THE PHOTOSYNTHETIC EFFICIENCY OF PHYCOCYANIN IN CHROOCOCCUS, AND THE PROBLEM OF CAROTENOID PARTICIPATION IN PHOTOSYNTHESIS

By ROBERT EMERSON and CHARLTON M. LEWIS

(From the Carnegie Institution of Washington, Division of Plant Biology, Stanford University)

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I

Chlorophyll in plant cells is regularly accompanied by other pigments. For many years there has been speculation as to whether the light absorbed by these so called accessory pigments is utilized for photosynthesis. Engelmann's skillful experiments (1883) on algae of various colors, with motile bacteria as an indicator of photosynthetic activity, have been the basis of a general belief that the light absorbed by certain accessory pigments (particularly fucoxanthin, phycoerythrin, and phycocyanin) is available for photosynthesis. This belief, although criticized by some who obtained conflicting evidence, has been strengthened by photosynthesis measurements with technique allegedly superior to that of Engelmann (*cf.* Harder, 1923). But a critical examination of both early and later work on this subject shows that the evidence is inconclusive, chiefly because the relative amounts of light absorbed in different wave lengths were not established. Comparison of the photosynthetic activity in different wave lengths can be decisive in establishing the part played by accessory pigments only if the absorbed energy in each wave length is known. Recent attention to the problem of the quantum yield of photosynthesis has resulted in development of techniques well suited to investigation of the activity of accessory pigments. Dutton and Manning (1941) have compared light absorption and photosynthetic activity in the diatom *Nitzschia closterium* in different parts of the spectrum, and found good evidence that the energy absorbed by the carotenoids, especially fucoxanthin, is used in photosynthesis. Recent measurements of the quantum yield of photosynthesis for various species of algae (Emerson and Lewis, 1941, p. 803) gave strong indication that the phycocyanin of the blue-green alga *Chroococcus* is active in photosynthesis. These measurements were all made in sodium light, $\lambda = 589$ mμ. In the green algae, practically all the absorbed energy of this wave length is absorbed by chlorophyll, but in the case of *Chroococcus* the phycocyanin is responsible for a large part of the total energy absorbed. Since the quantum yield for *Chroococcus* photosynthesis was in good agreement with the yields for green algae

it seemed probable that the energy absorbed by phycocyanin must be available for photosynthesis. In the present communication we report more conclusive evidence that this is the case. The energy absorbed by phycocyanin is used for photosynthesis with an efficiency closely approximating that of chlorophyll. Our results give slight indications of photochemical activity on the part of the carotenoids as well, but it appears that any photosynthetic yield from light absorbed by these pigments must be relatively low.

II

Material and Experimental Methods

The alga, a small species of *Chroococcus*, was obtained in pure culture from C. B. van Niel of Stanford University. The cells are about 2.5 to 3 μ in diameter (somewhat smaller than *Chlorella*). They are encased in a gelatinous sheath about half as thick as their own diameter. In spite of the sheath, cultures grow without serious clumping, and an inoculum of 5 c.mm. cells in 200 ml. of culture medium produces a growth of about 150 c.mm. cells in from 5 to 7 days, at a temperature of 30°C. The cultures are grown in continuous light from four closely spaced 60 watt incandescent lamps at a distance of 20 cm. from the bottoms of the culture flasks.

The composition of the culture medium is as follows:

per liter glass-distilled water,

$MgSO_4\ 7\ H_2O$	0.25	gm.
KH_2PO_4	1.00	"
KNO_3	1.00	"
Na_2CO_3 (anhydrous)	1.50	"
$Ca(NO_3)_2$	0.025	"
NH_4Cl	0.050	"
$Fe_2(SO_4)_3$	0.004	"

Manganese, boron, zinc, copper, and molybdenum were added from a solution of their salts, to give concentrations of 2, 1, 0.05, 0.01, and 0.01 parts per million respectively.

The cultures fail to grow if sodium or calcium is omitted from the medium. The addition of large amounts of potassium seems to be unnecessary. Successive cultures have been grown in the above medium with sodium salts substituted for both the potassium salts, and with amounts of potassium running from 1 to 10 parts per million. Growth is poor if potassium is omitted altogether, but as little as 1 p.p.m. is sufficient to support culture growth as well as does the control medium prepared with potassium salts.

The dry weight of 100 c.mm. of *Chroococcus* cells is about 10.5 mg., as compared to a corresponding value of about 20 for *Chlorella pyrenoidosa*. The lower dry weight of *Chroococcus* is probably due to the gelatinous sheath surrounding the cells, which prevents them from packing as closely as *Chlorella* cells, so that a cubic millimeter of cells represents a smaller amount of cell material in the case of *Chroococcus*. Cell counts showed about 4×10^7 *Chroococcus* cells per c.mm. of centrifuged volume, as compared to about 2.3×10^7 for *Chlorella*.

For measurements of photosynthesis, the cells were centrifuged out of their culture

medium and resuspended in freshly mixed carbonate buffer (85 parts M/10 bicarbonate to 15 parts M/10 carbonate). In preparing this mixture for experiments with *Chroococcus*, it was necessary to use sodium, or part sodium and part potassium salts. If the mixture was made from potassium carbonate and bicarbonate, as is the usual practice for *Chlorella*, the *Chroococcus* showed a rapid decline in both photosynthesis and respiration. We made a practice of mixing sodium bicarbonate and potassium carbonate for the measurements with *Chroococcus*. In this mixture the cells maintained fairly steady rates of respiration and photosynthesis for 2 to 3 hours. The proportions of the two constituents are less critical than for *Chlorella*. *Chroococcus* photosynthesis at light saturation seems to be relatively independent of carbon dioxide concentration, even down to quite alkaline carbonate mixtures. Efforts to measure photosynthesis in acid phosphate media were unsuccessful, apparently because the photosynthesis of *Chroococcus* was inhibited by the low pH. The inhibition was reversible on returning the cells to carbonate mixture.

