

ACTION SPECTRUM OF THE "SECOND EMERSON EFFECT"

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ABSTRACT A peak is found at 670 $m\mu$ in the action spectrum of the "second Emerson effect"¹ (22, 33), in the green alga *Chlorella pyrenoidosa*, and in the diatom *Navicula minima*; a shoulder appears in about the same location in the blue-green alga *Anacystis nidulans* and less clearly in the red alga *Porphyridium cruentum*. This peak (or shoulder) corresponds to an absorption band belonging to a form of chlorophyll *a in vivo*, which can be designated as "Chl *a* 670." Light absorption by this form can enhance the yield of photosynthesis caused by far red light (680 to 720 $m\mu$), as effectively as does light absorption by chlorophyll *b*, chlorophyll *c*, fucoxanthol, phycocyanin, or phycoerythrin. The action spectrum of the second Emerson effect in *Anacystis nidulans* shows three peaks attributable to phycocyanin, at 570, 600, and 640 $m\mu$. These correspond well to peaks on the curve, calculated by Emerson (17), which shows the fraction of total absorbed light absorbed by phycocyanin as function of wavelength. Intensity relation between the two participating beams has an important bearing on the second Emerson effect. In *Anacystis*, the "negative effect," described in (17), can be converted into a positive effect by change in this relation. In *Anacystis*, the saturation of photosynthesis occurs, at 700 $m\mu$, in weaker light and on a lower level than at 680 $m\mu$, and even more so, than at 600 $m\mu$. This may explain, at least in part, the negative Emerson effect observed in this alga.

INTRODUCTION

It has been—and still is—generally accepted that chlorophyll *a* is the primary sensitizer in photosynthesis (*cf.* Duysens (6) and Rabinowitch (32*a*)). Until recently, it was assumed that absorption of light by chlorophyll *a* is fully sufficient for photosynthesis, and that it gives a constant quantum yield throughout the spectrum. However, Emerson and Lewis (15, 16), and later Haxo and Blinks (26), noted that the quantum yield of photosynthesis drops in the far red region, well within the main red absorption band of chlorophyll *a* ("red drop"). Later, Emerson

¹ The second Emerson effect is the enhancement of the yield of photosynthesis caused by far red light, by simultaneous illumination with light of shorter wavelengths. The first one is the "burst" of carbon dioxide released by plants in the first minutes of illumination.

(7, 8) and Emerson and coworkers (10, 12, 13, 17) found that the quantum yield of photosynthesis attributable to far red light can be enhanced by supplying simultaneously substantial amounts of "supplementary" light of shorter wavelengths. ("Catalytic" amounts of supplementary light give no enhancement, contrary to the findings of Warburg (41).) Emerson (17) plotted the action spectrum of this enhancement (which became known as the "second Emerson effect") and found it to follow closely the curve showing the fraction of total absorbed light absorbed by the accessory pigments (be it chlorophyll *b* in *Chlorella*, fucoxanthol and chlorophyll *c* in *Navicula*, phycocyanin in *Anacystis*, or phycoerythrin in *Porphyridium*). Thus it appeared to Emerson (7, 8, 17) that excitation of chlorophyll *a* alone may not be enough to produce photosynthesis (or, at least, to do this with a high quantum yield); to obtain a full yield, it appeared necessary to excite simultaneously an accessory pigment, absorbing at shorter wavelengths.

The red drop begins much earlier in red than in green algae; but Emerson (7, 8, 12) noted that in this case, too, it starts when chlorophyll *a* begins to take up most (and ultimately all) of the absorbed light.

The conclusion that photosynthesis requires the excitation of an accessory pigment (in addition to that of chlorophyll *a*) seemed, however, to contradict the conclusions, drawn from experiments on sensitized fluorescence of chlorophyll *a* *in vivo* (cf. Duysens (6)), that accessory pigments contribute to photosynthesis to the extent to which their excitation is transferred, by resonance, to chlorophyll *a*. Franck (18) suggested that what is really necessary for full yield of photosynthesis, is balanced formation of two excited forms of chlorophyll *a*. Only one of these forms is obtained by absorption beyond the threshold of the red drop, while the other can be formed either by direct absorption by chlorophyll *a* at shorter wavelengths, or by resonance transfer of excitation energy from the accessory pigments. The difference between the two types of excitations is associated, according to Franck, with one part of chlorophyll *a* being present in the cells in a hydrophilic, and another part, in a hydrophobic surrounding. Lavorel (30), Krasnovsky (27, 28, 40), and Brody (3) suggested, as an alternative (or supplementary) interpretation, that the two forms of chlorophyll *a* are monomeric and dimeric, respectively. The development up to this point was summarized in a paper by Emerson and Rabinowitch (17).

French and coworkers (4, 19) confirmed, by differential spectroscopy, what was often surmised before—that several spectroscopically different forms of chlorophyll *a* exist in algae, flagellates, and leaves. Two forms of Chl *a* *in vivo* were noted also by Halldal (25) in *Anacystis nidulans*, Thomas and Marsman in *Porphyra* (39) and Thomas and Govindjee (38) and Cederstrand (5) in *Porphyridium cruentum*. Krasnovsky and coworkers (27, 28, 40) reported that two forms of chlorophyll *a* *in vivo* are photooxidized at different rates.

