CHANGES IN QUANTUM YIELD OF PHOTOSYNTHESIS IN THE RED ALGA *Porphyridium cruentum* CAUSED BY STEPWISE REDUCTION IN THE INTENSITY OF LIGHT PREFERENTIALLY ABSORBED BY THE PHYCOBILINS

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ABSTRACT This paper describes the relation between the quantum yield of photosynthesis in the red alga *Porphyridium cruentum*, and the spectral composition of light, changed by filtering white light through aqueous phycobilin solutions of increasing optical density. At sufficiently high densities of the filter solution, no measurable photosynthesis can be observed, although chlorophyll *a* molecules are still being excited at a significant rate, as can be proved by calculations from spectral distribution curves, and is confirmed by the occurrence of a "second Emerson effect" upon addition of orange light. An interpretation of this result, based on other experiments, will be given in a subsequent paper. A modification of the opal glass technique for reducing the effect of scattering when measuring absorption, was developed in connection with this research, and also is described in the paper.

INTRODUCTION

In the red region, the quantum yield of photosynthesis drops with increasing wavelength of the actinic light (*cf.* Emerson and Lewis (6); Haxo and Blinks (17)). Emerson and coworkers (1, 4, 8-11) found that this low quantum yield can be raised to approximately normal values by exciting, in addition to chlorophyll *a*, one or more of the auxiliary pigments. This phenomenon, known as the "second Emerson effect," has been interpreted by Emerson as evidence of a need for cooperation between the excited molecules of an auxiliary pigment and those of chlorophyll *a*. Ef-

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fective auxiliary pigments include chlorophyll *b*, phycobilins, and, among the carotenoids, at least fucoxanthol (*cf.* Emerson and Rabinowitch (11)).

According to Govindjee and Rabinowitch (15), light absorption in one of the chlorophyll *a* forms *in vivo*—the form which has an absorption peak at 670 mμ (chl *a* 670)—can complement, in green and brown algae, the light absorption above 680 mμ as effectively as light absorption in chlorophyll *b*. This suggests that what is really needed for high quantum yield of photosynthesis, is balanced excitation of two forms of chlorophyll *a*. In other words, chl *a* 670 acts as an "auxiliary pigment" to some other chlorophyll *a* form (or forms) (chl *a* 680 or chl *a* 690). Absorption above 680 mμ leads (in green algae) mainly or exclusively to one type of excitation, and therefore to a low quantum yield; the other type of excitation can be obtained either directly, by absorption in chl *a* 670, or indirectly, by energy transfer from an auxiliary pigment to chl *a* 670.

According to Emerson and Chalmers (10), the extent of short wave stimulation of the quantum yield in far red light depends on the intensity ratio between the "short-wave light" and the "long-wave light." This ratio must exceed a certain minimum value in order to produce any effect at all.

As to the nature of the two types of excitation, Franck (5, 12), suggested (*a*) excitation to the metastable triplet state, and (*b*) excitation to the fluorescent singlet state. Which excited state is obtained, depends on whether the chlorophyll molecules find themselves in a hydrophilic medium, in which case the primary excited fluorescent singlet state lives long enough to contribute to the photochemical process, or in a lipophilic medium, in which case the fluorescent state is converted practically instantaneously into the triplet state.

Another alternative was suggested by Lavorel (18) and S. Brody (2); namely, that the two types of excitations are those of chlorophyll *a* in the monomeric state and in the dimeric state, respectively. The first excited state is fluorescent, and the second non-fluorescent (probably because of immediate conversion into the metastable triplet state).

Emerson's measurements (6) were not extended far enough into the infrared to decide whether the yield of photosynthesis in the far red wing of the chlorophyll *a* absorption band declines to zero, or to a finite low value. These measurements ended at 700 mμ, where the quantum yield had a value of about 0.01; while chlorophyll *a* absorbs, *in vivo*, up to about 720 mμ. If the quantum yield could be reduced to zero while the absorption by chlorophyll *a* still remained finite, one could conclude that chlorophyll *a* (or, at least, the form of chlorophyll *a* responsible for absorption in the far red wing of the absorption band) is, in itself, entirely incapable of photosynthesis.