Rates of photosynthesis were based on manometric measurements of rate of pressure change during the second 5 minutes of alternate 10 minute periods of light and darkness. The buffering capacity of the carbonate mixture maintains a constant partial pressure of carbon dioxide, so the rates represent oxygen exchange alone. Most of the measurements were made at 20°C. The manometer and the general technique of making the measurements have already been described (Emerson and Lewis, 1939). Measurements were made with both thick and thin cell suspensions. The thick suspensions were dense enough to absorb all the incident light, so that a measurement of the incident intensity alone sufficed to establish the amount of light absorbed. The thin suspensions absorbed only about half of the incident light. The amount absorbed was determined immediately after the photosynthesis measurements, using the same cell suspension, light beam, and monochromator settings. For the measurement of light absorption, a sample of the suspension was transferred to an absorption cell of the same thickness (1.4 cm.) as the depth of the layer of cell suspension used in the manometer vessel for the photosynthesis measurements. (The effect of local irregularities in the depth of the cell suspension due to the rotary shaking of the manometer vessel was neglected.) The side walls of the absorption cell were of brass, nickel-plated on the inside to reflect scattered light. The intensity transmitted was measured by a photronic cell the active surface of which was larger than the back window of the absorption cell. The distance between active surface and absorption cell window was less than a millimeter. Thus even light which had been scattered through a considerable angle was registered by the photronic cell at nearly its full value. The incident intensity was measured, as usual, as that transmitted by a similar absorption cell filled with water instead of cell suspension.

Measurements of photosynthesis and light absorption were made in various wave length bands isolated from the light of a 1000 watt tungsten filament lamp by means of a large-aperture grating monochromator.[1] For measurements of photosynthesis in the red region of the spectrum, bands were used whose half-width, in-

[1] The monochromator was constructed with the aid of a grant from the National Research Council, and with valuable help and advice from members of the staff of the Mount Wilson Observatory. Details concerning the construction and light output of the monochromator are being prepared for publication elsewhere.

cluding nearly 75 per cent of the total energy, was from 6 to 10 mμ. In the blue, because of the lower output of the lamp, wider slits were used, giving band half-widths of 15 to 20 mμ. Spectral impurity due to scattered light from the various optical surfaces, principally that of the grating, was reduced by means of glass color filters which transmitted only the region of the spectrum close to the line for which the monochromator was set. Scattered light outside the band transmitted by the monochromator probably never exceeded 5 per cent of the total energy of the beam. For the quantum yield measurements, the entire light beam emitted by the monochromator was allowed to fall on the assimilating cells, or was concentrated on the sensitive surface of the bolometer for measurement of the energy received by the cell suspension. This was a modification of our former technique (Emerson and Lewis, 1939), in which only a small sample of the light beam was used for the energy measurements.

We gratefully acknowledge the cooperation of H. H. Strain in the extraction and separation of the various pigments, and in measuring the absorption of the extracts. The chlorophyll and carotenoids were extracted from the fresh, unground cells with methanol, transferred to ether, and the ether solution diluted with ethanol. The separation of pigment components and the determination of absorption spectra of the total extract in ethanol and of the chlorophyll and carotenoid fractions were carried out according to technique previously described by Strain (1938, pp. 125–132). The time allowed for saponification of the chlorophyll was 20–30 minutes. The phycocyanin was obtained in water solution by grinding the cells with silicon carbide ("crystolon") in a glass mortar at 0°C., precipitating twice with saturated ammonium sulfate, and redissolving the blue pigment by washing through a filter with distilled water.

When the carotenoids of *Chroococcus* were adsorbed on a sucrose column, the xanthophylls appeared to differ markedly from those commonly found in extracts of higher plants, and also in extracts of *Chlorella*. The carotenes, on the other hand, were apparently similar to those ordinarily encountered in leaf extracts. The unusual character of the xanthophyll-like pigments of the Cyanophyceae has been noted by several investigators (for example Tischer, 1938, and others cited by him). Unfortunately, in our work on *Chroococcus* not enough attention was devoted to the carotenoids to enable us to compare them with pigments isolated from related species by other investigators.

III

EXPERIMENTAL

The peculiar usefulness of the quantum yield as evidence concerning the part played by accessory pigments is that measurements of both photosynthesis and light absorption are involved. In different regions of the spectrum, the various plant pigments absorb different proportions of the total absorbed radiation. If some components are photochemically inactive in photosynthesis, then the observed quantum yield for the total radiation absorbed by all the components will vary with the wave length. A comparison of the wave

length dependence of the observed quantum yield with the fractions of total absorbed light which are absorbed by the different components may be expected to show which pigments are photochemically active in photosynthesis, and what are their relative activities. This reasoning implies the assumption that the yield for the fraction absorbed by any one pigment is independent of wave length over the range under consideration. This assumption will be discussed presently.

