STUDIES ON THE SECOND EMERSON EFFECT IN THE HILL REACTION IN ALGAL CELLS

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ABSTRACT This paper shows that the "second Emerson effect"[1] exists not only in photosynthesis, but also in the quinone reduction (Hill reaction), in *Chlorella pyrenoidosa* and *Anacystis nidulans*. The peaks at 650 mμ, 600 mμ, 560 mμ, 520 mμ, and 480 mμ, observed in the action spectrum of this effect in the Hill reaction in *Chorella*, are attributable to chlorophyll *b*; the occurrence of an additional peak at 670 mμ, 620 mμ, and of two (or three) peaks in the blue-violet region suggests that (at least) one form of chlorophyll *a* contributes to it. In analogy to suggestions made previously in the interpretation of the Emerson effect in photosynthesis, these results are taken as indicating that excitation by light preferentially absorbed by one (or two) forms of chlorophyll *a* (Chl *a* 690 + 700), needs support by simultaneous absorption of light in another form of chlorophyll *a* (Chl *a* 670)—directly or *via* energy transfer from chlorophyll *b*—in order to produce the Hill reaction with its full quantum yield. In *Anacystis*, the participation of phycocyanin in the Emerson effect in the Hill reaction is revealed by the occurrence, in the action spectrum of this effect, of peaks at about 560 mμ, 610 mμ, and 640 mμ; a peak at 670 mμ, due to Chl *a* 670, also is present.

INTRODUCTION

The discovery, by Emerson and Lewis (1), that the quantum efficiency of photosynthesis decreases well within the long-wave absorption band of chlorophyll *a*, posed the question whether excitation of chlorophyll *a* by itself can give a full yield of photosynthesis. Emerson and coworkers (2-9) discovered that if light of shorter wavelengths (< 680 mμ) is simultaneously provided, the quantum yield in the

[1] The second Emerson effect—to be abbreviated below as Emerson effect—is the enhancement of the quantum yield of a photochemical process produced, in plant cells or extracts, by far red light, by simultaneous illumination with light of shorter wavelengths (the first Emerson effect being the burst of carbon dioxide, produced by plants in the first few minutes of illumination).

"red drop" region is enhanced. This was called the (second) Emerson effect by Rabinowitch (reference 9). Its action spectra in various algae were found (*cf.* 7 and 9) to follow closely the curves showing the fraction of total absorbed light that is absorbed by the accessory pigments (chlorophyll *b* in *Chlorella,* phycocyanin in *Anacystis,* phycoerythrin in *Porphyridium,* and fucoxanthol in *Navicula*). These findings suggested that for photosynthesis to take place with full yield, at least one accessory pigment must be excited simultaneously with chlorophyll *a*; failure to do so appeared as the cause of the decrease in the quantum yield of photosynthesis in the red region (referred to as the red drop).

Franck (10) suggested a somewhat different interpretation: that the red drop occurs when light is preferentially absorbed by one form of chlorophyll *a*. Simultaneous excitation of another form (directly, by absorption of light < 680 mμ, or indirectly, by energy transfer from accessory pigments) is required for efficient photosynthesis.

Govindjee and Rabinowitch (11, 12) confirmed this surmise. They showed that in addition to preferential light absorption by fucoxanthol in *Navicula,* phycocyanin in *Anacystis,* phycoerythrin in *Porphyridium,* and chlorophyll *b* in *Chlorella,* preferential light absorption by a special form of chlorophyll *a* itself (Chl *a* 670), produces the Emerson effect. The peak at 670 mμ was most prominent in the action spectra of Emerson effect in *Chlorella* and *Navicula,* and less pronounced in those of *Anacystis* and *Porphyridium.*

There was, in past work, no direct evidence that the Emerson effect was not due, at least in part, to a light inhibition of respiration (rather than to an enhancement of photosynthesis), because the techniques employed (manometry (2-9,11,12) and polarography (13)) could not distinguish between positive changes in the rate of photosynthesis and negative changes in the rate of respiration during illumination. There was also no evidence as to the locus of the effect—in the oxygen-evolving or in the carbon dioxide–reducing phase of photosynthesis. It was thought that studies on the Hill reaction in quinone-poisoned non-respiring *Chlorella* cells could elucidate the above questions. To see whether accessory pigments (such as phycocyanin) do contribute to the Emerson effect in the Hill reaction, blue-green alga, *Anacystis,* was studied in addition to *Chlorella.* Preliminary results obtained with *Chlorella* were reported earlier (14); the detailed results of experiments with *Chlorella* and *Anacystis* follow in this paper. Experiments on cell-free leaf extracts from a higher plant, *Phytolacca,* will be published later (15).

