blue which is lower in our data than in Emerson's and which is possibly related to the low height of our action spectrum for photosynthesis in the same region.

The data presented on the magnitude of enhancement or its relation to rates of photosynthesis at 653 and 700 mμ hardly can be taken as absolute values. Our 700 mμ beam as presented by an interference filter provided a wider wavelength band than desired (11 mμ half-width). Further, we suspect that the magnitude of enhancement may be varied by the spectral character of illumination in previous cell history (*cf.* Blinks (1)). At the same time the data do provide at least an approximate measure of magnitude and relative contributions of the two wavelengths. For example it is clear that the Emerson effect requires substantial proportions of each wavelength, rather than being a catalytic phenomenon.

In interpreting enhancement there is a question not answerable from the data themselves as to the direction of the effect. Other evidence favors the view that the effect represents an increase in quantum yield of long wavelength (700 mμ) accomplished by simultaneous illumination of chlorophyll *b*. First, as noted by Emerson and Chalmers (7) it is the low quantum yield at long wavelength which is anomalous while the quantum yields at shorter wavelengths are near maximal. Second, the fractional absorption by chlorophyll *b in vivo* can never be very large, and enhancement of quantum yield

for light absorbed by chlorophyll *b* could scarcely be as great as the effects observed. These arguments reasonably eliminate the opposite possibility that enhancement results from action of long wavelength absorption (700 mμ) on chlorophyll *b*. However, neither argument eliminates a mutual interaction of such nature that maximum quantum yield requires certain limits of simultaneous chlorophyll *a* and chlorophyll *b* absorption. In this connection it should be noted that the data of Emerson and Lewis (8) on the quantum yield of *Chlorella* show a small but presumably significant minimum at 650 to 660 mμ and a more severe minimum (ascribed to carotenoid absorption) at 480 to 490 mμ.

There is a second question about enhancement upon which Emerson and Chalmers (7) also speculated. The question has to do with the apparent specificity to wavelengths longer than 685 mμ. Is the 685 to 710 mμ region peculiar with respect to chlorophyll *a* absorption because it is on the long wavelength side of the absorption band; or is the 685 to 710 mμ region peculiar because it is the only portion of the visible spectrum in which chlorophyll *b* absorption is negligible? The experimental evidence on *Chlorella* favors the first possibility. Emerson (5) failed to observe enhancement between wavelength pairs such as 436 and 644 mμ or 578 and 480 mμ. Our attempts to obtain an inverse action spectrum for $\lambda + 647$ also failed to show enhancement except when λ was 690 mμ or longer. However, the evidence is again subject to question. A difficulty is that *in vivo* chlorophyll *b/a* absorption ratio is always small; it is difficult to make any sizeable change in the ratio on adding two monochromatic beams unless one beam, as at 700 mμ, has a zero value for the ratio. Further, in our experiments, the 647 mμ reference wavelength (half-width 11 mμ) was not provided with high enough resolution by the interference filter employed. It appears likely that the answer would be best attained in other algae such as *Porphyridium* in which the main accessory pigment, phycoerythrin, has less overlap with chlorophyll *a* absorption.

The transition phenomena examined are related to the chromatic transients of Blinks (1, 2, 4) if we consider that phycoerythrin in *Porphyra* and chlorophyll *b* in *Chlorella* are analogous accessory pigments. Our work, as a confirmation and extension of Blinks's observations, has been no more than exploratory. Three important points are considered firmly established.

First, the transients are large. Following a transition from 700 to 650 mμ the peak of the upswing represents a transient rate of oxygen evolution typically more than 25 per cent of the steady rate. For the following 30 second period the area under the transient represents an average rate about 15 per cent higher than the steady rate. The transients are most simply examined when the alternating beams are matched in intensity to give equal steady rates of photosynthesis but, as also observed by Blinks, they do not depend upon exact matching.

Second, the transients are clearly related to chlorophyll *b* by their action spectrum.

Third, the transients are not at all peculiar to use of long wavelength (700 mμ) in one of the alternating beams. The transients shown in Fig. 2A may be repeated with varying magnitude but apparently identical character on transitions such as 630 → 647 → 630 mμ, 510 → 647 → 510 mμ, or 647 → 480 → 647 mμ. The only reasonable explanation now apparent is that the transients arise from a change in ratio of rate of supply of quanta to chlorophylls *a* and *b*. All our observations would fit the statement that, as in Fig. 2A, an upswing in rate of oxygen evolution accompanies increased *b*/*a* illumination ratio and a downswing accompanies a decreased *b*/*a* illumination ratio.

From these considerations we cannot escape the conclusion that there must be two kinds of photo events in photosynthesis, one of them specifically associated with chlorophyll *b* (or other accessory pigment). We cannot determine, and at this stage prefer not to speculate, whether the two events occur *via* different excited states of chlorophyll *a* (as proposed by Franck (9) for the Emerson effect) or whether they imply photochemical participation of chlorophyll *b* in initiating a different chain of succeeding reactions. A model proposed to account for the role of an accessory pigment will have to include consideration of fluorescence behavior, chloroplast structure, the phenomenon of enhancement, and the phenomenon of chromatic transients. On the latter we have not yet an adequate description.

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