Absorption Measurements

Direct measurement of the fraction of light absorbed by the various pigment components in the intact cell is not possible, but we have evidence that a fairly close estimate may be made from the absorption curves of the extracted pigments. Figs. 1 and 2 show the extent of the discrepancies between the absorption characteristics of the extracted pigments and of the intact cells. Fig. 1 shows the absorption spectra (plotted as $\log\left(\frac{Io}{I}\right)$) of the three pigment components of *Chroococcus*—chlorophyll, carotenoids, and phycocyanin. The solid curve represents the absorption of the total alcohol-soluble pigments, consisting of chlorophyll and carotenoids without phycocyanin. After saponification and removal of the chlorophyll fraction, the absorption of the carotenoids was measured. This is plotted as a broken line. The dotted line, calculated from the difference between the absorption of the carotenoids and that of the total extract, represents the absorption due to chlorophyll. From 570 to 700 mμ, where there is no absorption by carotenoids, the curve for chlorophyll is identical with the solid line for the total alcohol-soluble pigments. The absence of any maximum in the chlorophyll curve at 458 mμ, and the lack of apparent asymmetry in the red absorption band show that no appreciable amount of chlorophyll *b* is present. The absorption curve for phycocyanin dissolved in distilled water is shown by the curve drawn with alternating dots and dashes. The triangles are the observed points for our pigment, and the crosses are taken from the curve of Svedberg and Katsurai (1929), adjusted to give agreement with our curve at the maximum. The nearness of these points to our curve is evidence of the identity of the phycocyanin from *Chroococcus* and that which Svedberg and Katsurai isolated and crystallized from *Aphanizomenon flos aquae* (molecular weight about 200,000). The somewhat higher absorption of our curve in the blue may be due to the presence of a small amount of colloidal matter from the ground cells, which filtration failed to remove from the solution. In the photoelectric spectrophotometer used for all the curves in Fig. 1, even very slight light scattering would appear as absorption. For purposes of the subsequent discussion we have assumed that the absorptions measured in this way are correct. If the true values are lower

in the blue, this only adds emphasis to the discrepancy between light absorption and photosynthetic efficiency to be discussed below.

In order to compare the absorption by the pigments in the intact cells with the combined absorption of the extracted pigments, we have plotted them to-

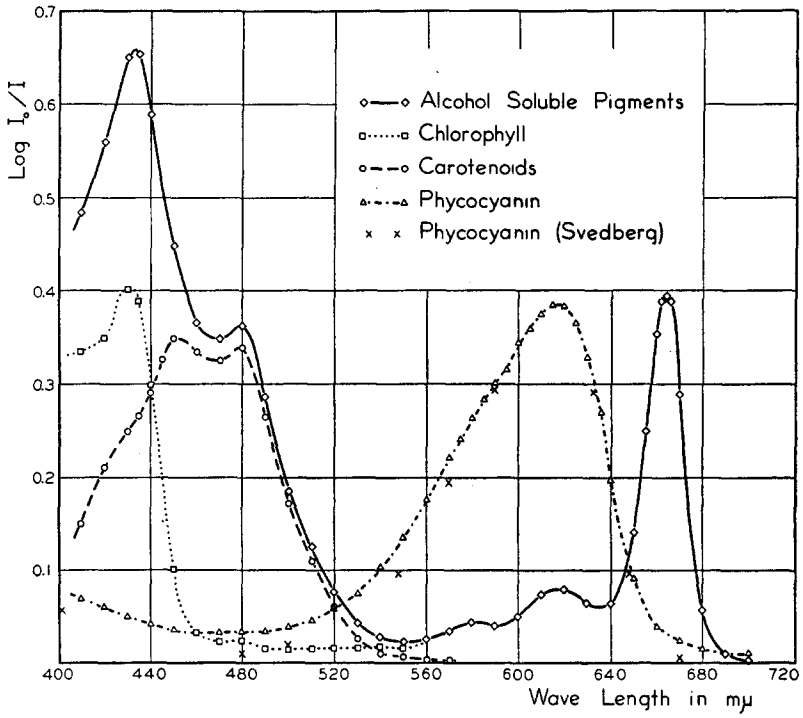

FIG. 1. The absorption spectra of the extracted pigments of *Chroococcus*. The solid line is for the total alcohol-soluble pigments, carotenoids, and chlorophyll. The broken line is for carotenoids alone, after saponification and removal of the chlorophyll. The dotted curve for chlorophyll represents the difference between the curve for the total alcohol extract and the one for carotenoids alone. In the red region, where there is no carotenoid absorption, the curve for chlorophyll is identical with the solid line for the total extract. The curve drawn with alternate dots and dashes is for an aqueous solution of phycocyanin.

gether in Fig. 2 (the two lower curves). The solid curve shows the absorption by a suspension of live cells, measured with a photronic cell by the method described in Section II. The broken curve shows the calculated combined absorption of the extracted pigments. In making this computation, certain adjustments have been made which must be kept in mind in order to make a fair comparison between the two curves. Each measured absorption curve

