THE DEPENDENCE OF THE QUANTUM YIELD OF CHLORELLA PHOTOSYNTHESIS ON WAVE LENGTH OF LIGHT [1]

Robert Emerson and Charlton M. Lewis

MEASUREMENTS OF the efficiency of photosynthesis in different wave lengths of light have recently been used as an approach to the problem of the physiological rôle of the pigments accompanying chlorophyll. Dutton and Manning (1941) concluded from their measurements with the diatom *Nitzschia closterium* that the light absorbed by fucoxanthin and other carotenoid pigments was available for carbon dioxide assimilation. Emerson and Lewis (1942) made similar measurements with the blue green alga *Chroöcoccus,* and concluded that light absorbed by the blue pigment phycocyanin gave a photosynthetic yield equal, within the experimental error, to the yield for light absorbed by chlorophyll. They found, on the other hand, that the quantum yield in the region of carotenoid absorption was much reduced, and regarded this as a clear indication that these pigments were not photosynthetically active with the full efficiency of chlorophyll and phycocyanin. However, when estimates of the proportion of light absorbed by the carotenoids were made from pigment extracts, and compared with the dependence of quantum yield on wave length, evidence was obtained that a fraction of the light absorbed by the carotenoids was probably available for photosynthesis. The same technique has been applied to the problem of carotenoid activity in the green alga *Chlorella pyrenoidosa,* and the results are reported in the present paper.

In the work with both *Nitzschia* and *Chroöcoccus,* the quantum yield for light absorbed by chlorophyll was measured in a wave length region where all or nearly all of the total light absorbed was absorbed by chlorophyll. This yield for light absorbed by chlorophyll was then compared with the yield for spectral regions where light absorption was divided between chlorophyll and accessory pigments, to show whether or not the energy absorbed by the accessory pigments was being used for photosynthesis. In making this comparison it was assumed that the yield for the fraction of light absorbed by chlorophyll did not vary with wave length, but remained the same even when a large fraction of the light was absorbed by other pigments. The Einstein photochemical equivalent law predicts that the primary photochemical action should be proportional to the number of absorbed quanta, so it might reasonably be expected that the quantum yield for light absorbed by chlorophyll should be independent of wave length, at least over the spectral range within which chlorophyll is capable of photosynthesis. But owing to the complexity of the plant's assimilatory mechanism, it seems appropriate to inquire to what extent the activity of chlorophyll bears out expectation.

Many investigators have accepted Warburg and Negelein's (1923) measurements of the efficiency of *Chlorella* photosynthesis in different parts of the spectrum as satisfactory proof of the constancy of the yield for light absorbed by chlorophyll. But these measurements were brought into question by the work of Manning, Stauffer, Duggar, and Daniels (1938) and Emerson and Lewis (1941) have shown that Warburg and Negelein's results are subject to certain errors inherent in their method, and that their value for the quantum yield of photosynthesis should, therefore, be rejected. Since the magnitude of the error arising from Warburg and Negelein's method may vary with wave length, their conclusions as to the dependence of quantum yield on wave length are also open to doubt. Further data on this point would have been desirable in any case, because their conclusions were based only upon measurements in the yellow mercury line (578 mμ) and in a broad band of red light from 610 to 690 mμ. Measurements were also made at 546 and 436 mμ, but at 436 the absorption was divided between chlorophyll and carotenoid pigments, and at 546 the measurements were regarded as somewhat uncertain because of the very dense cell suspensions required to absorb all the incident light in this region where chlorophyll absorption is low.[2]

The above considerations make evident the desirability of more precise information as to the dependence of quantum yield on wave length, particularly in the red region, where chlorophyll is the principal light-absorbing pigment. The absorption of light by the accessory pigments of *Chlorella* is restricted to a narrower range of the visible spectrum than is the case for *Nitzschia* or *Chroöcoccus,* leaving a broader region for study of photosynthesis in light absorbed by chlorophyll alone. If the yield for light absorbed by chlorophyll, and the dependence of this yield on wave length, can be established by measurements in a spectral region where chlorophyll alone absorbs all the light, this may make possible a more satisfactory analysis of measurements in other spectral regions, where absorption is divided between chlorophyll and other

[1] Received for publication October 6, 1942.

The experimental work reported in this communication was carried out during the years 1938–40 at the Carnegie Institution of Washington Laboratory of Plant Biology, Stanford University, Calif.

[2] The dense cell suspensions made the respiration readings unduly large in proportion to the photosynthesis. The apparent necessity of using such dense suspensions to achieve total absorption of the incident light may have arisen from the error associated with Warburg and Negelein's method of measurement. When this error was avoided, we found no indications of incomplete light absorption, even with much thinner suspensions than they used (cf. Emerson and Lewis, 1941, p. 801–802).

[The Journal for February (30: 83–163) was issued March 31, 1943.]
AMERICAN JOURNAL OF BOTANY, VOL. 30, NO. 3, MARCH, 1943.

pigments of uncertain physiological activity. This is the reason for the particular attention devoted to the red region in the present investigation.

In the course of the experimental work, two phenomena were encountered which raised special problems in the measurement of the quantum yield in certain portions of the spectrum. Exposure of cells to the blue-green region sometimes caused a considerable increase in the apparent rate of respiration. An unexpectedly sharp decline in the quantum yield was observed in the far red. Since these two observations are probably at least as significant as the original purposes of the work, they are discussed more fully than might appear to be necessary in view of their apparent treatment merely as difficulties of an experimental character.

The same general plan has been followed as in the corresponding work on *Chroöcoccus* (Emerson and Lewis, 1942). To obtain evidence concerning the activity of accessory pigments from measure-

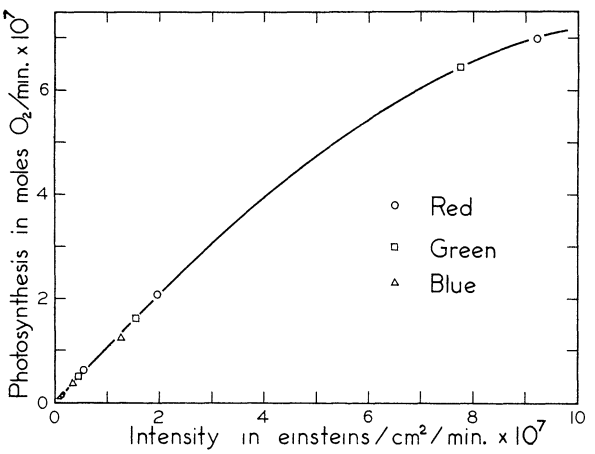

Fig. 1. Photosynthesis as a function of intensity at three different wave lengths, 660 mμ (circles), 560 mμ (squares), and 460 mμ (triangles). The area of cross section of the light beam was 15.4 cm.^{2} at the vessel. The intensities that were ordinarily used for measuring quantum yields varied from 0.1 to about 0.5 × 10^{-7} einsteins per cm.^{2} per minute.

ments of the quantum yield of photosynthesis at different wave lengths, it was necessary to supplement the quantum yield measurements with estimates of the proportions of light absorbed by the yellow and green pigments at each wave length. The quantum yield measurements have been made with dense suspensions of cells which absorbed all the incident light. The proportions of light absorbed by the yellow and green pigment components have been estimated from absorption measurements on extracted pigments. The conclusions drawn from a comparison of quantum yields and light absorption have been further supported by measurements with thin suspensions, which absorbed only about half of the incident light. From measurements with thin suspensions, the absorption spectrum of the photosynthetically active pigments has been plotted and compared with the absorption spectrum of the cells measured by direct methods.