MATERIALS AND METHODS

Two species of algae (*Chlorella pyrenoidosa,* Emerson's strain 3; and *Anacystis nidulans,* obtained from Professor Jack Myers at the University of Texas) were grown in inorganic culture media (for a summary of growing conditions, see Govindjee and Rabinowitch (12)).

The algal suspension in phosphate buffer (pH 6.8) was made up so that absorption

at the chlorophyll *a* peak (680 mμ) was about 60 per cent for *Chlorella* and 90 per cent for *Anacystis*. A solution of 12.5 mg recrystallized para-benzoquinone in 5.0 ml of 0.01 N sulfuric acid was prepared and 0.2 ml of it added to 6.8 ml of the algal suspension. Pure nitrogen (99.99 per cent) was conducted through the algal suspension for 15 minutes before quinone was added; the mixture was then transferred into the rectangular manometric vessel. A similar compensating vessel, used in the double manometer, received 7.0 ml of the phosphate buffer. The same gas (nitrogen) was passed for another 10 minutes through the manometer vessels. We did not add any carbon dioxide to the gas phase, but no attempt was made to remove all traces of this gas, and no test was made for its complete absence (see Warburg (16), Brown (17), and Stern and Vennesland (18) concerning a possible need of traces of carbon dioxide for the Hill reaction).

Evolution of oxygen in light was measured by a differential manometer (Emerson and Chalmers (19)) at 10°C. Measurements were made at intervals of 1 minute, with a precision of 0.01 mm, by using two cathetometers. The vessels were shaken all the time.

The Hill activity lasted for 4 to 5 hours and remained fairly constant throughout the set of experiments which covered a certain range of wavelengths. Corrections were made for declining activity, whenever such decline was observed.

Monochromatic light was obtained from the Emerson-Lewis grating monochromator (f. 1.5). The band-widths were 10 mμ in the 600 to 700 mμ region and about 20 to 30 mμ in the 400 to 600 mμ region.

When the action spectrum of the Emerson effect was measured, the Emerson-Lewis monochromator was used to provide the supplementary monochromatic beam, while the far red light was provided by a separate assembly. This assembly consisted of a 1000 watt tungsten lamp, infrared absorbing filter, lenses, a 45° reflecting prism, a light pipe of lucite, a stainless steel mirror, and an interference filter (Farrand 109556) or a combination of a Schott RG5 + RG8 and a red Corning glass filter (color specification No. 2-64) to give far red light of about 700 mμ (or > 680 mμ, *cf.* reference 12 for the transmission curves of the filters, and Thomas and Govindjee (20) for a diagram of the optical arrangement).

The intensity of far red light was kept constant throughout each set of measurements; that of the supplementary light was adjusted to give, by itself, about the same Hill activity at each wavelength.

The following sequence of measurements (each lasting for a period of 5 minutes) was used: dark, far red, dark, supplementary, supplementary + far red, supplementary, dark again, etc. In most cases there was no measurable dark reaction.

The absorption measurements were done either with the wet filter paper technique of Thomas and Govindjee (20) or with an integrating spectrophotometer.[2]

RESULTS AND DISCUSSION

1. *The Red Drop and the Quantum Yield of the Hill Reaction.* The red drop in the quantum yield of photosynthesis of green algae above 680 mμ was established by Emerson and coworkers (4). Ehrmantraut and Rabinowitch (21)

[2] The integrating spectrophotometer devised and constructed by Mr. Carl N. Cederstrand was used; the details of this instrument will be published later.

noticed that the quantum yield of the Hill reaction in *Chlorella* also declined between 669 mμ and 698 mμ, but a complete action spectrum of this reaction was not measured.