We tried to approach this problem by illuminating algae with white light, from which the wavelengths preferentially absorbed by the auxiliary pigments could be gradually removed. The red alga *Porphyridium cruentum* was chosen as object. Since red algae contain no chlorophyll *b*, the absorption bands of their major aux-

iliary pigments, the phycobilins, are well separated from those of chlorophyll *a*. It is therefore possible to vary the ratio of the amount of light absorbed by the auxiliary pigments to that absorbed by chlorophyll *a* by small increments over a wide range, with the help of light filters consisting of phycobilin extracts of increasing concentration. The results of this study are described below. For a preliminary note, see (21).

MATERIALS AND METHODS

Preparation of Algae. *Porphyridium cruentum* was grown in the medium described by M. Brody and Emerson (3) at about 22°C over ten 15 watt flourescent light tubes located about 15 cm below the bottom of the flasks. An inoculum of about 100 μl of cells in 200 ml of the medium, contained in 300 ml culturing flasks, was used. Air containing 5 per cent carbon dioxide was bubbled through the cultures, which were continuously shaken. In order to reduce respiration, the light intensity was decreased during the last 2 days previous to the experiment, by using only two out of the ten fluorescent tubes. After 6 or 7 days of culturing, the cells were harvested by centrifugation and washed twice in Warburg buffer No. 11, with NaCl added in the following proportion: 16 gm $NaHCO_3$ + 1 gm Na_2CO_3 + 15.2 gm NaCl, in 1 liter of glass-distilled water. The suspension was diluted by the same medium, to a concentration giving about 30 per cent absorption of white light in 1 cm path.

Preparation of Phycobilins for Filters. Large amounts of aqueous phycobilin extracts were prepared from *Porphyra perforata.*[1] The fronds were rinsed with tap water, washed, and stored in distilled water, at room temperature in the dark for 5 or more days. The cells died during this period, and released the major part of the phycobilins, but no chlorophyll or carotenoids. By decanting this extract, washing the algae with distilled water once more, and storing them under the same conditions for 5 more days, an extract of phycoerythrin free from phycocyanin could be obtained.

The extracts were filtered through a Seitz filter, using filter sheets No. 3. Next, following Fujiwara (14), the phycobilins were precipitated by adding a solution containing 44 weight per cent of reagent-grade ammonium sulfate. After filtration, the precipitates were washed with the same ammonium sulfate solution and redissolved in glass-distilled water. In this way, highly concentrated pigment solutions could be obtained. They were dialyzed against glass-distilled water for at least 12 hours. Some grayish white precipitation was formed, and removed by centrifugation. The clear, intensely colored solutions were stored in a refrigerator. Upon standing, more grayish white precipitate was formed. Therefore, the pigment solution was recentrifuged before each experiment. In light, the extracted pigments showed some bleaching. Hence, optical density had to be checked before each experiment.

Phycocyanin extracts were prepared from the blue-green alga *Anacystis nidulans* by supersonic treatment and subsequent removal of chlorophyll by fractional precipitation with ammonium sulfate. They were stored in the refrigerator and repeatedly checked for absorption changes.

Calibration of Filters. The required densities of the "phycobilin filter" were obtained either by diluting the original preparation, or by using several cuvettes containing undiluted solution. The filters were placed in the actinic white light beams. For

[1] Our thanks are due to Dr. L. R. Blinks for kindly providing us with these algae.

practical reasons, zero order light passed through the Emerson grating monochromator was used. A 225 watt ribbon filament lamp served as light source. If necessary, the light intensity could be reduced by wire screens. Care was taken to weaken the non-absorbable infrared radiation by using a 6 inch water filter together with two American Optical Company heat-absorbing glass filters; an approximate correction was applied for the residual amount of radiation beyond 720 mμ. This correction was determined by plotting the product of the emission curve of the lamp and of the transmission curve of the filters, and computing the area of the graph at wavelengths exceeding 720 mμ in per cent of the total area from 400 to 2000 mμ. For white light, the correction was 37 per cent. A similar factor was determined also for light transmitted by each of the phycobilin filters used. Orange "background light" could be supplied by a 1000 watt air-cooled lighthouse incandescent lamp, run at about 700 watts and provided with two heat-absorbing filters in