Experimental methods.—Most of the techniques used have been fully described in earlier communications (Emerson and Lewis, 1939, 1941, 1942), and need only be mentioned briefly. Cells were grown in the usual inorganic nutrient medium prepared in glass distilled water. Manganese, boron, zinc, copper, and molybdenum were added in concentrations of 0.5, 0.25, 0.025, 0.005, and 0.05 parts per million respectively. Cultures were grown in continuous incandescent illumination at a temperature of about 20°C. An inoculum of about 1 cmm. cells in 200 ml. of medium produced a growth of about 300 cmm. of cells in from five to seven days. Cultures of about this density were harvested for the photosynthesis measurements. The cells were centrifuged out of the culture medium, washed twice in a mixture of 85 parts M/10 sodium bicarbonate and 15 parts M/10 potassium carbonate, suspended in the same mixture, and pipetted into the manometer vessel. Twenty-five ml. of fluid containing about 300 cmm. cells was the usual filling for the manometer vessel. This concentration of cells was usually sufficient for total absorption of the incident light, except for the green and infrared regions of the spectrum, where denser suspensions were used. It was sometimes necessary to make a small correction for transmitted light, based on visual estimation of the per cent transmitted. The reliability of such estimates has been discussed in an earlier paper (1939, p. 819–20). Further experience has shown that when the transmitted light is less than 5 per cent, visual estimation introduces no serious error. For measurements of the "active absorption," where it was desired to work with suspensions absorbing only about half the incident beam, about 40 cmm. cells were used in 25 ml. of carbonate mixture. The carbonate mixture is strongly buffered for carbon dioxide and at 20° maintains a constant concentration of about 0.2 millimoles per liter. Rates of photosynthesis were calculated from changes in oxygen pressure, measured manometrically with a double cathetometer reading in hundredths of a millimeter.

The photosynthesis measurements were made at 20°C., with light from a 1000-watt tungsten filament lamp, passed through a large grating monochromator. The monochromator and lighting system, constructed with the aid of a grant from the National Research Council, will be described in a separate publication (Lewis and Emerson, in preparation for publication). Photosynthesis could be measured in the red region with band half-widths as narrow as 50 Å (width to half the maximum intensity), and in blue with half widths of about 160 Å. Scattered radiation outside the band being transmitted by the monochromator was reduced by means of colored glass filters transmitting only in the region of the band being used. Scattered infrared radiation was reduced by a 1-cm. layer of a saturated solution of ferrous ammonium

sulfate. By a simple change of a mirror and lens in the light path, it was possible to project the entire light beam emitted by the monochromator either into the vessel containing the assimilating cells, or onto the receiving surface of a platinum bolometer for measurement of radiation intensity.

We are again indebted to H. H. Strain for extraction of the cell pigments, separation of the yellow components by saponification and removal of the chlorophyll, and measurement of the absorption spectra both before and after separation. (For a description of the technique of extraction and separation of the pigments, cf. Strain, 1938, p. 125–132.) The absorption due to chlorophyll was computed as the difference between the absorption before and after removal of the chlorophyll. We measured the absorption spectrum of live cells just as in the work on *Chroöcoccus,* with a photronic cell placed in contact with the window of an absorption cell containing the algal suspension.

DEPENDENCE OF YIELD ON LIGHT INTENSITY.—Before proceeding to a comparison of quantum yields and absorption spectra, we must mention certain experiments showing the dependence of the quantum yield measurements on light intensity and rate of respiration. The results of these experiments lend support to the validity of the technique used for measuring quantum yields.

It has already been shown (Emerson and Lewis, 1941, fig. 6) that the quantum yield for *Chlorella* in sodium light ($\lambda = 589$ mμ) is practically independent of light intensity over a considerable range. Eichhoff (1939), however, has claimed that the shape of the curve for dependence of photosynthesis on light intensity is different in white light and in red light. Therefore, it seemed necessary to investigate whether the measured quantum yield was independent of intensity over the intensity range used for several wave lengths in the visible spectrum. Figure 1 shows measurements of rate of photosynthesis plotted against light intensity in red, green, and blue light, up to the highest intensities obtainable with the monochromator. Much more energy was available in the red region (points plotted as open circles) than in the blue region (triangle points). Measurements in green are plotted as squares, and run nearly up to the maximum intensity used in red. Since the purpose of this experiment was only to show how nearly the rate of photosynthesis was a linear function of light intensity in the three different wave lengths, the three sets of measurements were made with different cultures, and should not be used for comparing quantum yields in different wave lengths. All the measurements fall pretty close to the same line, lending no support to Eichhoff's suggestion that intensity dependence is a function of wave length. The figure shows that for the low intensities used for efficiency measurements (about 5×10^{-8} einsteins/cm.^{2}/min.), the yield may be considered independent of intensity in all three wave length regions.