Fig. 1 shows the quantum yield of the Hill reaction in *Chlorella* at 10°C as a function of wavelength in the red spectral region. Correction was made for the incompleteness of absorption, by measuring the absorption curve of the same

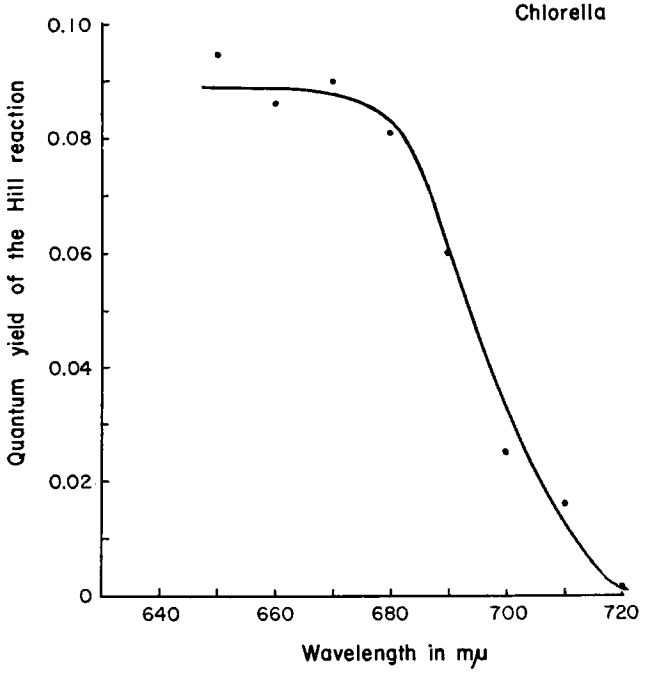

FIGURE 1 Red drop in the action spectrum of the Hill reaction (quinone reduction) in *Chlorella pyrenoidosa*, at 10°C.

suspension in Cederstrand's integrating spectrophotometer. The quantum yield was calculated according to the following equation:

$$\Phi = \frac{K_0 \cdot V}{22.4 \cdot R \cdot k \cdot A \cdot}$$

in which Φ, measured as $\frac{\text{(number of moles of oxygen produced)}}{\text{(number of einsteins of light absorbed)}}$, is the quantum yield, V the rate of oxygen production (in millimeters pressure change per hour), K_0 the so called "vessel constant" (a function of temperature) which converts pressure changes (in millimeters) into volume changes (in microliters), 22.4 the constant used to convert microliters of oxygen to micromoles of

oxygen, R the reading (in microvolts) of the bolometer used to measure the intensity of the incident radiation, k (a function of wavelength) the factor converting R into the number of microeinsteins incident per hour on the surface of the algal suspension (for details of the evaluation of this factor we refer to Appendix 2 and Fig. 33 in the doctoral thesis of Govindjee (22)) and A (also a function of wavelength) is the fraction of incident light absorbed in the vessel.