Govindjee and Rabinowitch (22) and Rabinowitch and Govindjee (33) reported in a preliminary note that absorption in the form "Chl *a* 670" enhances the yield of photosynthesis produced by absorption in other chlorophyll forms (Chl *a*

680 and Chl *a* 690). These results are described in more detail in the present paper. It also presents evidence concerning the "negative Emerson effect" (17), and new features of the action spectrum of the second Emerson effect in *Anacystis* and *Porphyridium*.

Rajni Govindjee *et al.* (24) recently observed the second Emerson effect also in the photoreduction of quinone by *Chlorella* cells; while Govindjee *et al.* (21) reported that the yield of fluorescence of chlorophyll *a* in a mixture of far red and blue light is smaller than the average of the yields in the two separate beams. These observations provide additional evidence of differences in the immediate consequences of excitation of the several forms of Chl *a* *in vivo*, and of their mutual interaction.

MATERIALS AND METHODS

The studied organisms were *Chlorella pyrenoidosa* Chick strain 3, *Navicula minima* Grun, *Porphyridium cruentum*, and *Anacystis nidulans*. All four species were cultured in 300 ml Erlenmeyer flasks with sealed-in tubes for passing air (or air containing 5 per cent carbon dioxide). These flasks were charged with 200 ml of culture medium, plugged with cotton, and sterilized in an autoclave for 10 to 30 minutes at 15 pounds per square inch before inoculation. Care was taken to use always the same amount of inoculum from the same stock when working with a given species. All the cultures used were single strain cultures free from bacteria and molds. Details of their growing conditions are given in Table I.

Cells were harvested and washed twice in cold (10°C) buffer (Warburg's buffer No. 11 with added NaCl for *Porphyridium*; buffer No. 9 for *Chlorella*, *Anacystis*, and *Navicula*). Cell suspensions were made up to 7 ml in their respective buffers. We adjusted them to identical optical density of 0.500 at the chlorophyll *a* absorption peak (rather than to identical cell density). Measurements were made in closed differential manometers (*cf.* Emerson and Chalmers (9), for detailed description of the method). The levels of the liquid in both arms of the manometer were read simultaneously to hundredths of a millimeter, using two cathetometers. Seven readings were taken at intervals of 1 minute in each measuring period; the first three were discarded because the steady rate was reached only after 2 minutes. The water bath was maintained at 10°C.

"Far red" light was obtained, in the earlier experiments, by means of a combination of sharp cut-off filters (Schott RG5, Schott RG8, and a Corning filter). Fig. 1 shows the resulting transmission curve. In later experiments we used instead an interference filter (Farrand No. 109556), with maximum transmission at 700 m μ (half-band width, 16 m μ).

The optical system used to isolate far red light was described by Thomas and Govindjee (see Fig. 1, (38)). "Supplementary" light was obtained from the Emerson-Lewis monochromator (f 1.5) with a 225 watt tungsten ribbon filament lamp operated at full capacity. Both the supplementary light, and the far red light were projected at the manometer vessel from below. The following sequence of readings was used in most of the experiments: *Dark, Far Red, Dark, Far Red, Supplementary, Supplementary + Far Red, Supplementary, Supplementary + Far Red*. The action of the far red light was checked at the end of each experiment; but if there was reason to think that this action

TABLE I

SUMMARY OF CULTURING CONDITIONS

	<i>Chlorella pyrenoidosa</i>	<i>Navicula minima</i>	<i>Anacyclus nidulans</i>	<i>Porphyridium cruentum</i>
Strain isolated by	Emerson	Tanada	Kratz and Allen	Lewin
Inoculum, in μ l/200 ml	15-20	100	15-20	100
Culture medium described by	Emerson and Chalmers* (11)	Tanada (36)	Kratz and Myers (29)	Brody and Emerson (1)
Illumination	4 tungsten lamps and 1 white fluorescent ring	10 white fluorescent rods	4 white fluorescent rods and 1 tungsten bulb	10 fluorescent rods. See Thomas and Govindjee (38)
Wattage of each lamp or rod	60 watt tungsten and 32 watt white fluorescent	15 watt white fluorescent	40 watt white fluorescent and 100 watt tungsten	15 watt white fluorescent
Distance from the bottom of flask	6 inches (tungsten) 4 inches (fluorescent ring)	6 inches	7½ inches	6 inches
Axis to axis distance of the fluorescent rods	Distance between the axes of the tungsten bulbs = 3 inches	1½ inches	3½ inches	2¼ inches
No. of days culture flasks were on line	2-3	3-4	2-3	6-7
Temperature of culture bath	20°C ± 2°C	25°C ± 2°C	27°C ± 2°C	19°C ± 2°C
Gas mixture	5% CO ₂ in air	Filtered room air or 5% CO ₂ in air	Filtered room air or 5% CO ₂ in air	5% CO ₂ in air
Growth (x-fold)	40	20	40	40

FOOTNOTES TO TABLE I

* $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 1.017×10^{-3} M; KH_2PO_4 : 13.79×10^{-3} M; CaCO_3 : 0.1598×10^{-3} M; KNO_3 : 12.38×10^{-3} M; Na_2HPO_4 : 0.2498×10^{-3} M; KCl : 0.335×10^{-3} M; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 2.05×10^{-3} M; A_3 : 0.2 ml/200 ml; ABC : 0.1 ml/200 ml.

[A_3 contains $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$: 1.8 gm/l; H_3BO_3 : 2.86 gm/l.]