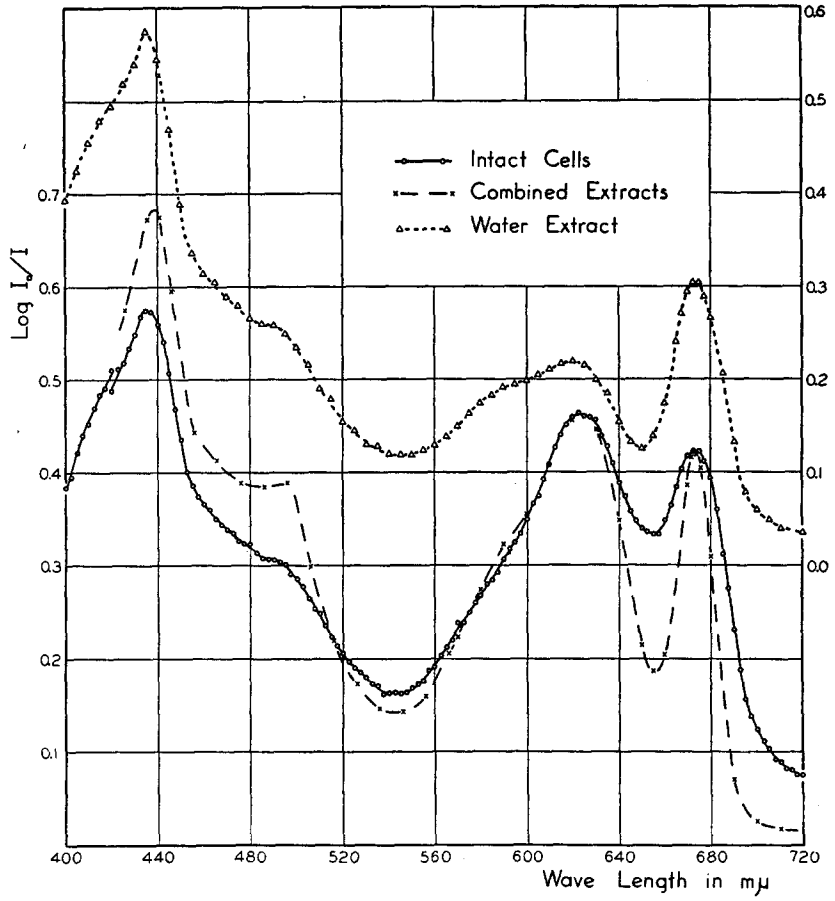

FIG. 2. Comparison of the sum of the absorption spectra of the extracted pigments, with the absorption spectrum for intact *Chroococcus* cells. The curves are based on a cell concentration of 1.26 c.mm. cells per ml. of suspension, in a layer 1.4 cm. thick. The solid curve is for the intact cells. The broken curve is for the total extracted pigments, obtained by addition of the curves for individual components shown in Fig. 1, after making adjustments in the positions of the maxima as explained in the text. The dotted curve, displaced upward on the ordinate axis to avoid confusion of the other two curves, is for an aqueous extract of ground cells. The scale for the dotted curve is on the right.

shown in Fig. 1 was shifted as a whole toward longer wave lengths an amount which appeared to make the individual maxima correspond best with the respective maxima for the live cells. In the case of chlorophyll, this shift was different in the red and blue regions, being 10 mμ in the red, and 6 in the blue

(the change being made arbitrarily at 580 mμ). The carotenoid curve was shifted 14 mμ, and that for phycocyanin 6 mμ. The chlorophyll and carotenoid curves in Fig. 1 are directly comparable with the curve for the live cells in Fig. 2, because aliquot portions of the same culture were used, and extraction and separation were carried out quantitatively. On the assumption that Beer's law applies to our conditions, the measurements made in solution were so corrected that they apply to the same light path and concentration of pigment per unit volume as prevailed in the cell suspension. (However, the exact agreement of the curves in Fig. 2 at the red chlorophyll maximum must be considered accidental.) The extraction and separation of phycocyanin, on the other hand, were not made quantitatively. The curve in Fig. 1 represents an arbitrary concentration, chosen to give agreement between the two curves in Fig. 2 at the phycocyanin absorption maximum. This is the only adjustment involved in the calculated curve aside from the wave length shifts mentioned above.

A comparison of the curves in Fig. 2 for the observed absorption of the intact cells, and for the absorption calculated from the curves for the combined extracts (the solid and broken curves respectively), shows that in the region of carotenoid absorption the calculated curve is too high. This is perhaps an indication that these pigments absorb somewhat more in solution than in the live cells. The chlorophyll absorption band in the red is obviously much broader in the intact cells than in solution, so that for wave lengths near this band the absorption measured in solution is significantly lower than for live cells. It is not clear to what extent this applies also to the blue band of chlorophyll, and to the carotenoid band.

It is noteworthy that the absorption curve measured for a scattering suspension can give such good agreement with that of the extracted pigments in homogeneous solution. This favorable result may be due in part to the absence of plastids in the *Chroococcus* cells, and consequent simpler arrangement of pigments than prevails in the higher algae. In *Chroococcus* the pigments appear to be uniformly distributed throughout the protoplast. In the case of *Chlorella*, where the pigments are concentrated in plastids, comparison of the absorption spectrum of the live cells with that of the extracted pigments gives far less satisfactory agreement. Further evidence of the relative simplicity of pigment arrangement in *Chroococcus* is obtainable from an examination of water extracts. It is well known that water extracts from ground cells of higher green plants are difficult to analyze spectroscopically, although Smith (1941) has shown that satisfactory absorption curves can be made with extracts in aqueous digitonin or other detergents. But if *Chroococcus* cells are ground in a glass mortar with an equal volume of crystolon and then diluted with distilled water and centrifuged, a fairly clear extract of the combined pigments is obtained without resort to detergents. The absorption curve of such an extract is included in Fig. 2 (dotted curve). The sharpness of the absorption bands is comparable with that of Smith's digitonin extract. The aqueous extract was not perfectly clear,

so the absorption spectrum was measured with the photronic cell. The plotted values, displaced upward on the ordinate scale to avoid confusion of the other two curves (scale on the right), refer to a pigment concentration arbitrarily chosen to give agreement with the blue chlorophyll maximum of the live cells. The extract was prepared from a sample of the same culture used for the other two curves in Fig. 2. In the aqueous extract of pigments the positions of the maxima of the alcohol-soluble pigments remain unaltered, as compared to the corresponding maxima for the