Fig. 1 shows that the maximum quantum yield of the Hill reaction found in our

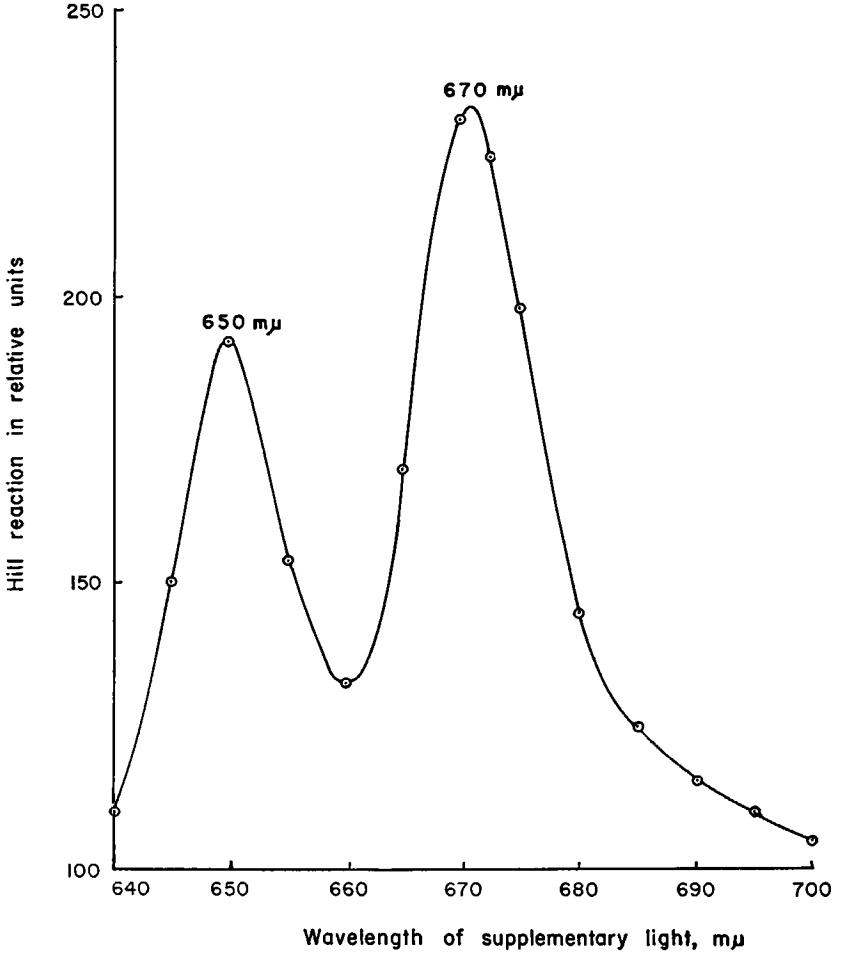

FIGURE 2 The 670 mμ peak (due to Chl *a* 670) in the action spectrum of the Emerson effect in the Hill reaction in *Chlorella pyrenoidosa*. Action due to far red light (using Schott red cut-off filters R-G8 and R-G5 combined with a Corning filter C.S. No. 2-64; $\lambda > 680$ mμ) was 3.0 μl O_2 /hr. / 60 μl cells. Ratio of the action of far red light alone to action of supplementary light alone was 1:6. (This spectrum was confirmed by using an interference filter, $\lambda_{max} = 700$ mμ, to produce far red light.)