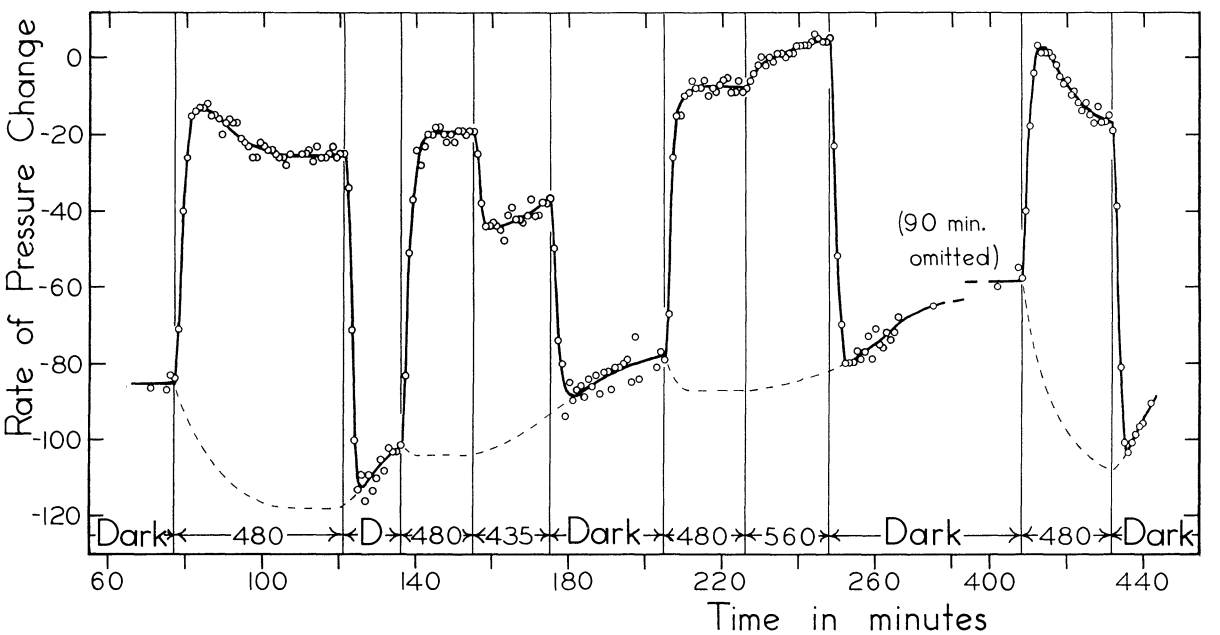

Fig. 2. Evidence for an effect of light on rate of oxygen consumption. The rate of pressure change (due to oxygen exchange) is based on readings of the manometer at one-minute intervals and is plotted in hundredths of a millimeter of manometer fluid per minute. Vertical lines indicate the times at which the light was turned on or off. Light periods are indicated at the bottom of the graph by giving the wave length in mμ of the light used. The cells were in the dark for about 75 minutes preceding the beginning of observations. The vessel contained 260 cmm. of cells in 25 ml. of carbonate buffer. The vessel constant K_{O_2} was 2.106. Intensities used were 8.0×10^{-8} einsteins per cm.^{2} per minute at 480 mμ; 4.1×10^{-8} at 435; and 7.3×10^{-8} at 560.

THE CORRECTION FOR RESPIRATION AND THE DEPENDENCE OF OXYGEN CONSUMPTION ON WAVE LENGTH OF LIGHT.—A small effect of light on respiration is generally admitted to be a probable source of error in all measurements of photosynthesis. According to Gessner (1938) the magnitude of the effect depends very much on previous dark adaptation, but it does not seem to be directly dependent on accumulation of the products of photosynthesis, because ultraviolet irradiation, which caused no photosynthesis, nevertheless resulted in small subsequent increases in respiration similar to those observed after exposure to visible light. Mothes, Baatz, and Sagromsky (1939) report that if infrared radiation is carefully filtered out, then exposure to visible light has no effect on subsequent respiration. Although these and similar observations suggest only minor effects of light on respiration, we have found that there is a fairly narrow range of wave lengths which may cause such large increases in rate of oxygen consumption that they easily become observable and constitute a serious obstacle to the correct measurement of photosynthesis. In the following discussion we refer to these effects of light on oxygen consumption as effects on "respiration," simply because the dark correction applied to measurements of photosynthesis is conventionally designated as "respiration." We do not wish to imply that the physiological nature of the extra oxygen consumption is the same as ordinary respiration, because we have no evidence whatever on this point.

Figure 2 shows the behavior of the rate of pressure change when a suspension of cells was exposed to light of 480 mμ, where the effect is about maximal. (The rates shown represent oxygen exchanged expressed in hundredths of a millimeter of the manometer fluid per minute.) The observations begin after 75 minutes in darkness, when the rate of respiration has dropped to a steady value of −85 per min. Exposure to light causes a change to −12 as a result of photosynthesis, but instead of maintaining this new rate, as we should expect from measurements at other wave lengths (cf. Emerson and Lewis, 1941, fig. 5), the rate changes in the course of a few minutes to −25 per minute. This might be interpreted as a fall in rate of photosynthesis, but when the suspension is darkened after 44 minutes of light exposure, the respiration is found to be much larger than it was before illumination, −112 instead of −85, suggesting strongly that the alteration in rate of pressure change during the early part of the light period was due to an increase in respiration rather than a decrease in photosynthesis. By the end of the 15-minute dark period the rate of respiration has dropped back to about −100, and is still dropping rapidly. At this point, light of 480 mμ is given again, and this time a fairly steady rate of pressure change is attained almost at once, showing that a stationary state has been reached, and the respiration neither rises nor falls in response to the light. The rest of the curve in figure 2 shows responses to other treatments, the probable course of respiration during the light periods being drawn as a dotted line. The course of this line is estimated from the drift in rate of pressure change during the light exposures, on the assumption that the rate of photosynthesis is constant, and that the changes are due to respiration. Exposures to 435 or 560 mμ do not appear to interrupt the slow decline in respiration which is evident during the dark periods. Another exposure to 480 mμ just before the change to 560 suggests another rise in respiration following the drop which had occurred during and after the exposure to 435 mμ. This time the rise is much smaller than it was with the first exposure to 480. However, after a dark period of two and a half hours the effect is rather larger than with the original exposure. It will be noted that all these changes in respiration are superimposed on a gradual downward trend which persists during the whole course of the observations.

Other experiments were made to establish the approximate limits of the effect. Starting at the red end of the spectrum, there was no clear influence of light on respiration down to about 530 mμ. A ten-minute exposure to this wave length caused about a ten per cent increase in respiration, and shorter wave lengths gave increasing effects to about 470. The effect was still prominent at 460, but by 435 mμ it was small or perhaps entirely absent. The maximum observed increase in respiration was from the last exposure shown in figure 2, which caused a rise of over 70 per cent. For such large effects, intensities considerably above those ordinarily used for the quantum yield measurements were required.

The rates of respiration of different samples of cells were not equally sensitive to light of the region near 480 mμ. Cells grown over neon tubes, instead of the usual incandescent lamps, showed particularly large increases of respiration in response to light in this region. The cells grown in neon light developed a smaller amount of chlorophyll in proportion to the yellow pigments, and were distinctly yellow-green in color. With certain other samples of cells, exposure to light of 480 mμ caused only negligible increases in respiration. In the experiments with *Chroöcoccus* cells (Emerson and Lewis, 1942) no specific effects of blue light on respiration were observed.

The observations discussed above were made on thick suspensions of cells. With thin suspensions the rate of respiration appeared to be altered to approximately the same extent, but the effect was not ordinarily noticeable because of the much lower rate of pressure change due to respiration.