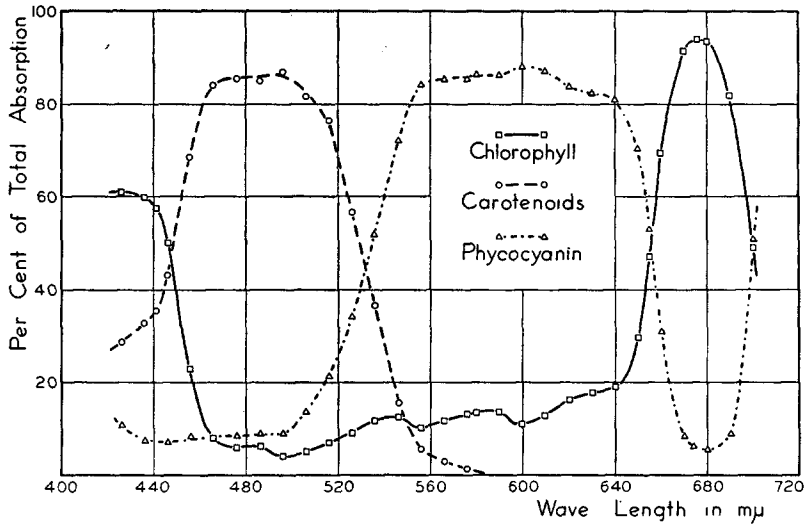

FIG. 3. These curves show what percentage of the total absorbed light is absorbed by each of the three pigment components, and how the percentage absorbed by each component varies with wave length. The solid curve is for chlorophyll, the broken curve is for the carotenoids, and the curve drawn with alternate dots and dashes is for phycocyanin. These curves are based on the measurements with extracted pigments. The values for total light absorbed were taken from the points of the broken curve in Fig. 2. To find the per cent absorbed by each component, the values plotted in Fig. 1 were used, after making the same adjustments in positions of the maxima as were used in deriving the broken curve in Fig. 2.

intact cells. The absorption band of phycocyanin, however, is shifted, opening up a deep minimum at 650 mμ, which is particularly striking when the absorption is observed visually. The secondary maximum at 590 mμ, barely visible in the isolated pigment, is quite strongly developed. This may be related to the reduced level of phycocyanin relative to the other pigments present in the aqueous extract.

The good general agreement between the two lower curves of Fig. 2 indicates that, at least in *Chroococcus*, extraction of the pigments results in no fundamental change in their absorption spectra. The data of Fig. 1 have therefore been used to calculate the per cent of the total absorbed light which is absorbed

by each of the three pigment components, using the wave length shifts mentioned above. Fig. 3 shows how the percentage of light absorbed by each component varies with wave length. The values plotted in this figure are compared in the next section with the dependence of the quantum yield on wave length, in order to show how much of the photosynthetic activity is due to light absorption by the accessory pigments.

Fig. 3 is clearly an inexact picture of the percentage of light absorbed by the pigment components in the intact cell, since the curves in Fig. 2 show certain discrepancies between intact cells and extracted pigments. Even where the combined absorption of the pigment extracts agrees well with the absorption of the cells, there is no certainty that relative absorption of the components can be calculated correctly from the measured absorption of the extracts. It is always possible that the process of extraction increases the absorption of one pigment and decreases that of another, in such a way as to produce no net change in the total absorption. While such uncertainties are a distinct limitation of the method, at present there appears to be no simple way of avoiding them.

Quantum Yield

The quantum yield for *Chroococcus* photosynthesis is plotted against wave length in Fig. 4 (solid line). Eight separate runs were made, in general on different days and with cells from different cultures. The points of each set are identified by distinguishing characters on the graph. Although careful control of culture conditions made it possible to obtain cells with very nearly the same characteristics from different cultures, the reproducibility was not perfect. It was assumed that a slightly lower activity of a particular culture would lower the quantum yield at different wave lengths by the same factor, so that a uniform correction could properly be applied. One set of values in Fig. 4 has been multiplied by 1.05, and another by 1.13, to give a better fit with the other points. One culture gave yields about 20 per cent below the others, and has been omitted. The other five sets are presented as they were obtained. In some runs the series of wave lengths was gone over twice, in other cases only once. A small correction was sometimes made for the observed decline in activity of the cells during the 2 or 3 hours of a run. Of the 36 points, three, belonging to different sets, appear to be definitely out of line with the others, and have been arbitrarily omitted from consideration in drawing the curve.