[ABC contains 5 ml A_3 , 1 ml B_9 , and 5 ml C_{10} made up to 100 ml.]

[A_3 contains $(\text{NH}_4)_2\text{MgO} \cdot \text{O}_4 \cdot 4\text{H}_2\text{O}$: 0.188 gm/l; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.22 gm/l; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.079 gm/l.]

[B_9 : refer to Brody and Emerson (1).]

[C_{10} contains As_2O_3 : 6.61 mg/l; SrSO_4 : 10.49 mg/l; HgCl_2 : 6.77 mg/l; PbCl_2 : 6.71 mg/l; LiCl : 30.55 mg/l; RbCl : 14.2 mg/l; $\text{K}_2\text{TiF}_6 \cdot \text{H}_2\text{O}$: 5.0 mg/l; NaSeO_4 : 11.96 mg/l; $\text{Be}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$: 103.7 mg/l; uranyl nitrate : 10.0 mg/l.]

Special Care.

Chlorella. (a) The illumination was reduced to two 15 watt tungsten bulbs for the final 24 hours preceding the manometric experiments as advised by Emerson (14) to reduce the rate of respiration. (b) Culture flasks were shaken once a day to avoid settling of algae on the bottom of the flask.

Navicula. (a) On the last day, the culture flask "saw" essentially only one 15 watt fluorescent light. (b) A magnetic rod wrapped in teflon was sterilized with alcohol, dried by keeping it near a flame and aseptically introduced into the flask on the last day. The culture flask was left overnight (12 to 16 hours) on the magnetic stirrer. Both (a) and (b) were necessary to get homogeneous cultures having convenient rates of respiration for manometry.

Anacystis. Culture flasks were shaken once a day; the illumination was reduced to one 40 watt fluorescent rod on the last day.

Porphyridium. (a) On the last 2 days, illumination was reduced to a single 15 watt fluorescent rod. (b) Cultures were shaken continuously by a wrist action shaker to prevent settling of the algae and to get homogeneous cultures.

was not constant, it was checked repeatedly during the experiment. The light action of far red light in the presence of accessory light of various wavelengths was expressed in per cents of that of far red light alone. Values above 100 mean a "positive" second Emerson effect; values lower than 100 suggest a "negative" effect. The results are plotted in Figs. 2, 4, 6, 8, and 9.

Absorption measurements were done either with the integrating sphere of Cederstrand (5); or by the wet filter paper technique described by Thomas and Govindjee (38), or by a modified technique of Shibata, using oiled filter paper instead of opal glass (35).

RESULTS AND DISCUSSION

A. Different Photochemical Activity of Two Forms of Chlorophyll a in vivo. Supplementary monochromatic light used in these experiments was varied between 640 and 700 $m\mu$, by increments of 10 $m\mu$, the band width being 10 $m\mu$. Fig. 2 shows the resulting action spectrum of *Chlorella*. In the range studied, there are two sharp peaks: at 650 and 670 $m\mu$. The former was noted by Emerson and coworkers (10, 17) and ascribed to chlorophyll *b*; it was also observed by

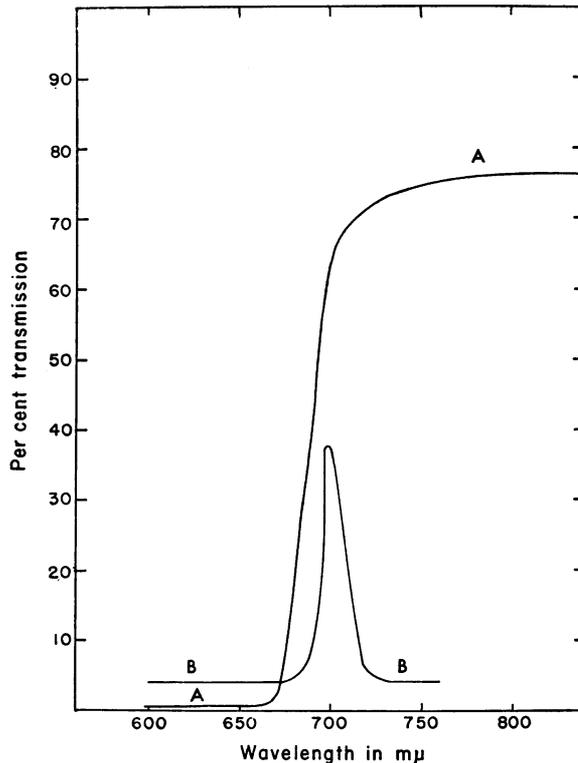


FIGURE 1 *A*, transmission curve of the combination of Schott glass filters RG5, and RG8, and a red Corning filter. *B*, transmission curve of a Farrand interference filter (No. 109556) used to produce far red light ($\sim 700 m\mu$).

Myers and French (31). The peak at 670 $m\mu$ was not noted before. The ratio of the heights of the two peaks changed from culture to culture (between 0.86 and 1.20) probably owing to variations in the relative amounts of chlorophyll *b* and chlorophyll *a* 670.

When the absorption spectra of the same cultures were measured, two peaks, which must be both due to chlorophyll *a*, could be seen, at 670 to 672 $m\mu$ and at 678 to 680 $m\mu$ respectively (Fig. 3).