Since the effect is limited to wave lengths where a considerable proportion of the absorbed light must be absorbed by the carotenoids, a photochemical acceleration of respiration by the yellow pigments is suggested. The large responses with the yellow-green cells grown in neon light support this suggestion. But a strictly photochemical effect of

this kind would not be expected to carry over into the ensuing dark period, nor should it be so slow in starting as indicated by figure 2.

Various modifications of experimental procedure were tried in order to avoid, as far as possible, the errors in photosynthesis measurements which would arise from such large effects of light upon rate of respiration. We found that with wave lengths producing such effects, a conditioning exposure of about twenty minutes usually sufficed to bring the respiration to a fairly steady rate. Subsequent measurements of rate of photosynthesis by means of the usual alternating ten-minute periods of light and darkness then gave no indications of serious errors due to changes in rate of respiration during illumination.

It may be that other wave lengths of light have smaller effects on respiration which remain undetected because they do not persist long enough into the subsequent dark periods to be evident. Since all our work on quantum yields, and indeed most quantitative work on photosynthesis, is based on the assumed constancy of respiration during light exposure, one must keep in mind the possibility that minor changes in respiration may bring about small and perhaps systematic errors in the computed rate of photosynthesis. Under the conditions of the quantum yield measurements, the rate of respiration tends to decline slowly over a period of several hours. We have found that some decline takes place regardless of whether the cells are kept in light or darkness or whether they are exposed to the ten-minute alternations of light and darkness used for the photosynthetic measurements. The decline is generally slower when some illumination is given. At first we thought it possible that much time could be saved by measuring the rate of respiration only at the beginning and end of a series of exposures to different wave lengths of light, and interpolating values of respiration on the assumption that the

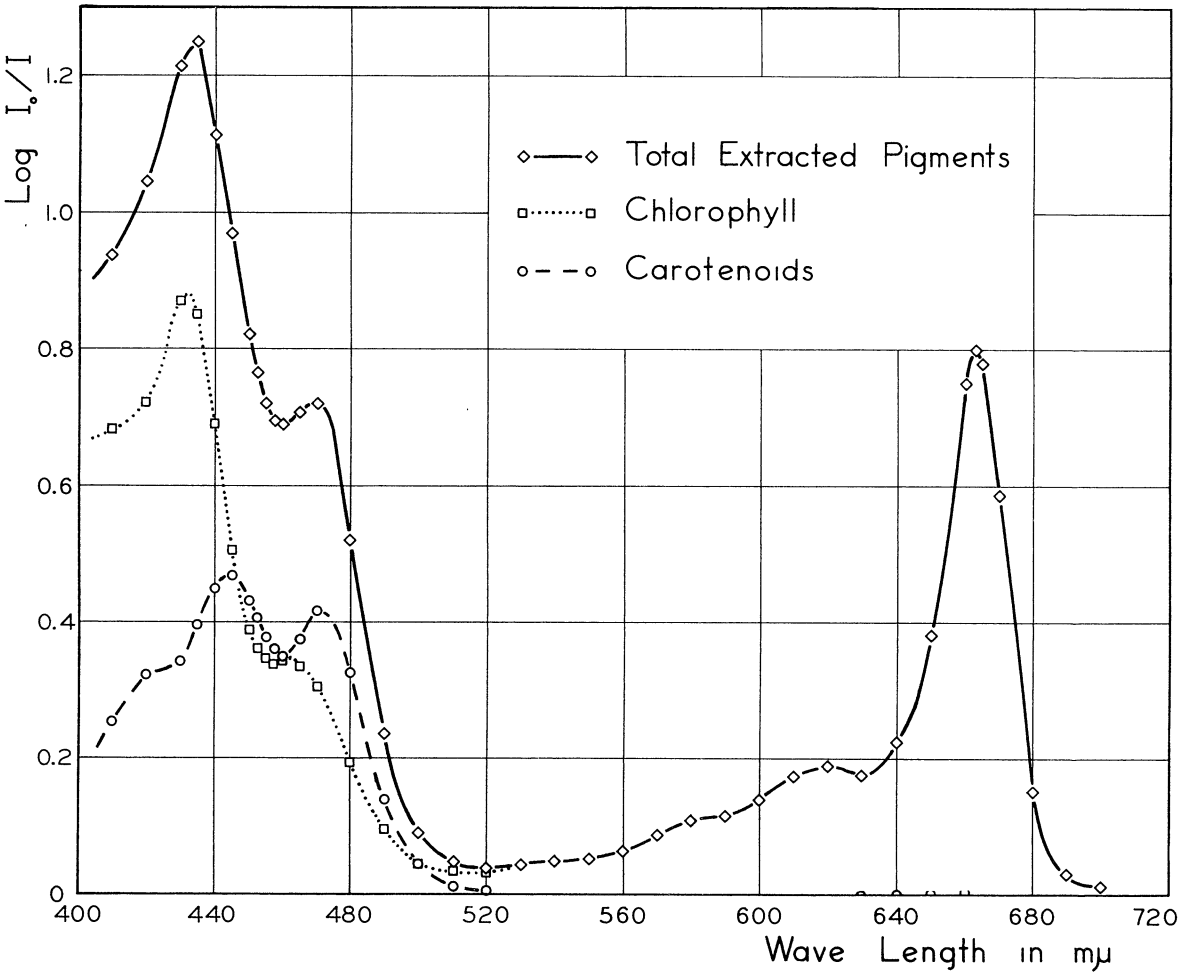

Fig. 3. Absorption spectra of ethanol solutions of pigments extracted quantitatively from *Chlorella* cells. The solid line is for total pigments; the broken line for the carotenoid fraction, after saponification and separation of the chlorophyll; and the dotted line for the chlorophyll fraction, obtained by subtracting the curve for carotenoids from that for total pigments. At wave lengths longer than 520 mμ the spectrum for chlorophyll coincides with that of the total extract. Complete absence of chlorophyll from the carotenoids after saponification is indicated by the four points of the carotenoid curve near 650 mμ.

rate of decline had been linear. But measurements made in this way were unsatisfactory, because the rate of decline was not the same during continuous light as with alternating light and dark periods, and a series of exposures to different wave lengths seemed always to be accompanied by a certain number of unpredictable small irregularities in respiration. We, therefore, used alternating ten-minute periods of light and darkness for most of the measurements. It was often necessary to discard the measurement during the first exposure to a new wave length or intensity, because such changes seemed to be followed by small changes in respiration, so that the rate of respiration before exposure to a new wave length was not strictly comparable with the rate after the exposure.

There is good evidence that, with alternating ten-minute periods of light and darkness, the dark readings ordinarily lead to a close estimate of the rate of respiration during the light exposures. The quantum yield has been found to be the same at 0°, 10°, and 20°, although the rate of respiration changed by a factor of 5 over this range of temperatures. The relative constancy of the quantum yield in a number of different organisms, as compared to wide variability in rate of respiration, is further evidence that errors due to the method of estimating the respiration correction must be small (cf. Emerson and Lewis, 1941, tables 2 and 6). But in limiting cases, such as at very low light intensities or toward the infrared where photosynthesis becomes very small, it may sometimes be impossible to distinguish between actual photosynthesis and small effects of light on respiration.