Considering first the red end of the spectrum, Fig. 4 shows that the quantum yield is essentially constant from 690 mμ to about 570 mμ, aside from the small maximum at 680. It is uncertain whether this maximum is real, or is the result of a higher activity of the particular culture used for this region. The difference in photosynthetic efficiency over the entire range does not in any

case appear to be more than about 10 per cent. Fig. 3 shows that at 676 mμ phycocyanin absorbs 6 per cent of the total light absorbed, chlorophyll 94 per cent; while throughout the region from 640 to 560 the relationship is nearly reversed, with phycocyanin absorbing over 80 per cent, and chlorophyll less than 20 per cent. Even allowing wide margins for the uncertainty in estimating absorption in the live cell from the measurements on extracted pigments, it is clear that the light absorbed by phycocyanin must be available for photo-

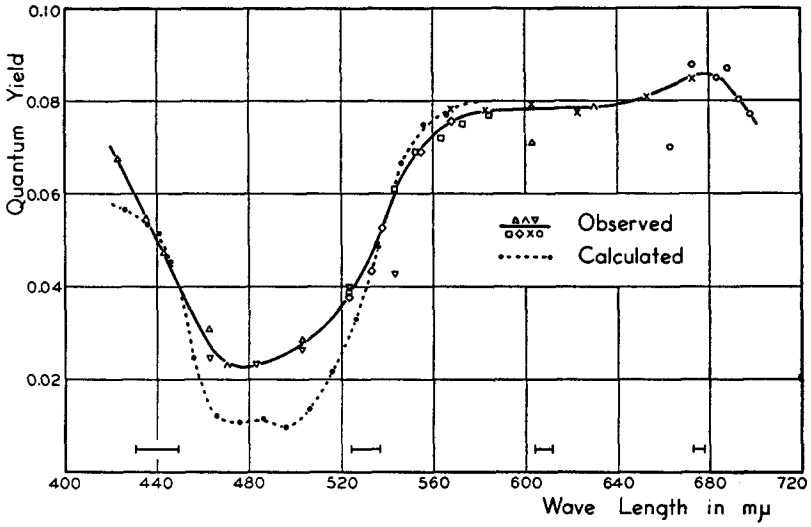

FIG. 4. The quantum yield of *Chroococcus* photosynthesis. The solid line is drawn through the experimental points, the values obtained in different runs being distinguished by different characters. The dotted curve shows the expected dependence of the quantum yield on wave length, on the assumption that the yield for light absorbed by chlorophyll and phycocyanin is 0.08 at all wave lengths, and that the light absorbed by the carotenoids is not available for photosynthesis.

synthesis with an efficiency of the same order of magnitude as that absorbed by chlorophyll.

Proceeding to the short wave length half of Fig. 4, the most obvious explanation for the sharp decline in yield beyond 570 mμ would be that the carotenoids are here absorbing light but not contributing the energy to photosynthesis. To test this hypothesis, we have calculated the expected photosynthetic yield for the total energy absorbed by the live cells, using the figures for relative absorption of the three pigment components, as plotted in Fig. 3 from the curves for the extracted pigments. The quantum yield for light absorbed by chlorophyll or by phycocyanin is assumed to be 0.08, and the yield for light absorbed by carotenoids zero. The resulting curve for yield as a function of wave

length is shown by the dotted line in Fig. 4. Qualitatively this is in fair agreement with the solid line drawn through the experimental points, a clear indication that the light absorbed by the yellow pigments is relatively ineffective in photosynthesis. But quantitatively the calculated yield is too low by a factor of two in the region of lowest efficiency. This is definitely outside the experimental error of the quantum yield measurements, and appears to be an unreasonable discrepancy to attribute to the assumptions involved in calculating the relative amounts of light absorbed by the pigment components.

On the other hand, if the above calculation is modified by assuming that the yield for light absorbed by the carotenoids is one-fifth that for chlorophyll and phycocyanin, instead of zero, then each point on the calculated curve is raised one-fifth of the distance between its present location on the dotted curve and the value 0.08. Agreement with the experimental curve is then entirely satisfactory, the minor discrepancies being less than one would expect in view of the imperfect agreement between calculated and measured absorption for the live cells shown in Fig. 2. The uncertainties involved indicate that no great significance should be attached to the precise value assumed in this case for the quantum yield for light absorbed by the carotenoids. But the good general agreement obtained suggests that a small part of the energy absorbed by the carotenoids may be available for photosynthesis.

Measurements with Thin Suspensions

As a check of the results with thick suspensions and in order to get additional information, a few runs were made with suspensions absorbing roughly half of the incident light. The photosynthesis and incident energy were measured in the same way as before, and the fraction of the incident light absorbed by the suspension was determined as a function of wave length directly after each run, in the apparatus described in Section II. The resulting values for the quantum yield were in general agreement with those obtained with totally absorbing suspensions, tending to confirm both the latter and the technique of absorption measurements. Though the absorption in different regions varied by a factor of more than two, and the quantum yield by a factor of more than three, the calculated quantum yield showed, within 10 per cent, the same dependence on wave length as the solid curve in Fig. 4.

These measurements with thin cell suspensions may also be presented in another way, to distinguish between that portion of the absorbed light which is absorbed by pigments photochemically active in photosynthesis, and that which is absorbed by inactive pigments. The usefulness of a distinction between "active" and "inactive" pigments depends in part on two simplifying assumptions. These are that the quantum yield for light absorbed by any one pigment is independent of wave length, and that the yields for the various accessory pigments are either zero or equal to the yield for light absorbed by

chlorophyll. (The possibility of generalizing the discussion to include intermediate and even varying efficiencies is obvious.)