These results provide clear evidence of the existence and a specific photochemical function of a form Chl *a* 670 *in vivo*. This function is not shared by Chl *a* 680. (There is evidence of the existence of a third form, Chl *a* 690; but our action spectra did not permit us to differentiate between the contributions to photosynthesis due to Chl *a* 680 and Chl *a* 690. In order to obtain a full yield of photosynthesis

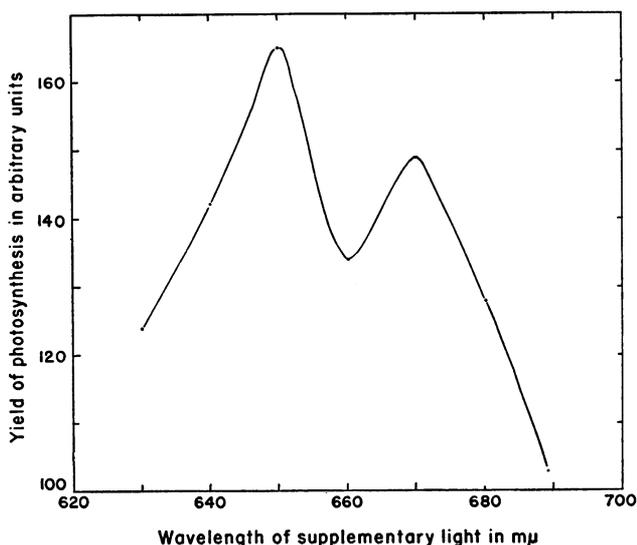


FIGURE 2 Action spectrum of the second Emerson effect in *Chlorella pyrenoidosa*. Light action of far red light alone is taken as 100. Light action of the far red light alone was 3.0 $\mu\text{l}/\text{hour}$ and the ratio of far red:supplementary light actions was 1:1. Far red light was $> 680 m\mu$. Similar results were obtained by using light that was $\sim 700 m\mu$.

from excitation of Chl *a* 680, the Chl *a* 670 must be also excited in appropriate proportion, and failure to do so is the reason for the red drop. It can be postulated that when Chl *a* 670 alone is excited, it transfers enough energy to Chl *a* 680 to achieve balanced excitation of both forms; but the reverse transfer is negligible for energetic reasons. Therefore, as soon as quanta become preferentially absorbed by Chl *a* 680, the quantum yield begins to decline (*cf.* Franck (18) and Emerson and Rabinowitch (17)).

After having observed the 670 $m\mu$ peak, we reexamined the original curves of Emerson and coworkers and noticed that Emerson's figure for *Chlorella* (17) already suggested the existence of this peak.

Fig. 4 shows the action spectrum of *Navicula minima*. We clearly recognize a peak of 670 $m\mu$ (Chl *a* 670), in addition to the peaks at 630 $m\mu$ (Chl *c*) and 540 $m\mu$ (fucoxanthol). The position and shape of the fucoxanthol band in the action spectrum should be analyzed in terms of relative contribution of this pigment to the total light absorption by the cells at different wavelengths. Emerson's calculation (17) suggests only one peak in the latter curve, at 535 $m\mu$; but this result cannot be reliable because of the strong change the absorption curve of fucoxanthol undergoes *in vivo* compared to its shape *in vitro*.

The absorption spectrum of *Navicula* (Fig. 5) again shows evidence of two peaks attributable to chlorophyll *a*. The ratio of the optical densities due to Chl *a* 670 and Chl *a* 680 appears higher in *Navicula* than in *Chlorella*; it remains to be seen whether this is a general rule, or only applies to the cultures used by us.

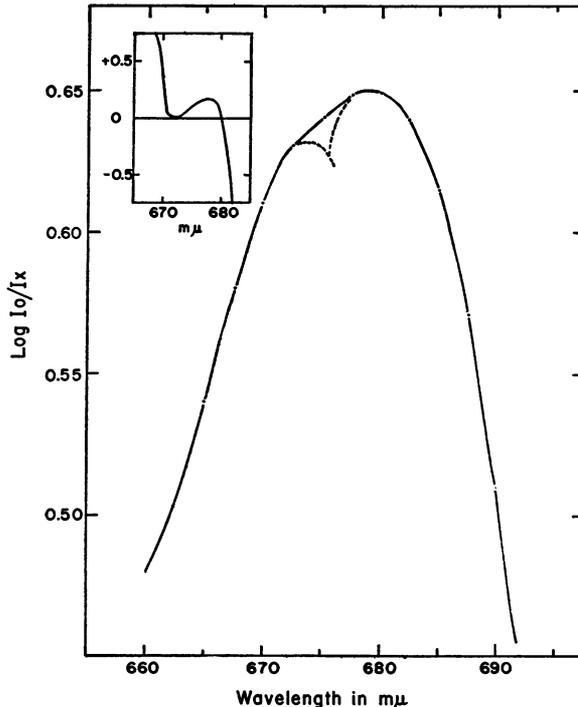


FIGURE 3 Absorption spectrum of a culture of *Chlorella pyrenoidosa*, measured by Beckman spectrophotometer using the oiled filter paper technique. Dotted lines are drawn to emphasize the existence of two peaks of chlorophyll *a*. Insert shows a first derivative absorption spectrum of *Chlorella* (a different culture). All samples measured showed a double peak of chlorophyll *a*.