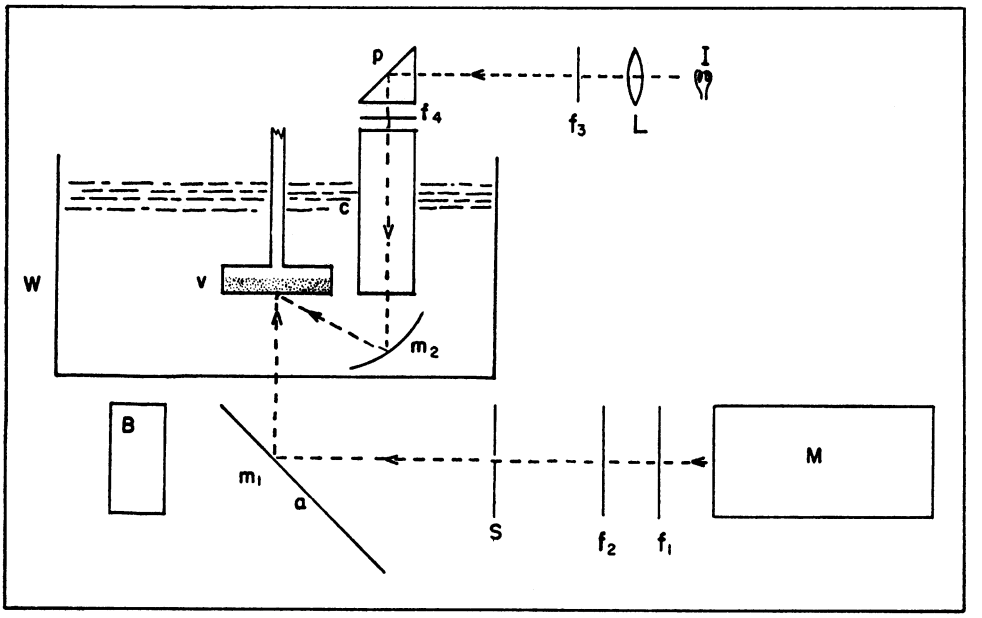

FIGURE 1 Scheme of the optical system. *M*, monochromator; f_1 and f_3, *infrared*-absorbing filters; f_2, "phycobilin filter"; f_4, orange filter; *S*, exit slit; m_1, glass mirror, movable around axis *a*; *B*, bolometer; *W*, water bath; *v*, vessel with algal suspension; m_2, stainless steel mirror; *c*, lucite column; *p*, reflecting prism; *L*, lens, *I*, lamp.

addition to a 3 mm thick Corning glass filter No. 3480. A diagram of the arrangement is shown in Fig. 1. Light energy was measured with a bolometer as described by Emerson and Chalmers (7). True (*i.e.*, scattering-free) light absorption of the algal suspension in white light, as well as in light transmitted by the phycobilin filters, was determined in an integrating sphere instrument, built by Mr. C. Cederstrand in our laboratory, which will be described in a separate paper.

Measurement of Oxygen Liberation. Photosynthetic oxygen evolution was measured in the differential manometer described by Emerson and Chalmers (7). The experimental vessel contained 7 ml of cell suspension. The temperature was 10°C. Readings were made every minute. As a rule, each measuring period included 8 readings; the first 3

were discarded and a mean value was computed from the next 5. If the readings were not stationary after a 3 minutes' adjustment period, this period was extended until the measurements were constant, and the 5 subsequent readings were used. At least 4 such sets of measurements were made in each case (2 in darkness, alternating with 2 in light) to calculate the light action. Mean errors were computed according to the formula:

$$\text{M.E.} = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Determination of Absorption Spectra. A modification of the opal glass or oiled filter paper technique of Shibata *et al.* (19), was developed to measure the absorption spectrum of algal suspensions. Whatman filter paper No. 1 was cut into strips which just fitted into 1 cm wide Beckman absorption cuvettes. One strip was soaked in a dense