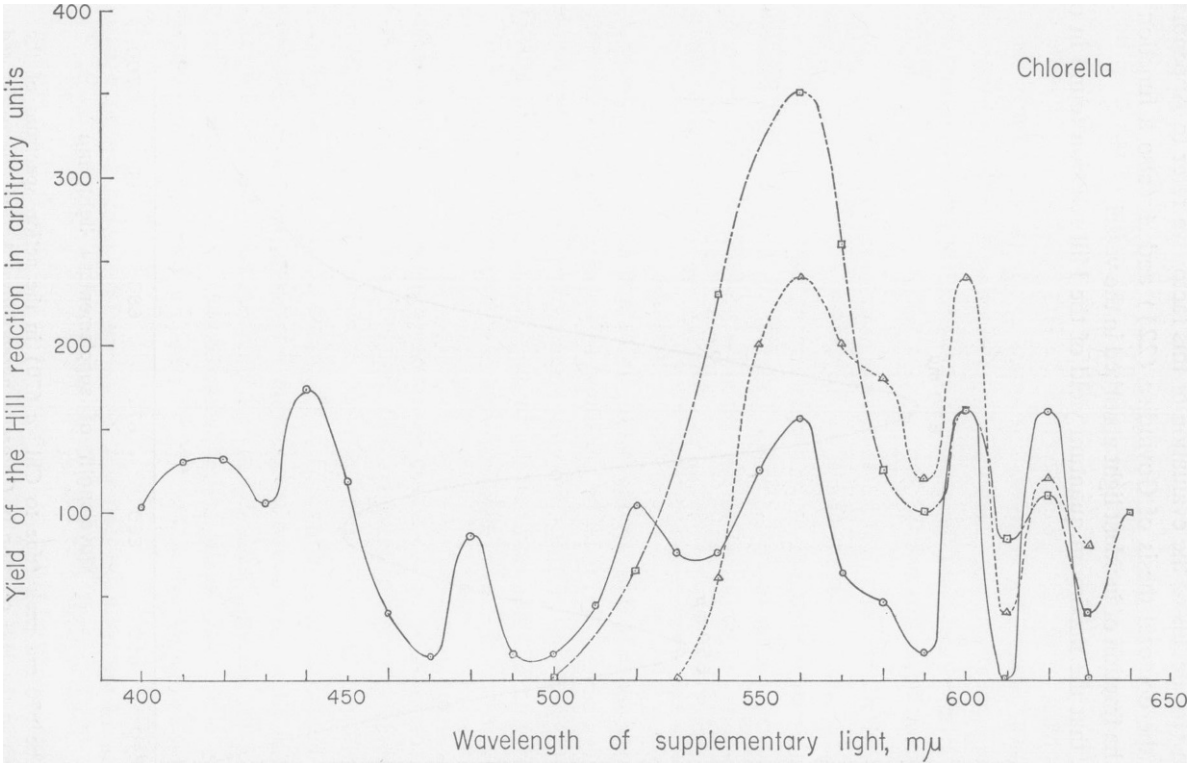

FIGURE 3 Complete action spectra of the Emerson effect in the Hill reaction in *Chlorella pyrenoidosa* (3 experiments). The peaks ascribed to chlorophyll *b*, 480 mμ, 520 mμ, 560 mμ, and 600 mμ; peaks ascribed to Chl *a* 670, 410 to 420 mμ, 440 mμ, and 620 mμ. Action due to far red light alone (> 680 mμ) was 2.5 μl 0_2/ hr./60 μl cells. Ratio of the action of far red light to supplementary light was about 1:1.5.

cultures of *Chlorella* was about 0.09. The decline in the Hill reaction activity began at about 680 mμ. The quantum yield fell to half the maximum value at about 695 mμ. The existence of the red drop in the Hill reaction in *Chlorella* was clearly confirmed by this experiment. It will be noted that the curve published in our preliminary report (14) fell more steeply than the one presented here; but an accurate correction for incomplete absorption could not be made in the earlier experiments.

2. *Role of the Chlorophylls in the Hill Reaction.* Figs. 2 and 3 show the action spectrum of the Emerson effect in the Hill reaction in *Chlorella* cells. This spectrum was obtained as follows. The action of the far red light in the presence of supplementary light was calculated by subtracting the action of the supplementary light, given alone, from the action of combined supplementary and far red light. The Emerson effect was defined as:

$$\epsilon = \frac{\text{Action of far red light in presence of supplementary light}}{\text{Action of far red light alone}} \times 100$$

and plotted against the wavelength of the supplementary light.

Fig. 2 shows the action spectrum of the Emerson effect in the Hill reaction in *Chlorella* (a two-day-old culture) in the region 640 to 700 mμ. The curve represents an average of five experiments. In the 640 mμ to 700 mμ region, there are two distinct peaks. That at 650 mμ is due to chlorophyll *b* (*cf.* Fig. 7 in (9)), while the peak at about 670 mμ, found before in the action spectra of the Emerson effect in photosynthesis (12), must be attributed to a special form of chlorophyll *a*. The absorption spectrum of the *Chlorella* suspension (Fig. 4) shows evidence of bands at 650 mμ, 673 mμ, and 680 mμ in the red region. The peaks at 673 mμ and 680 mμ must belong to two distinct forms of chlorophyll *a* ("Chl *a* 670" and "Chl *a* 680").

Fig. 3 is a continuation of the action spectrum of a two-day-old *Chlorella* into the shorter-wavelength region, between 400 and 640 mμ. The peaks at 480 mμ, 560 mμ, and 600 mμ, also present in Emerson's curve for the same effect in photosynthesis, can be ascribed to chlorophyll *b*. The peaks at 620 mμ and 520 mμ were not noticeable in Emerson's action curve (Fig. 7 in (9)), nor in that by Govindjee (22). Since the 520 mμ peak did appear in Emerson's "fractional absorption curve" (9), its occurrence in the action spectrum was to be expected. This peak also must be due to chlorophyll *b*. The 620 mμ peak can be assigned to a vibrational band of Chl *a* 670.

The locations of the peaks at 410 to 420 and 440 mμ could not be determined very precisely, because wide slits had to be used in the blue-violet region. These peaks can be tentatively assigned to the Soret band of Chl *a* 670, as suggested by Govindjee (22) in the case of photosynthesis.

Fig. 5 shows the action spectrum of the Emerson effect in the Hill reaction in *Anacystis* cells. A peak at 670 mμ can be clearly noted in this spectrum, too.