Fig. 6 represents the action spectrum of the second Emerson effect in the blue-green alga *Anacystis nidulans*. The main peak is clearly due to phycocyanin; the results in the chlorophyll absorption region are not very clear, and vary from culture to culture. In nearly all experiments with this alga, at least a shoulder could be noted in the 670 to 680 $m\mu$ region. In one experiment, (Fig. 6C) a peak was present at 665 $m\mu$, in another (Fig. 6D) at 675 $m\mu$. This suggests that the Chl *a* 670

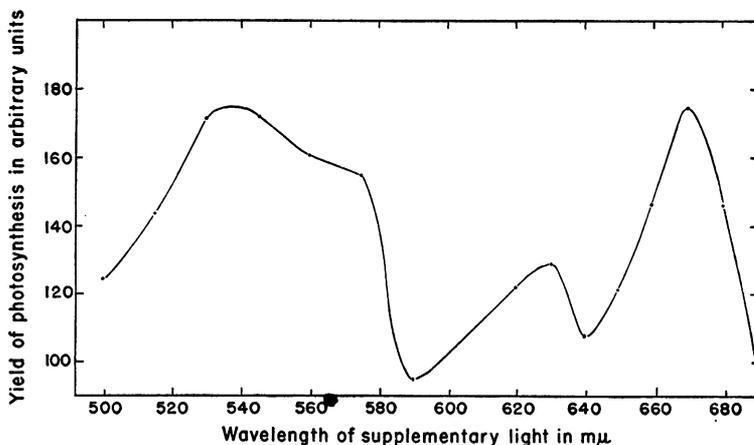


FIGURE 4 Action spectrum of the second Emerson effect in *Navicula minima*. Light action of far red light alone is taken as 100. Measurements were made only in the region 600 to 700 $m\mu$; the action spectrum of Emerson (17) is shown from 500 $m\mu$ to 600 $m\mu$ (we have confirmed the approximate shape of this section). Light action of the far red light alone was 8.0 μ l/hour and the ratio of far red light to supplementary light actions was 1:2. Far red light was $> 680 m\mu$. Similar results were obtained by using light that was $\sim 700 m\mu$.

form may exist and be active also in this species. The variability of the action spectrum of *Anacystis* in this region (670 to 700 $m\mu$) may be related to the fact that *Anacystis* often shows in this region a negative Emerson effect (to be discussed later); but the nature of the relation we do not yet understand.

Fig. 7 shows the absorption spectrum of *Anacystis nidulans*. In addition to the phycocyanin maximum at 627 $m\mu$, it shows an apparently triple band of chlorophyll *a*, with peaks located at approximately 672, 675, and 680 $m\mu$. However, not in all cultures was such a triple peak observed.

Fig. 8 represents the action spectrum of the red alga *Porphyridium*; it shows a less clear shoulder at about 675 $m\mu$.

Red algae, such as *Porphyridium*, exhibit a red drop much earlier than *Chlorella*, *Navicula*, and *Anacystis* (at $\sim 650 m\mu$ instead of at $\sim 680 m\mu$). Despite the early red drop, Brody and Emerson (2) found, in *Porphyridium*, measurable photosynthesis in monochromatic light even at 680, 690, and 700 $m\mu$ (confirmed by us). The reason for earlier drop may be that the ratio of absorptions by Chl *a* 670 and Chl *a* 680 in these organisms falls earlier than in *Chlorella* below the minimum

required for full photosynthesis. This hypothesis should be checked by quantitative analysis of the red absorption bands of the different algae and calculation of the relative contributions of several Chl *a* components to the total absorption. The amount of Chl *a* 670 in red algae may be sufficient to serve as acceptor for energy transfer from auxiliary pigments, but not enough for significant direct absorption. The absorption spectrum of *Porphyridium* (see Figs. 2, 4 (38)) suggests a doublet nature of the chlorophyll *a* band in this alga.

Alternatively, the difference in the position of the red drop may be due to spatial separation of Chl *a* 670 and Chl *a* 680 in *Porphyridium*, with phycoerythrin being closely associated with "Chl *a* 680" (along the lines of Duysens' original theory).

Phycocyanin is not prominently present in *Porphyridium*, but there is indication in our experiments of its presence in the existence of several peaks in the 600 to 660 m μ region in the action spectrum of the second Emerson effect (Fig. 8). However, the amount is too small to raise the yield to its maximum value; otherwise, the red drop in *Porphyridium* would have occurred in the same region as in *Anacystis*, (*i.e.* at 680 m μ).

B. Photochemical Activity of Phycobilins in the Second Emerson Effect.

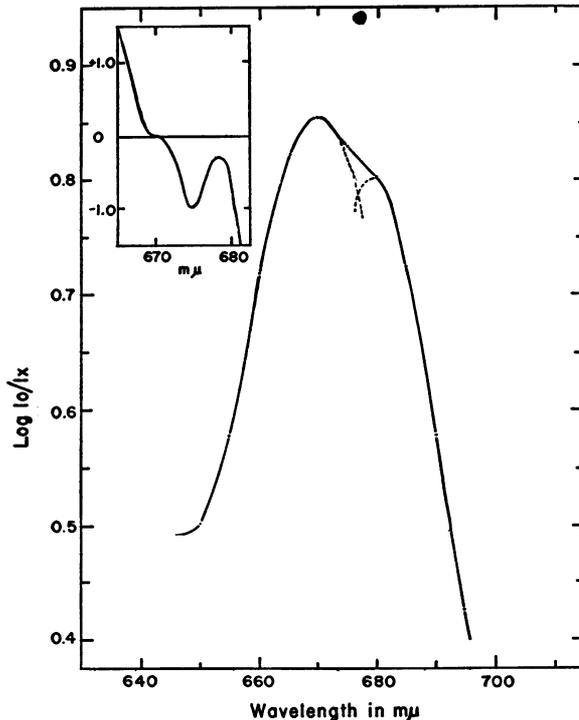


FIGURE 5 Absorption spectrum of *Navicula minima* obtained with Cederstrand's integrating sphere spectrophotometer. Dotted lines are drawn to emphasize the presence of two peaks of chlorophyll *a*. Insert shows a first derivative absorption spectrum of *Navicula*. All samples measured showed a double peak of chlorophyll *a*.