Comparison of absorption spectra of extracted pigments and intact cells.—Figure 3 shows a plot of the absorption spectra of the pigments extracted from *Chlorella*. The curve drawn with a solid line is for the alcoholic extract, containing both chlorophyll and carotenoids. The

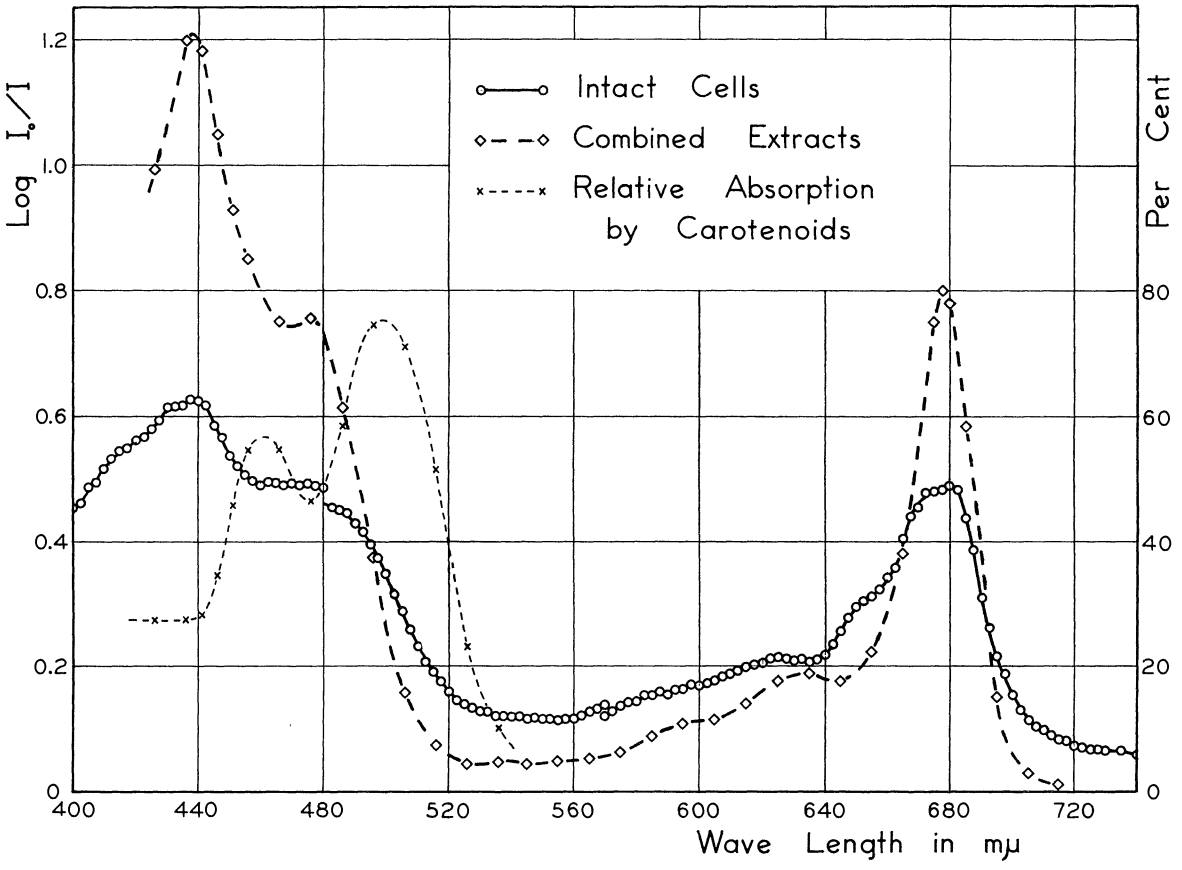

Fig. 4. Comparison of the absorption spectra of the extracted pigments and of intact *Chlorella* cells. The solid curve shows the absorption due to a suspension of intact cells 1.4 cm. thick and containing 0.96 cmm. of cells per ml. The spectrum of the extracted pigments, given by the broken line, was derived from figure 3 by combining the chlorophyll and carotenoid curves after shifting each one toward the red an amount intended to compensate for the wave length shifts accompanying extraction. All curves for extracted pigments represent a pigment concentration that corresponds quantitatively to the concentration of live cells used in obtaining the solid curve. (The two breaks in the latter curve are at points where the cell suspension was stirred and the filters on the monochromator changed.) The crosses show calculated values for the fraction of the total absorbed light that is absorbed by the carotenoids, based on the curves for the extracts after introduction of the wave length shifts. The ordinate scale for these points is at the right.

broken curve shows the absorption by carotenoids after saponification and removal of the chlorophyll. A few measurements were made on the carotenoid solution at about 650 mμ, and the absence of significant absorption in this region shows the effectiveness of the technique used for the removal of the chlorophyll. The dotted curve from 400 to 520 mμ shows the difference between the curve for the carotenoids and the one for the total pigments, and represents absorption due to chlorophyll. Beyond 520 practically all the absorption is due to chlorophyll.

Figure 4 shows a comparison of the absorption by the extracted pigments (broken curve) with the absorption by a suspension of the living cells (solid curve). Both curves represent the same concentration of pigment per unit cross section of the light beam, and they were made with samples of cells from the same suspension. The curves for the extracted pigments have been shifted to make the positions of the maxima coincide as nearly as possible with the maxima of the intact cells. It seems reasonable to make such adjustments before comparing the absorption spectra, to avoid differences due merely to the shift of the bands which is known to accompany extraction. For the *Chlorella* curves, the red chlorophyll maximum has been shifted 15 mμ toward longer wave lengths, and the blue maximum 6 mμ, the change in amount of shift coming at 540 mμ. The carotenoid curve was shifted 14 mμ toward the red. These adjustments were made before adding the curves to give the broken line in figure 4.