The measurements of photosynthesis and light intensity with the thin suspensions show the yield per quantum of incident energy. We call this ϕ' to distinguish it from ϕ, the conventional symbol for the yield per quantum of absorbed energy. The value of ϕ' varies with wave length, and depends not only on the fraction of the incident light which is absorbed by the cell suspension, but also on the fraction of the absorbed energy which is absorbed by pigments active in photosynthesis. This latter fraction is not measurable directly, but can be estimated as follows. We will use ϕ_o to denote the yield for energy absorbed by chlorophyll, a pigment which we are sure is photochemically active in photosynthesis. The value of ϕ_o may be assumed, or it may be measured directly at some wave length not appreciably absorbed by the other pigments present. According to the assumptions mentioned above, the value of ϕ_o is independent of wave length, and applies also to the energy absorbed by any other pigments besides chlorophyll which may be active in photosynthesis. Therefore at any wave length where ϕ' has been measured, we can use the value of ϕ_o to find the fraction of the incident energy which is absorbed by photosynthetically active pigments. This fraction is $\frac{\phi'}{\phi_o}$, the "active absorption" for the particular suspension of cells on which ϕ' was measured. It is to be compared with the total absorption, measured directly on the same cell suspension with the photronic cell. To express total absorption in the same units as active absorption, we will call I_o the incident energy, and I' the absorbed energy (the difference between the incident and transmitted energy). Then $\frac{I'}{I_o}$ is the total absorption, that fraction of the incident energy which is absorbed by all the pigments present in the suspension, both active and inactive. Comparison of $\frac{I'}{I_o}$ with $\frac{\phi'}{\phi_o}$ should show what fraction of the absorption is attributable to active pigments and what to inactive pigments. Reference to the absorption spectra of the extracted pigments may then show which are the active and which the inactive pigments.

Fig. 5 shows a plot of measurements of both $\frac{I'}{I_o} \times 100$ (solid curve) and $\frac{\phi'}{\phi_o} \times 100$ (broken curve), against wave length. Three experiments are represented, each covering a different portion of the spectrum. For the run between 600 and 700 mμ, the figure of 0.075 was used for ϕ_o, the yield for light absorbed by chlorophyll. This value was measured with a thick suspension of cells from the same culture as the one used for the thin-suspension measurements. For the other two runs, no direct determinations of ϕ_o were made, and

a value of 0.070 was assumed, in order to bring the curves for active and total absorption roughly together at 583 mμ.

In the long wave length section of Fig. 5, the curve for total absorption shows a maximum near 670 mμ due to chlorophyll, and another one near 620 mμ

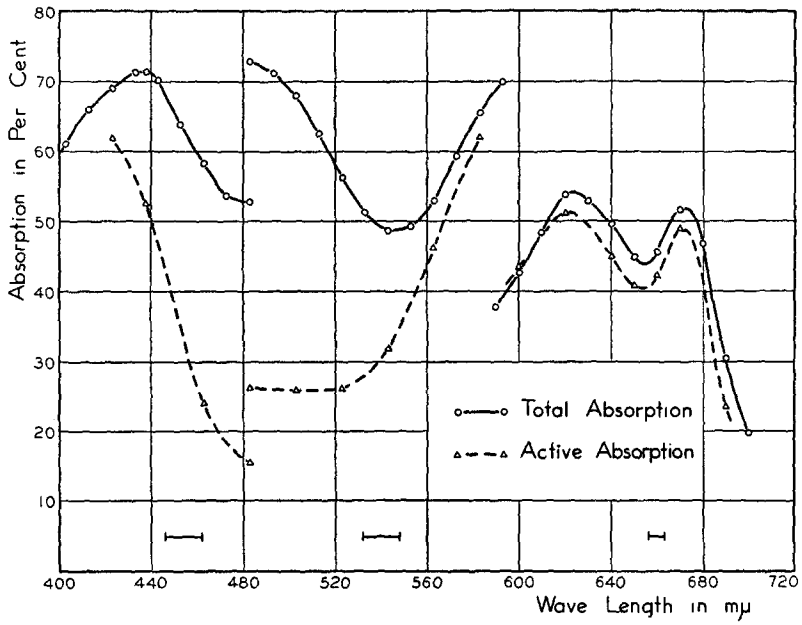

FIG. 5. Comparison of absorption spectrum and "action spectrum." The three parts of the figure represent three different runs, each covering a portion of the spectrum. For the blue region a concentration of 1.02 c.mm. cells per ml. of suspension was used, for the green region 1.67 c.mm., and for the red region 0.75 c.mm. The short horizontal line in each section shows the half-width of the band used for the run represented. The solid line shows the per cent of incident light absorbed by a suspension of live *Chroococcus* cells. The broken curve shows the per cent of the incident light which is absorbed by photosynthetically active pigments, on the assumption that all the light used for photosynthesis gives a quantum yield equal to the observed yield for the light absorbed by chlorophyll.

due to phycocyanin. The curve for active absorption shows two maxima which correspond closely to the ones for total absorption. The active absorption is nearly equal to the total absorption, showing that the same fraction of the incident energy which is absorbed is also used for photosynthesis. This is clear evidence that radiation absorbed by phycocyanin, as well as that absorbed by chlorophyll, is used for photosynthesis. There is no indication that the quantum yield for phycocyanin is any lower than for chlorophyll.

Our interpretation of this evidence, taken together with that in thick suspensions, is that the two yields are the same within 10 per cent, and probably within 5 per cent.