3. *Role of Phycocyanin in the Hill Reaction in Anacystis.* Thomas and

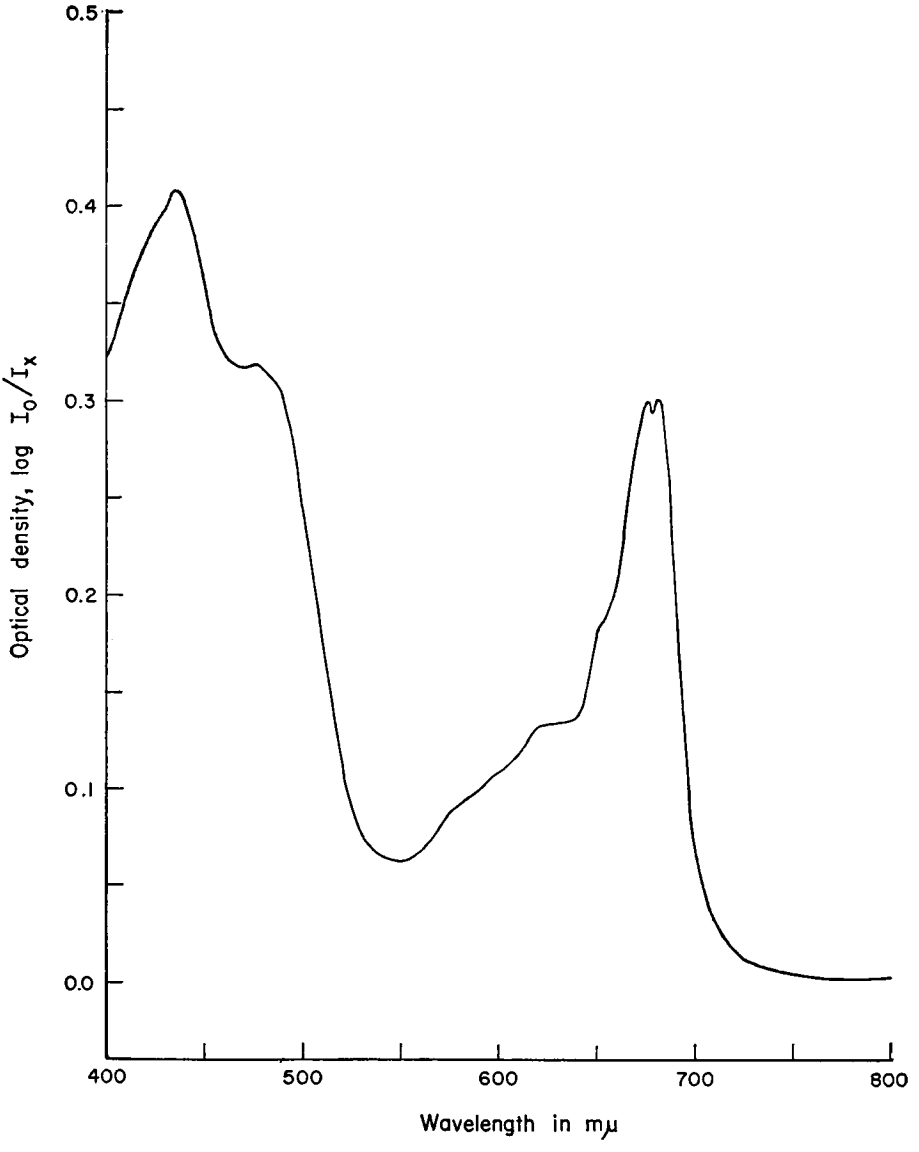

FIGURE 4 Doublet structure of the red chlorophyll *a* absorption band of *Chlorella pyrenoidosa* measured by Beckman DU spectrophotometer using the wet filter paper technique (20). Note the peaks at 673 mμ and 680 mμ.