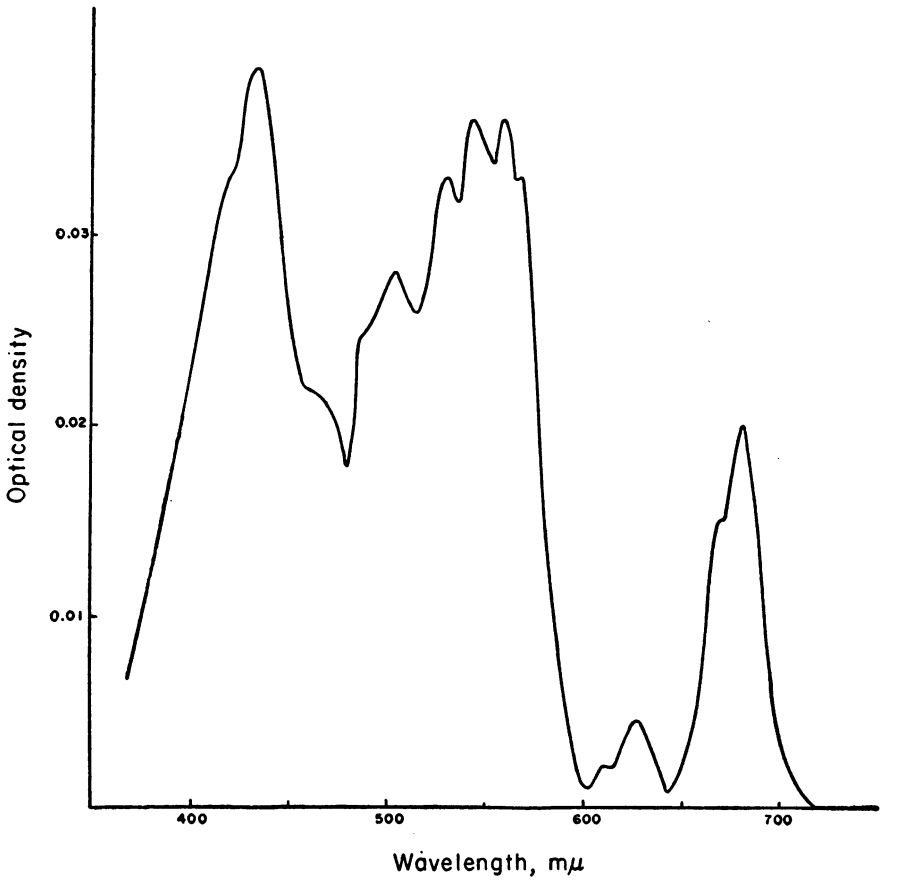

FIGURE 2 Absorption spectrum of *Porphyridium cruentum.*

cell suspension and adjusted to the inner side of the wall of the empty cuvette facing the phototube. One or two drops of the suspension were added at the bottom of the cuvette in order to prevent evaporation. Then, the cell was closed. The meniscus of the liquid

layer was well below the light beam. A cuvette containing a similar paper strip soaked with distilled water (or culture medium) was used as a blank. The scattering by the filter paper strips varied slightly. Since measurements in the integrating sphere showed that the absorption of *Porphyridium* is zero at 800 mμ, all experimental curves were shifted, up or down, so as to make the absorption value at 800 mμ equal to zero. This served as an approximate correction for variations in the scattering power of filter strips. Clearly, this method cannot be used for quantitative purposes; but it enables detailed spectral characterization with only a few drops of a highly scattering suspension (*cf.* Fig. 2).