In the region of carotenoid absorption the curves behave quite differently. Below 560 mμ, as the total absorption turns sharply upward, the active absorption continues to decline. After going through a broad minimum from 520 to 480 mμ, it finally rises gradually as the blue absorption band of chlorophyll is approached, and at 423 mμ nearly rejoins the curve of total absorption. The area between the two curves, which from the point of view outlined above represents absorption by inactive pigments, may evidently be associated with carotenoid absorption. However, the active absorption in Fig. 5 never reaches a lower fraction of the total absorption than 29 per cent. Calculation based on absorption of the extracted pigments indicates that throughout the region from 466 to 500 mμ chlorophyll and phycocyanin together account for less than 15 per cent of the total absorption. The discrepancy, amounting to a factor of two, is as before not easily attributable to errors in the measurement of photosynthesis or radiant energy. It is possible that throughout the region of low efficiency the process of extraction alters the absorption by the pigments much more than was assumed. The discrepancy would be eliminated if we should assume that in the plant chlorophyll absorbs about three times as much, or that the carotenoids absorb only half as much, as in solution. The curves in Fig. 2 make either of these hypotheses seem unlikely, but a more moderate effect of extraction on both pigments is certainly compatible with the absorption measurements. Due to this possibility no more definite conclusions can be drawn than from the experiments with thick suspensions. Both groups of experiments suggest either that certain of the carotenoid pigments of *Chroococcus* are photochemically active in photosynthesis, or that all are active with an efficiency much lower than that of chlorophyll and phycocyanin. Any attempt to distinguish between these two possibilities by the present method would be difficult because of the similarity between the absorption spectra of the various carotenoid pigments.

In presenting the experiments with both thick and thin suspensions, we have made use of the assumption that the quantum yield for light absorbed by any one pigment is independent of wave length. It is appropriate to mention some experimental evidence in support of this assumption. In the case of the green alga *Chlorella pyrenoidosa*, which contains no phycocyanin, practically the entire absorption of light at wave lengths longer than about 570 mμ can be attributed to chlorophyll. Measurements of the quantum yield of *Chlorella* photosynthesis at different wave lengths (the results of which are being prepared for publication) have shown that from about 570 to 680 mμ, the yield is essentially constant. Franck and Herzfeld (1937, p. 251) state that the yield of chlorophyll fluorescence is constant from 400 to 650 mμ. If their

interpretation of the significance of fluorescence is correct, then constancy of the yield of fluorescence favors the conclusion that the photosynthetic yield for light absorbed by chlorophyll must be constant, not only in the region where chlorophyll alone absorbs light, where the experiments with *Chlorella* indicate that it is constant, but also at wave lengths where other pigments compete with chlorophyll for the absorption of light and make direct measurements impossible. But the extension of the assumption of constant photosynthetic yields for each pigment to organisms other than *Chlorella*, and pigments other than chlorophyll, must be regarded as only a working hypothesis.

It must be emphasized that these reservations concern only the conclusions drawn in regard to the carotenoid pigments. The experiments reported here establish beyond reasonable doubt that the light absorbed by phycocyanin is used for photosynthesis with an efficiency approximating that of the light absorbed by chlorophyll. But whether the phycocyanin acts by transferring the energy it absorbs to chlorophyll, which then carries on photosynthesis in the same way as if it had absorbed the light initially, or whether the phycocyanin can carry out carbon dioxide assimilation independently of chlorophyll, is a question to which our experiments give no answer.

SUMMARY

The absorption spectra of the principal pigment components extracted from *Chroococcus* cells have been measured, and their sum compared with the absorption of a suspension of living cells. The agreement was sufficiently close so that it was concluded the absorption spectra of the extracted and separated pigment components could be used to obtain estimates of the relative absorption of the various components in the living cells.

The quantum yield of *Chroococcus* photosynthesis was measured at a succession of wave lengths throughout the visible spectrum, and the dependence of yield on wave length was compared with the proportions of light absorbed by the pigment components. This comparison showed beyond reasonable doubt that the light absorbed by phycocyanin is utilized in photosynthesis with an efficiency approximately equal to that of the light absorbed by chlorophyll. The light absorbed by the carotenoid pigments of *Chroococcus* seems for the most part to be unavailable for photosynthesis. The results leave open the possibility that light absorbed by the carotenoids is active in photosynthesis, but with an efficiency considerably lower than that of chlorophyll and phycocyanin. It is also possible that the light absorbed by one or a few of the several carotenoid components is utilized with a high efficiency, while the light absorbed by most of the components is lost for photosynthesis.

CITATIONS

Dutton, H. J., and Manning, W. M., 1941, *Am. J. Bot.*, **28,** 516.
Emerson, Robert, and Lewis, C. M., 1939, *Am. J. Bot.*, **26,** 808.
Emerson, Robert, and Lewis, C. M., 1941, *Am. J. Bot.*, **28,** 789.
Engelmann, T. W., 1883, *Bot. Z.*, **41,** 1.
Franck, J., and Herzfeld, K. F., 1937, *J. Chem. Physics*, **5,** 237.
Harder, R., 1923, *Z. Bot.*, **15,** 305.
Smith, E. L., 1941, *J. Gen. Physiol.*, **24,** 565.
Strain, H. H., 1938, Leaf xanthophylls, *Carnegie Institution of Washington, Pub. No. 490.*
Svedberg, T., and Katsurai, T., 1929, *J. Am. Chem. Soc.*, **51,** 3573.
Tischer, J., 1938, *Z. physiol. Chem.*, **251,** 109.