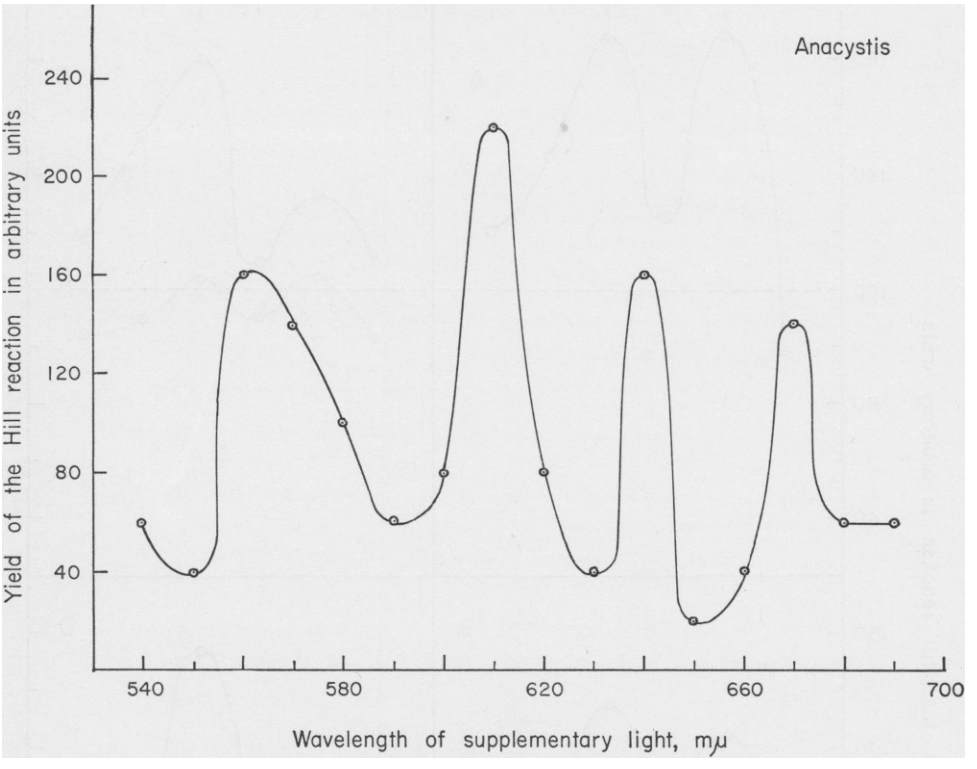

FIGURE 5 Action spectrum of the Emerson effect in the Hill reaction in *Anacystis nidulans*. Peaks due to phycocyanin, 560 mμ, 610 mμ, and 640 mμ. Peak ascribed to Chl *a* 670, 670 mμ. Action of far red light was 1.2 μl O_2/ hr./ 60 μl cells. Ratio of action of the far red light to that of the supplementary light, 1:1.5.

DeRover (23) showed that phycocyanin can serve as sensitizer for the Hill reaction; but there have been no previous data on its involvement in the Emerson effect. Fig. 5 shows, beside a peak at 670 mμ (to be ascribed to Chl *a* 670), three other peaks, at 560 mμ, 610 mμ, and 640 mμ. These peaks coincide approximately with peaks in the calculated curve showing the fraction of total absorbed light that is absorbed by phycocyanin (Fig. 6 in (9)). Analogous results were found for *Anacystis* in photosynthesis measurements (*cf.* Fig. 6 in (9), and Fig. 6 in (12)).

4. *The Negative Emerson Effect in the Hill Reaction.* It was observed in this study that the Emerson effect as measured by ϵ often falls below 100, suggesting inhibition instead of enhancement. We call this the "negative" Emerson effect. It is clearly noticeable in Figs. 3 and 5. Negative Emerson effects have been noticed previously also in photosynthesis (*cf.* Emerson and Rabinowitch (9), and Govindjee and Rabinowitch (12)). It was suggested (12, 22) that lower saturation level of photosynthesis at 700 mμ may explain the negative Emerson effect,