RESULTS AND DISCUSSION

Absorption Spectrum of Porphyridium cruentum. The absorption spectrum of a sample culture of *Porphyridium cruentum,* obtained in the above described manner, is shown in Fig. 2. As evident from the absorption around 625 mμ,

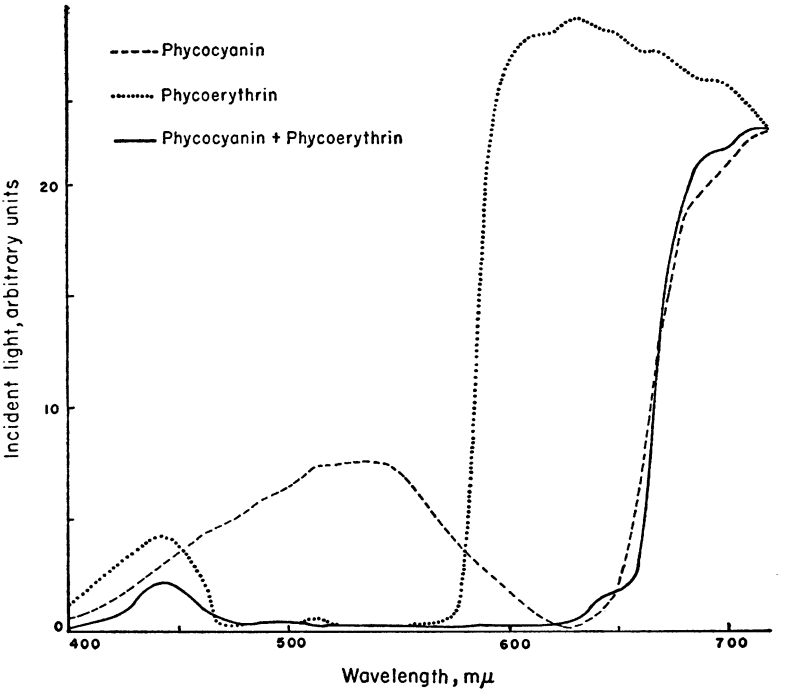

FIGURE 3 Composition of actinic light after passing an aqueous solution of phycocyanin from *Anacystis nidulans,* a solution of phycoerythrin from *Porphyra perforata,* and a mixture of both phycobilins from the latter species.

Porphyridium cruentum contains only a very small amount of phycocyanin. (Part of the absorption in this region is due to a minor band of chlorophyll *a.*)

A distinct shoulder can be noted in Fig. 2 on the short wave side of the main

chlorophyll *a* band. In this case, the shoulder and the main maximum are at 670 and 682 mμ, respectively; in other cultures, their locations varied between 670 and 674 mμ for the first, and 678 and 682 mμ for the second. The occurrence of such a shoulder in the red absorption band of chlorophyll *a in vivo* has also been noted and discussed by Halldal (16), French and Young (13), and Thomas and Marsman (20). Leaving aside specific interpretation of the two forms of chlorophyll *a in vivo,* which must be postulated to account for the two bands, we will designate them, regardless of the exact location of the absorption bands, as chl *a* 670 and chl *a* 680, respectively.

Transmission Characterization of the Filters. Fig. 3 shows the composition of the light transmitted by "phycobilin filters" of the highest density used. The product of the emission curve of the lamp and the per cent transmission curve of a filter was plotted for this purpose *versus* wavelength. With the phycoerythrin filter, the incident light consists mainly of wavelengths longer than 580 mμ, and of a weak band around 440 mμ. With the phycocyanin filter, this light is composed of wavelengths longer than about 650 mμ, plus a broad band with a maximum at 520 to 540 mμ, in the region where phycoerythrin absorbs most strongly. With both phycobilins, the incident light consists nearly exclusively of wavelengths longer than about 660 mμ. If the energy of distribution of the latter is multiplied by the absorption coefficient of chlorophyll *a* in *Porphyridium,* (*cf.* Fig. 4), it becomes evident that chl *a* 680 is excited by this light much more efficiently than chl *a* 670.