In the case of the corresponding curves for *Chroöcoccus,* which were obtained by making similar adjustments (Emerson and Lewis, 1942, fig. 2), the absorption curve for the sum of the extracted pigments was sufficiently similar to the curve for the intact cells to justify the conclusion that the absorption characteristics of the individual components had been altered but slightly by extraction, and, hence, that the relative amounts of light absorbed by the pigments in the living cells could be estimated from the relative amounts they absorbed in the extracted condition. The same cannot be said of the *Chlorella* curves. Absorption by the intact

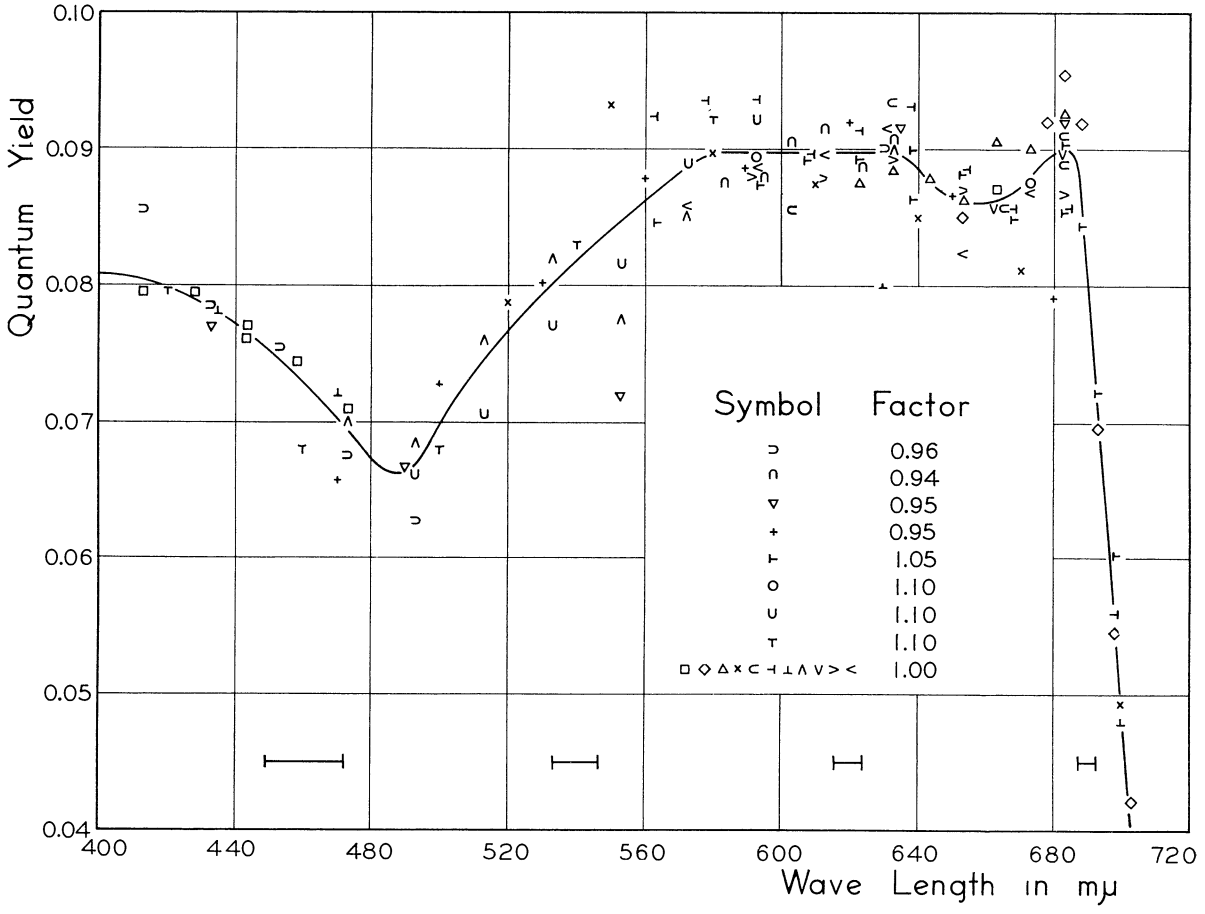

Fig. 5. The quantum yield of photosynthesis as a function of wave length for *Chlorella.* The points obtained on each of nineteen separate runs are indicated by a distinct symbol. For eight of these runs arbitrary adjustments have been made by multiplying all the values obtained in each run by a factor close to unity. The factors used are given in the figure. The band half widths that were commonly used in the various parts of the spectrum are indicated by horizontal lines of corresponding length.

cells is twice as high as absorption by the extracted pigments in the green region, where absorption is minimal, and the relationship is reversed in the red and blue maxima, making an over-all difference of about a factor of 4 between the two curves. One must conclude that the absorption characteristics of the *Chlorella* pigments have been substantially altered by extraction, and that it may not be possible to obtain a useful estimate of the proportions of light absorbed by the components in the cell from an examination of the absorption curves of the extracted components. Nevertheless we have included in figure 4 a lightly drawn curve to show the percentage of total absorbed light which is absorbed by the carotenoids. This curve is derived from the curves for the separated components after making the adjustments to compensate for shifts in the absorption bands due to extraction, and represents the best available estimate of the light absorption due to carotenoids in the intact cells.

Since we are concerned here with the special problem of establishing the distribution of light absorption between the pigment components in the living cell, no specific reference need be made to the voluminous literature on the absorption spectra of extracted plant pigments. In an investigation, the purposes of which were similar to our own, French (1937) used the absorption curves of pigment extracts to identify the absorption maxima of the red and green pigments in living *Spirillum rubrum* cells.

Dutton and Manning (1941) in their work on *Nitzschia* also based estimates of distribution of absorption in the intact cell upon measurements made on pigment extracts, and emphasized the uncertainty inherent in the method. They made allowance for a uniform shift in the absorption bands due to extraction, but made no quantitative comparison of absorption by extracts and by suspensions of live cells.

The unsatisfactory outcome of our comparison between absorption by extracted pigments and absorption by intact cells in the case of *Chlorella,* as compared to the relatively good agreement shown earlier by the curves for *Chroöcoccus,* is probably associated with the higher level of complexity of the *Chlorella* cells. The *Chroöcoccus* cells show no internal structure whatever, the pigments apparently being uniformly distributed throughout the protoplast. In *Chlorella,* on the other hand, the pigments are concentrated in the chloroplast, a highly specialized structure which occupies only a small part of the total volume of the cell. This greater structural complexity is perhaps an indication of greater chemical complexity as well.

The optically less homogeneous *Chlorella* cells probably also scatter light more strongly than the *Chroöcoccus* cells, but the differences between the solid and broken curves in figure 4 do not appear to vary regularly with wave length, and, therefore, should not be attributed primarily to scattering. The light-scattering by suspensions of *Chlorella* cells can be much reduced by suspending the cells in glycerine, and this results in a slight sharpening of the absorption maxima, but the changes are not such as to bring the curve for the intact cells into appreciably closer agreement with the curve for extracted pigments. This is an indication that our photronic cell method of measuring absorption for the cell suspensions is fairly satisfactory from the standpoint of integrating the scattered light. Further evidence against the existence of serious errors due to scattering is mentioned in connection with figure 7.