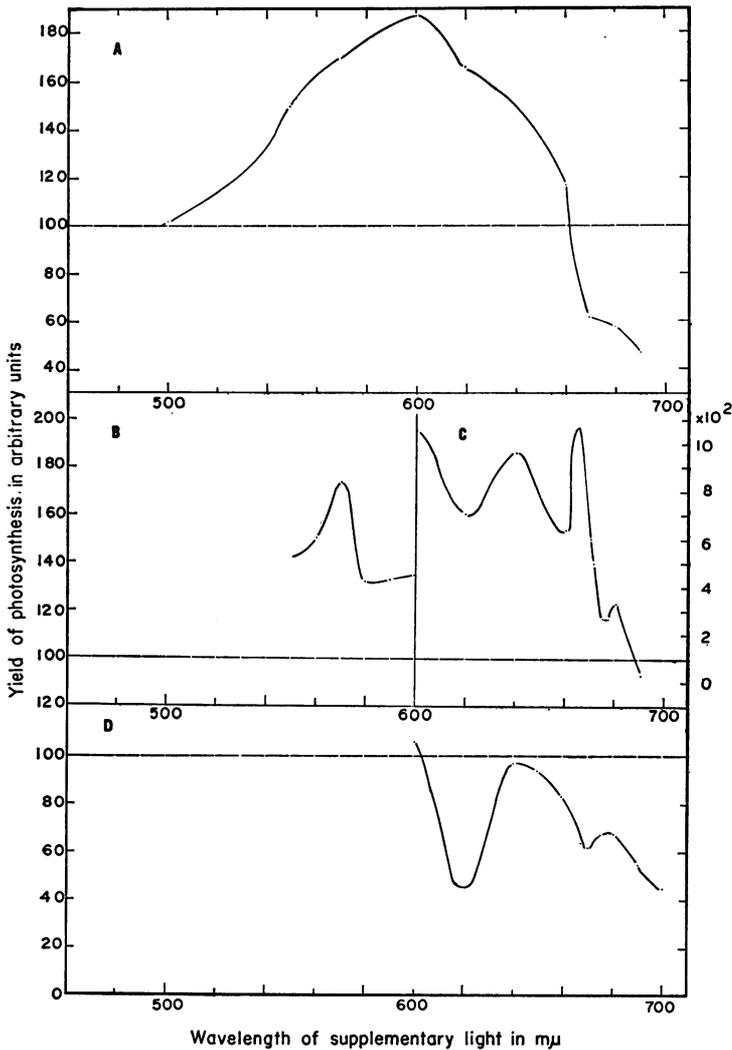


FIGURE 6 Action spectra of the second Emerson effect in *Anacystis nidulans*. *A*, intensity of far red light comparable to that used by Emerson (10, 17). Light action of the far red light alone was $8.5 \mu\text{l}/\text{hour}$ and the ratio of far red to supplementary light actions was 1:1. Curve drawn to suggest a triple peak, although this particular experiment does not justify it. Note points which confirm Emerson's findings. *B*, a different intensity of far red light; note peak at $570 \text{ m}\mu$. Light action of far red light alone was $1.5 \mu\text{l}/\text{hour}$ and the ratio of far red to supplementary light actions was 1:2. *C*, intensity of far red light lower than in *A*, *B*, and *D*. Light action of far red light alone was $0.25 \mu\text{l}/\text{hour}$ and the ratio of far red to supplementary light actions was 1:20. Nearly all values are positive; note peaks at 640 and $665 \text{ m}\mu$. *D*, intensity of far red light higher than in *A*, *B*, and *C*. Light action of far red light alone was $15.0 \mu\text{l}/\text{hour}$ and the ratio of far red to supplementary light actions was 1:1. All values are negative, and there are peaks at 640 and $675 \text{ m}\mu$. (Scale of ordinates is different for different curves. Far red light was $\sim 700 \text{ m}\mu$.)