Quantum Yield in Relation to Filter Density. Fig. 5 shows the results of six experiments on the relation between the per cent light transmission by "mixed phycobilin" filters of various optical densities, and the quantum yield of photosynthesis, expressed in per cent of that in white light. The plotted mean values are obtained by averaging the results of six single experiments. The vertical lines show the range of variations of the individual results. Since differences in photosynthetic activity of the studied cultures are coresponsible for this scattering of results, the errors are, as a rule, higher than those calculated for each single experiment. For pressure changes of 10 μl/hour or more, the variation did not exceed 3 per cent.

It is apparent from Fig. 5 that the relationship between the quantum yield and the percentage of white light transmitted by the phycobilin filters of various densities cannot be described by a single linear function. As the transmission is decreased from 100 to 80 per cent, ϕ does not change. Reducing the transmission further, a linear decrease of ϕ occurs until the light intensity is down to 20 per cent. If the density of the filter is increased still further, the rate of decline of ϕ is accelerated, until the quantum yield is practically zero at a filter density still allowing about 5 per cent of the light to pass.

The nature of the relationship shown in the graph may be explained qualitatively in terms of the dependence of the quantum yield on the intensity ratio of the auxiliary light and of the far red light, noted by Emerson and Chalmers (10). When using

phycobilin filters transmitting 100 to 80 per cent, the proportion of the light absorbed by the phycobilins in the algal cells, may be high enough to make this ratio exceed the critical value suggested by Emerson's results. Further reducing this proportion by applying filters with 80 to 20 per cent transmission causes ϕ to decline linearly with decreasing filter transmission. When, by using filters of a still higher density, a certain minimal value of this ratio is passed, the auxiliary light becomes unable to produce any effect, and the yield drops sharply. Depending on whether light absorbed exclusively by chlorophyll *a* (or a certain form of chlorophyll *a*) does or does not produce photosynthesis with a finite quantum yield, ϕ should drop to a certain small but constant value, or decline to zero. Fig. 5 suggests that the latter may be true. Using a mixed phycobilin filter absorbing 95 per cent of incident

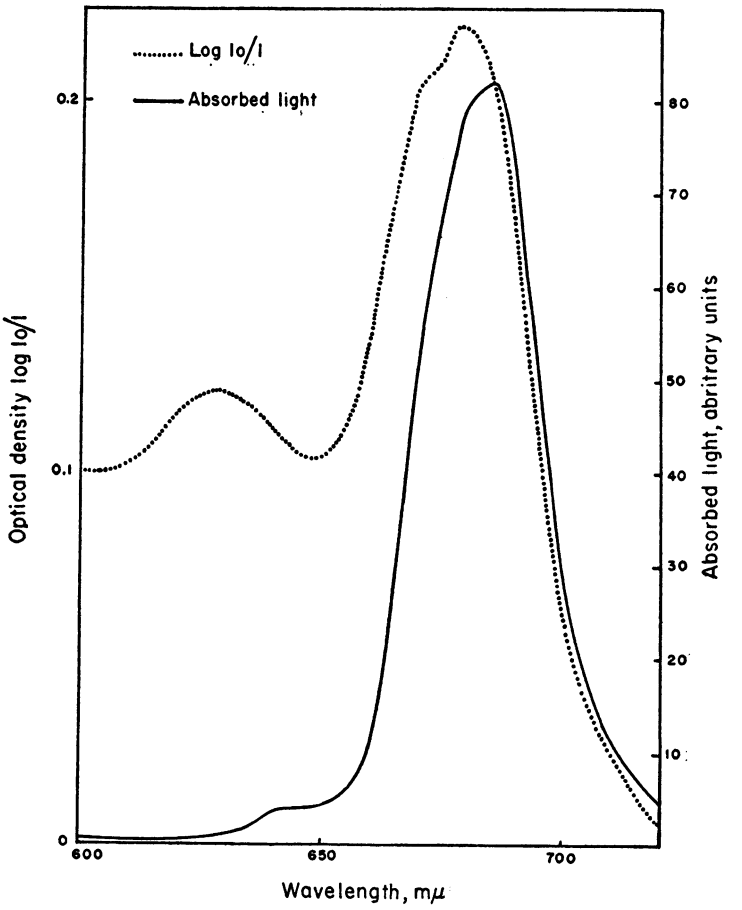

FIGURE 4 Absorption curve of chlorophyll *a* in the living cell, and composition of light absorbed by chlorophyll from light filtered through densest filter of mixed phycobilins.