The chief purpose of the absorption measurements was to obtain an estimate of the distribution of light absorption between the yellow and green pigments, for comparison with the dependence of quantum yield on wave length. It is unfortunate that in the case of *Chlorella* the absorption characteristics of the pigments are so much altered by extraction, that the measurements on the extracted pigments give only an uncertain picture of the proportions of light absorbed by the pigment components in the living cell. But in order to obtain from the quantum yield measurements evidence concerning the possible participation of the yellow pigments in photosynthesis, some estimation of the proportion of light they absorb must be made. Since the curves plotted in figure 4 appear to be the best estimate available at present, we have used them, in the following section, for comparison with the dependence of quantum yield on wave length.

QUANTUM YIELDS.—Nineteen sets of measurements of the quantum yield of photosynthesis as a function of wave length have been plotted together in figure 5. Each set of points, distinguished by a different symbol, represents one series of measurements made with a freshly-harvested culture. The almost inevitable variability in the physiological activity of a series of cultures grown over a period of more than two months and the slight modifications made in the technique of measurement as the work progressed were held responsible for a slight variability in the observed quantum yield at any given wave length. But the dependence of yield on wave length appeared to be essentially the same in spite of this variability in the magnitude of the yield. In order to plot all the measurements in a single figure without obscuring the dependence of yield on wave length because of the scatter of the points, it seemed necessary to make some adjustment in those sets of points which fell uniformly above or below the level of the majority of measurements. The need for such an adjustment arises chiefly from the fact that some sets of measurements covered nearly the entire spectral range under investigation, while others were limited to a narrower region. Of the nineteen sets of measurements, four were moved upward by a small factor and four were moved downward. In no case was the adjustment larger than ten per cent. The other eleven sets have been plotted just as they were measured, without adjustment. The adjustments do not

appear to alter the conclusions to be drawn from the observations, and they bring out the dependence of yield on wave length somewhat more clearly.

Figure 5 shows that the yield is roughly constant from 580 to 685 mμ. From 685 toward the infrared, the yield drops sharply. This is discussed in greater detail in connection with figure 6. From 580 toward the violet the yield declines to a minimum at about 490, and rises again toward 400 mμ. As in the case of the *Chroöcoccus* measurements, the minimum near the maximum of the relative absorption by the yellow pigments is qualitatively what would be expected if the light absorbed by the yellow pigments were not available for photosynthesis. The fact that the decline begins as near the red as 580 mμ would indicate that carotenoid absorption is already becoming appreciable. The extracted carotenoids, however, show very slight absorption at wave lengths longer than 520. The above interpretation of the decline in quantum yield would, therefore, require that in the plant the carotenoid absorption is not only shifted as a whole 14 mμ toward the red as compared with the extract, but is also broadened to such an extent that the tail of the band extends an additional 45 mμ. This sort of broadening is to be expected, but hardly to so marked a degree.

Figure 4 indicates that between 490 and 510 mμ the yellow pigments absorb over 70 per cent of all the light absorbed, but the yield drops only from a maximum of about 0.090 in the red to a minimum of 0.066, a decrease of less than 30 per cent. This decrease is too small to agree quantitatively with the interpretation that the light absorbed by the carotenoids is inactive in photosynthesis. At 420 mμ the yellow pigments still appear to absorb about 30 per cent of all the light absorbed, but the yield is only about 10 per cent below the value in red, where there is no carotenoid absorption.

These comparisons would be more significant if the curves for the extracted pigments agreed better with the absorption of the intact cells. Since this agreement is so poor it must be admitted in the first place that the dotted curve for the percentage of absorption by carotenoids may be only a very rough picture of the relationship in the living cells. Hence, it is possible to do little more than speculate as to the significance of the variations in quantum yield with wave length. Possibly certain of the carotenoids are capable of playing a photochemical part in carbon dioxide assimilation, and others are not. Perhaps all are active, but with a relatively low efficiency. Another, though less probable, possibility is that the carotenoids are all photochemically inactive in photosynthesis. In this case the ratio of carotenoid to chlorophyll absorption near 500 mμ would have to be only about one-third as great in the intact cells as when measured in solution. Further experimental work with cells containing different proportions of carotenoid pigments and chlorophyll might be expected to help in solving this problem.

THE LOW YIELD IN THE FAR RED.—The abrupt decline in yield in the far red starts at 685 mμ, where chlorophyll absorption is still strong, and where there are no known bands of pigments present in sufficient concentration to compete seriously with chlorophyll for the absorption of light. Before we consider possible interpretations of this decline in yield toward the infrared, it seems necessary to consider certain potential sources of error peculiar to measurements in this region. As already stated in the section on methods, the quantum yield measurements are based on total absorption of the incident light by the assimilating cells. Visual inspection has been found to be a sensitive test for totality of absorption, but toward the infrared the sensitivity of the eye diminishes to the vanishing point, so transmission of the incident light can no longer be detected this way. Direct measurement of the absorption by the photronic cell method, which we have found so useful in the visible region, is not feasible in the infrared, both because of the lower sensitivity of the cell, and because when the absorption is small the error caused by scattered light becomes great. This is clearly shown in table 1 which gives the percentage of the incident light

TABLE 1. *Scattered reflection from a cell suspension, and from a suspension of India ink.*

Wave length	Per cent of light scattered	
	India ink	Cell suspension
450	0.76	0.62
500	0.64	0.79
550	0.52	1.24
600	0.49	0.75
650	0.48	0.61
700	0.4	1.8
730	0.5	9.3

which was scattered back toward the source of illumination by a thick suspension of cells. The measurements were made with the photronic cell facing an illuminated suspension of cells, and placed just enough to one side to avoid light reflected from the window of the vessel. For comparison, similar measurements with a suspension of india ink are shown. In both cases readings are given as per cent of those obtained when a piece of white matte paper was placed over the window of the vessel. As already noted by Warburg and Negelein (1922), the scattering of light from a cell suspension back toward the source of illumination is small with visible light. At the red and blue absorption bands it is scarcely greater for the cells than for the ink. In the green where absorption is less, the light penetrates deeper into the suspension so that more cells contribute to the scattered light, and this is somewhat greater. Toward the infrared, as the absorption falls to a very low value, scattering takes place from all the cells of the suspension, and amounts to almost ten per cent of that scattered from white paper. At wave lengths toward the visi-

ble from 700 mμ, where absorption is high and scattering low, the photocell appears to give quite satisfactory measurements of absorption. But toward the infrared from 700, accurate measurements of absorption would clearly require better integration of the scattered light than can be expected of such a simple arrangement as we have used.