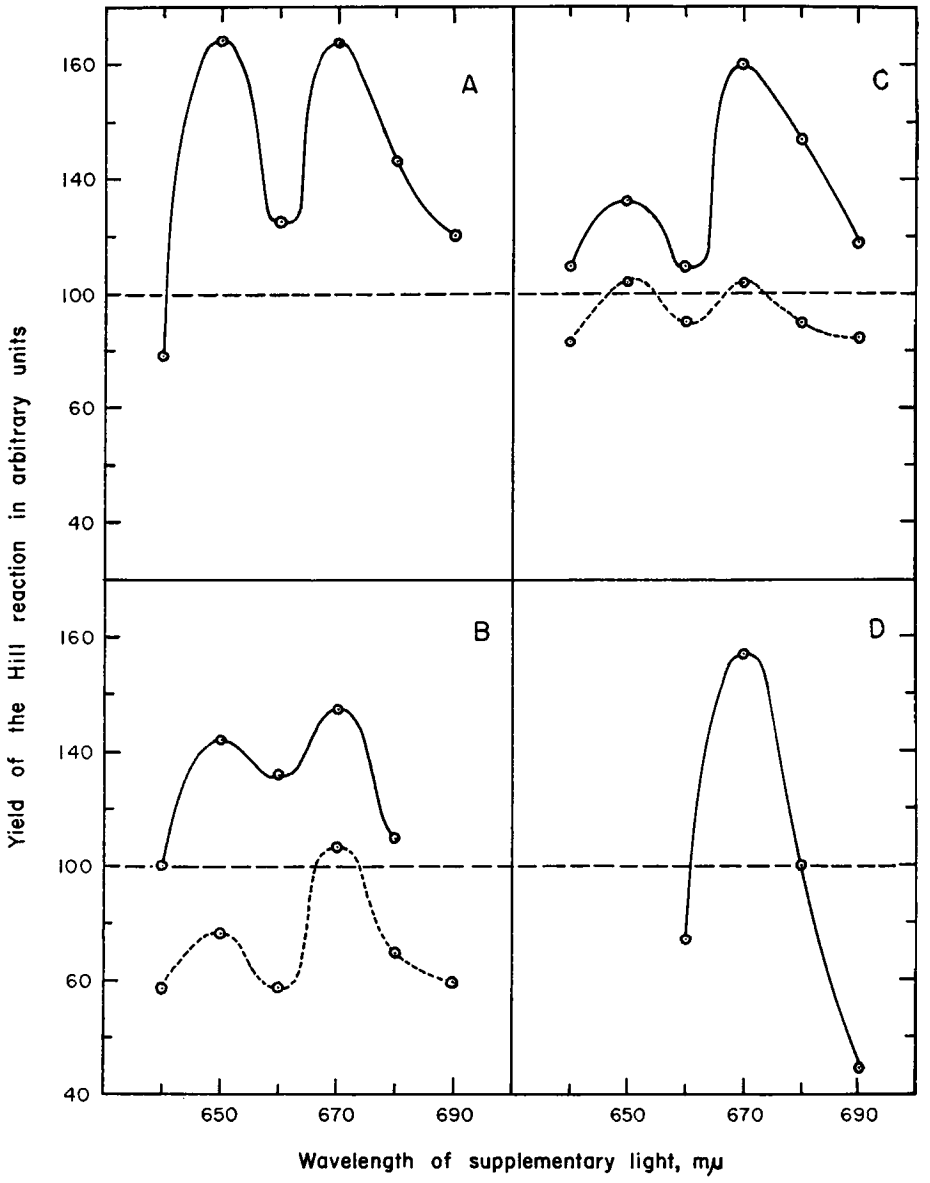

FIGURE 6 Effect of the absolute values and of the ratio of the actions of the far red and the supplementary light on the sign of the Emerson effect (solid line curves in figures *A*, *B*, *C*, and *D*.) Effect of increasing the absolute light actions keeping the ratio of the actions of far red light and supplementary light constant (Figs. *B* and *C*, dashed curves). *A*, Action of far red light, 2.0 μl 0_2 / hr. Ratio of the actions of far red and of supplementary light, 1:1. *B*, Action of far red light, solid line curve, 2.4 μl 0_2 / hr.; dashed line curve, 6.2 μl 0_2 / hr. Ratio of the actions due to far red and supplementary light, 1:2. *C*, Action due to far red light, solid curve, 2.4 μl 0_2 / hour; dashed curve 6.2 μl 0_2 / hr. Ratio of the actions of far red and of supplementary light, 1:3. *D*, Action of far red light, 4.0 μl 0_2 / hr. Ratio of the actions of far red and supplementary light, 1:4.

at least partly. For this, we must assume that when light of a wavelength at which the negative effect is observed (and which by itself, gives a normal saturation level) is added to light of 700 mμ, (whose saturation level is low), the saturation level of the combined beam remains low. This assumption remains to be proved. The same explanation obviously could apply also to the negative Emerson effect in the Hill reaction in *Chlorella.*

When the intensity of the far red light is sufficiently low, the Emerson effect is always an enhancement, $\epsilon > 100$. If the far red light intensity is raised, negative effects, $\epsilon < 100$, appear (*cf.* solid line and dashed curves in Fig. 6*B* and 6*C*). This is in agreement with the suggested interpretation. No attempt has yet been made to study the relation of the light intensity and the rate of photosynthesis in the combined beam and compare them with those of the separate monochromatic beams.

The negative Emerson effect requires further investigation before a definitive explanation can be proposed. The usual hypothesis suggests that the saturation of photosynthesis is due to a rate limitation imposed by the available amount of a certain "limiting" enzyme. It obviously needs revision because the saturation level in the 700 mμ region is different from that in shorter-wave light (12, 22). An interpretation of this phenomenon is needed before we will be able to predict what the saturation level will be in a mixed beam.

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* Govindjee in references 11, 12, 20, and 22 has no initial.