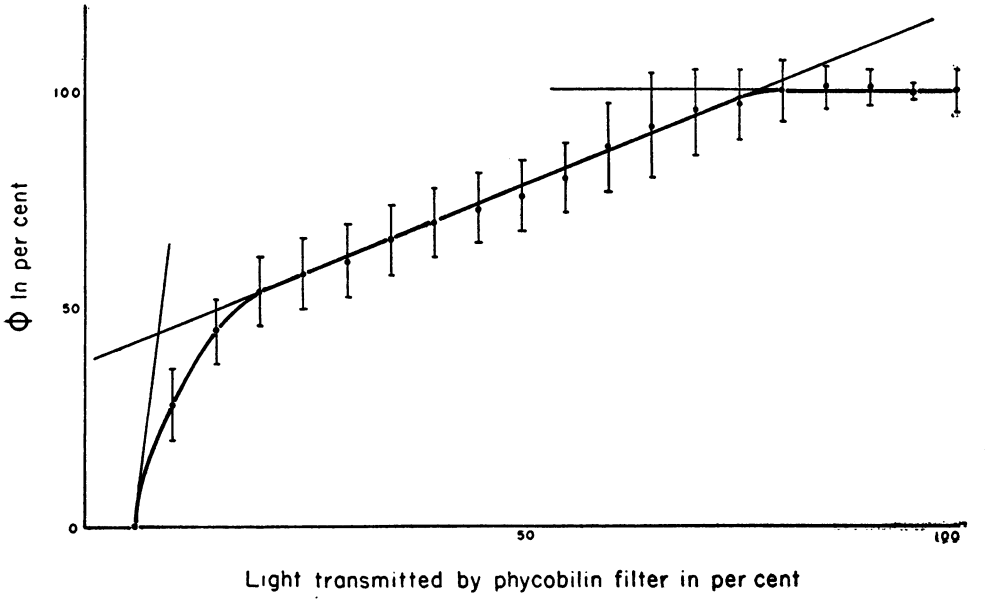

FIGURE 5 Relation between the proportion of incident white light transmitted by mixed phycobilin filters of various optical densities, and the relative quantum yield.

white light (referred to below as PB-95), no photosynthetic activity could be measured at all. The intensity incident on the vessel still amounted to 2 to 4 μeinsteins/hour, and chlorophyll *a* was markedly excited by the incident light. This is strikingly demonstrated by the occurrence of the Emerson effect, shown in Table I. Without orange background light, photosynthetic oxygen evolution was below the measuring range. When orange light was added, a measurable rate of oxygen production had to be attributed to light transmited by PB-95.

Two additional experiments were made in which, instead of the PB-95 filter,

TABLE I

EMERSON EFFECT ON PHOTOSYNTHETICALLY INACTIVE LIGHT TRANSMITTED BY A PB-95 FILTER

Experiment No.	Oxygen evolution attributable to light transmitted by PB-95	
	Without background light	With orange background light
	μl/hr.	μl/hr.
a	−0.60	+1.20
b	−0.60	+2.40
c	0.00	+2.40
d	+0.53	+1.14
e	−0.54	+1.17
Mean	−0.24 ± 0.25	+1.66 ± 0.30

either a phycocyanin or a phycoerythrin filter of comparable optical density at the absorption maxima was used. In these experiments, photosynthesis was clearly measurable—obviously due to transmission of some short wave light.

The conclusion which one could be inclined to draw from these results, that in the studied organism, chlorophyll *a* (or the form of chlorophyll *a* absorbing above 680 mμ) is by itself unable to produce photosynthesis, contradicts the observation of M. Brody and Emerson (4) (confirmed by us) that photosynthesis does occur when the same algae are irradiated with narrow spectral bands, up to 700 mμ. This disagreement will be discussed in a succeeding paper.

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