Fig. 6 shows the action spectrum of the second Emerson effect in *Anacystis nidulans*. It confirms the conclusions of Emerson and coworkers that light absorbed by phycocyanin (or rather, by the several phycocyanins) can raise the yield in far red light. The phycocyanin peak is not single but often triple (Fig. 6), corresponding to three maxima in the curve showing the fraction of light absorbed by phycocyanin as function of wavelength (17). These peaks lie at 570, 600, and 640 m μ . In *Porphyridium* (Fig. 8) we noted in two experiments a new peak (not noted by Emerson and coworkers) at 650 m μ . It cannot be due to Chl *b*—which is absent—but may be due to phycocyanin or allophycocyanin. The height of this peak relatively to that of the peak at about 510 m μ (not shown in Fig. 8; cf. Emerson (10,

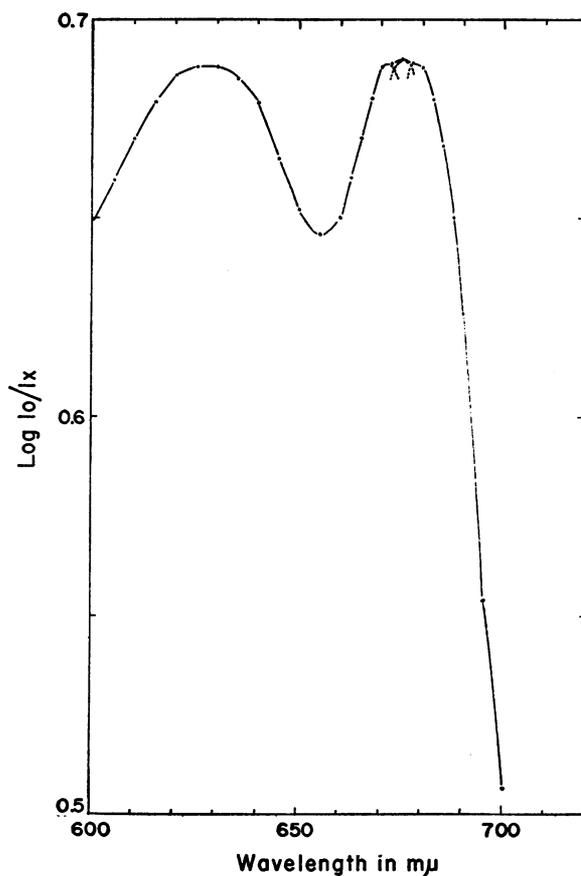


FIGURE 7 Absorption spectrum of *Anacystis nidulans* measured by Beckman spectrophotometer, using oiled filter paper technique. The spectrum was confirmed by wet filter paper technique. All samples did not show the triple peak. Some samples had two peaks and some appeared to have only one. This may be due to variability in the relative amounts of the three forms in different samples.

17)) is usually 1:6. We do not know the exact absorption spectrum of the phycocyanin in *Porphyridium* and therefore cannot calculate the fraction of light absorbed by this pigment. The 650 m μ peak was not observed in two other experiments on *Porphyridium*; this could be due to the very low concentration of phycocyanin in some cultures.

C. Negative Emerson Effect. The action spectrum of *Chlorella* (Emerson and Rabinowitch (17); and Fig. 2) shows no negative effects in *Chlorella*. However, some of our experiments with this alga did show a negative effect when 680 to 700 m μ supplementary light was used (see Fig. 9). We asked ourselves whether it could be associated with an early saturation of photosynthesis in 700 m μ

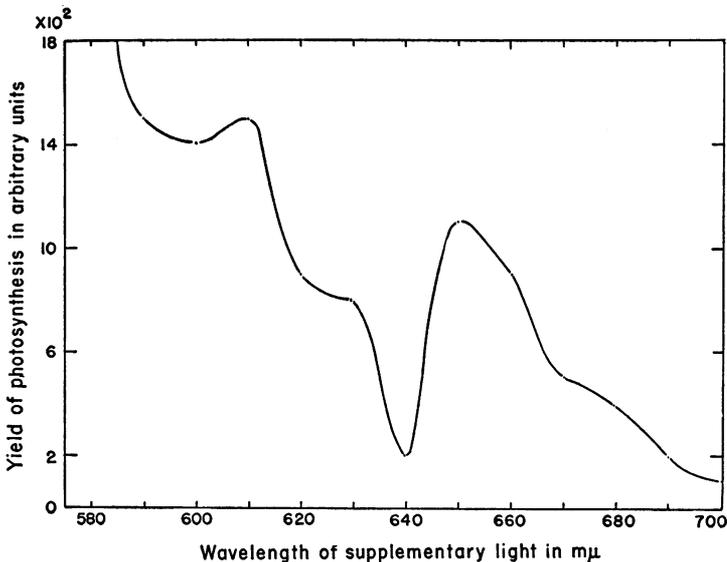


FIGURE 8 Action spectrum of the second Emerson effect in *Porphyridium cruentum*. Intensity of far red light used in this experiment was very low. Only two experiments of this type were made. Light action of the far red light alone was 0.09 μ l/hour and the ratio of far red to supplementary light actions was 1:60. Far red light was \sim 700 m μ .

light. It was at first difficult to believe that saturation could occur, at certain wavelengths, below compensation; but this turned out to be the case. Data in the literature did not indicate (*cf.* Rabinowitch (32*b*)) that saturation levels can vary with wavelength. However, preliminary results with *Anacystis nidulans* (33) and the findings of French (20) with *Chlorella*, revealed that the saturation levels at 700 m μ are much lower than at 680 m μ ; while the saturation levels at 600 m μ are much higher than at 680 m μ (Fig. 10). The negative effects could be generally associated with early light saturation of photosynthesis in one of the two beams. We noticed that by reducing the intensity of far red light, a positive Emerson effect can be

obtained in the spectral region where negative effects were observed at the higher intensities (*cf.* Table II and Fig. 6 for *Anacystis*).

Rajni Govindjee (unpublished data), working on the second Emerson effect in the Hill reaction, also found that by reducing the light intensity in the region where the initially observed effect was negative, its sign could be changed.

Relation of the negative Emerson effect to light saturation is plausible. Owing to early saturation, addition of a certain amount of far red light (700 $m\mu$) to background light of the same wavelength, naturally causes a negative effect (*i.e.*, the total light action is smaller than the sum of the light actions of the two separate beams). It seems plausible that the same effect may result from combination of two beams of different wavelength, one of which is characterized by early saturation. However, if the two beams have different primary photochemical functions—and this is what we have postulated as our working hypothesis—this conclusion becomes uncertain. Obviously, a satisfactory explanation would have to begin with explaining how the saturation level of photosynthesis can be different in light of different wavelengths, since this fact contradicts the simple hypothesis that saturation level of photosynthesis is the measure of the maximum rate of a certain physical process or enzymatic reaction unrelated to the primary photochemical process.

The discovery (23, 34, 37, 38) of the inhibitory effect of extreme red light (740

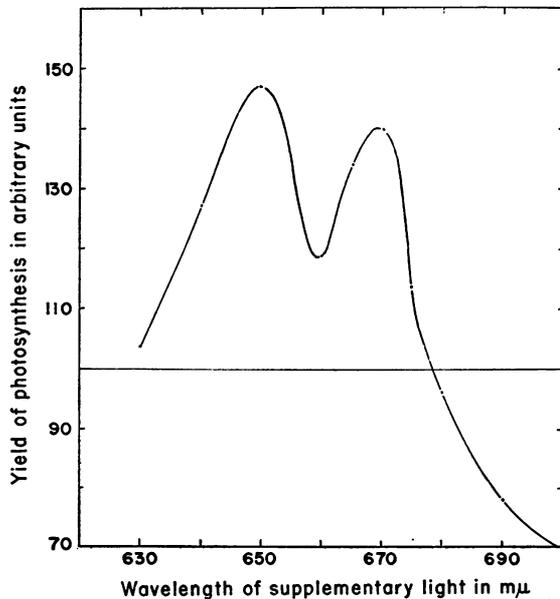


FIGURE 9 Action spectrum of second Emerson effect in *Chlorella pyrenoidosa*. Note negative Emerson effect in 680 to 700 $m\mu$ region. Light action of the far red light alone was 15.0 μ l/hour and the ratio of far red to supplementary light actions was 1:3. Far red light was \sim 700 $m\mu$.

TABLE II
CHANGE FROM POSITIVE TO NEGATIVE EMERSON EFFECT
WITH INCREASING LIGHT INTENSITY

A	B	C	D = C - B	A:B	100 × D/A
Light action of far red light alone	Light action of supplementary light (680 mμ)	Light action of far red light + 680 mμ light given simultaneously	Light action of far red light in presence of supplementary light	Ratio of far red light action: Supplementary light action	effect (Far red light action alone = 100)
μl/hr.	μl/hr.	μl/hr.	μl/hr.		
1.89	2.34	5.52	3.18	1:1.2	168
3.42	2.16	5.22	3.06	1:1.0	90
7.38	7.64	13.50	5.86	1:1.10	80
9.90	13.68	20.15	6.47	1:1.4	66
12.24	13.86	21.80	7.94	1:1.11	65
18.00	19.80	26.80	7.00	1:1.10	39

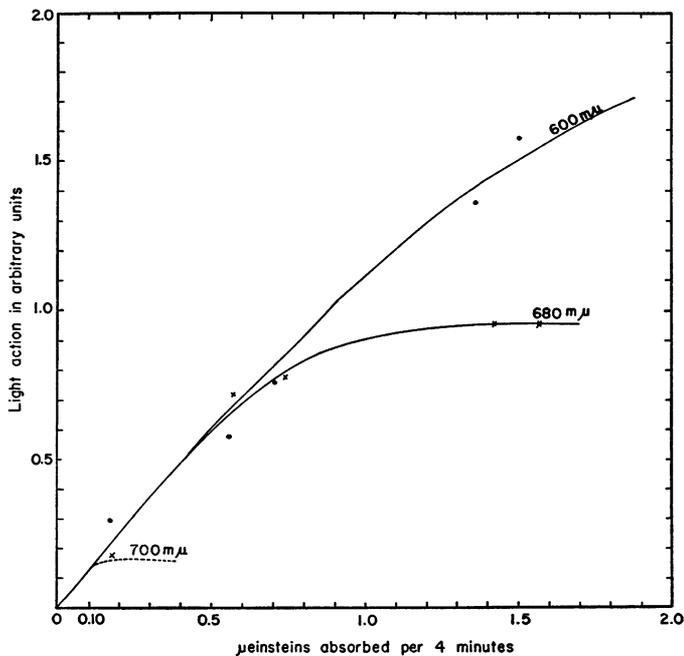


FIGURE 10 Light curves of *Anacystis nidulans* in light of 600, 680, and 700 mμ. Different saturation levels are approached at these three wavelengths. Initial slope of 700 mμ curve could not be determined with certainty. Saturation at this wavelength was reached at a very low level as indicated by dotted line. Abscissae are energies in μeinsteins per 4 minutes per 14 cm².

to 750 m μ) on the rate of photosynthesis in far red light (680 to 700 m μ) suggests a possible relation between this effect and the negative Emerson effect observed when monochromatic light > 670 m μ is added to "far red" light obtained by means of sharp cut-off filters (and therefore containing some of the "inhibitory extreme red" light).

The negative Emerson effect obviously requires further study.

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