Without measurement of the scattered light, it is nevertheless possible to test whether a given cell suspension transmits an appreciable amount of the incident beam. The yield of photosynthesis per unit of *incident* light may be measured with different

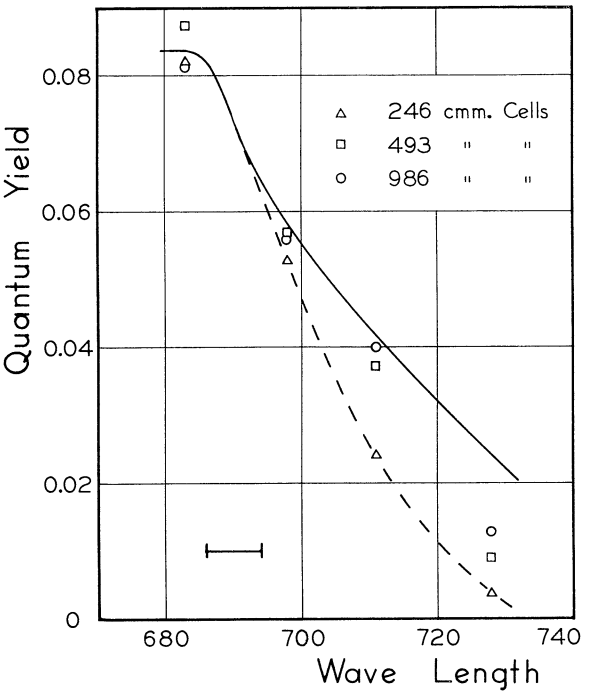

Fig. 6. Decline in quantum yield in the far red. The points show the apparent quantum yield with three different suspension densities, calculated on the basis of incident light. The solid curve shows an estimate of the true yield, based on the assumption that differences between values obtained with different suspension densities are due to differences in absorption. The volume of cells used in each case (in 25 ml. of fluid) is given in the figure.

suspension densities. At low light intensities the yield is independent of suspension density, apart from dependence of absorption on density. If a thick suspension gives a higher apparent yield than a thinner one, then absorption of the incident beam by the thinner one must be incomplete. If the thin suspension gives a yield equal to that of the thicker one, then presumably absorption is equally good in both cases. Figure 6 shows measurements of the quantum yield with three different suspension densities, covering the region of the steep decline shown in figure 5. The wave length scale has been expanded, so the slope appears less steep. The results with the three suspension densities are plotted with distinguishing symbols. The figure shows that at 683 and 698 mμ the thinnest suspension gave a yield practically equal to the yield for the thickest suspension. But beyond 698 the yield for the thinnest suspension falls more and more below that for the others, a clear indication of incomplete absorption of the incident light by the thin suspension. At 728 mμ, the measurements show that even the thickest suspension could not be depended upon to absorb all the incident radiation. The lower curve drawn in figure 6 shows how the yield might have been thought to depend on wave length if the measurements had been made only with the 246 cmm. suspension, which had been adequate for total absorption at 690 mμ. The upper curve shows the estimated actual yield, making a generous allowance for the incomplete absorption as revealed by the observed dependence of yield on suspension density. These measurements show beyond doubt that both the absorption of light and the quantum yield drop very rapidly between 685 and 730 mμ. Probably there is a similar decline in the yield for *Chroöcoccus* (Emerson and Lewis, 1942, fig. 4) in this part of the spectrum, but measurements with different suspension densities were not made, so the observed decline may be due in large part to incomplete absorption.

The most plausible interpretation of the sharp drop in quantum yield of photosynthesis toward the infrared seems to be that the light quanta of this spectral region no longer provide sufficient energy for the photochemical primary process. This seems reasonable if, as has sometimes been suggested, the capacity of chlorophyll to effect photosynthesis is linked with the energy level required for the emission of fluorescence (cf. Franck and Herzfeld, 1937, p. 251; also Franck, French and Puck, 1941). Experiments made by Vermeulen, Wassink and Reman (1937) give considerable support to this suggestion. They measured the fluorescence spectrum of chlorophyll, and found that with three widely separated wave lengths of exciting light, the fluorescence band was always the same. They found the quantum yield of fluorescence was constant with increasing wave lengths up to 624 mμ. The maximum of the principal fluorescence band is generally given as about 685 mμ for live cells (cf. Dhéré, 1937), and measurements with exciting light closer to this than was used by Vermeulen et al. would be unsatisfactory because of the increasing difficulty of distinguishing between the fluorescent light and scattered light from the exciting source. It is, therefore, impossible to say how long the wave length of the exciting light may be and still raise chlorophyll to the energy level necessary for the emission of fluorescence. But if the fluorescence maximum is at 685 mμ, one would expect that wave lengths longer than this would excite fluorescence of this band with rapidly decreasing efficiency, and that this might be reflected in a decreased quantum yield of photosynthesis. The wave length at which the photosynthetic yield begins to drop is very close to 685 mμ.

We have obtained no clear evidence of photosynthesis at wave lengths longer than the series in figure 6. Measurements also were made at 760 and 800 mμ, with very dense suspensions of cells and high intensities of radiation. There were consistent differences in rate of pressure change between "dark" periods and periods of exposure to radiation. These differences amounted to about one per cent of the respiration rate and were too small to justify the conclusion that the infrared radiation had caused photosynthesis. The pressure differences could have been accounted for either by a small inhibitory effect of infrared radiation on respiration or by photosynthesis due to the small amount of scattered visible light coming from the optical surfaces of the monochromator. In some instances a filter of 0.5 per cent iodine in carbon tetrachloride was used to absorb scattered visible radiation, but a trace of deep red was still transmitted. This impurity was not measured, but had it contained as much as 1.5 per cent of the total energy of the beam, which seems quite probable, it would account for the observed effect. The lack of clearly demonstrable photosynthesis at 760 and 800 mμ does not necessarily indicate that the quantum yield has decreased to zero, since the absorption by chlorophyll may be vanishingly small. Extracted chlorophyll in most solvents shows no appreciable absorption beyond 700 mμ. Van Gulik (1915), however, reports that chlorophyll in carbon bisulfide shows quite appreciable absorption in this part of the infrared. Possibly chlorophyll in carbon bisulfide gives a closer approximation to the absorption by chlorophyll in live cells, in which case the quantum yield in the infrared must be very small.

Eichhoff (1939) reported no diminution in the yield of photosynthesis in infrared out to 800 mμ. Our observations afford no support for his conclusion, and we wonder if his results may not have been due to scattered visible radiation. Though he measured the energy distribution of the radiation photographically, it is not clear whether he made sufficient allowance for the fact that an appreciable minimum exposure is necessary to produce measurable darkening of an emulsion.

In using the dependence of quantum yield on wave length (fig. 5) as evidence concerning photochemical activity on the part of the carotenoids, we assumed that the yield for the fraction of the light absorbed by chlorophyll did not vary with wave length. Constancy of the observed quantum yield in spectral regions where all the absorbed radiation is absorbed by chlorophyll would support this assumption. However, the decline in yield beyond 685 mμ (fig. 6) should not be regarded as contrary evidence, even though in this region no other pigments compete appreciably with chlorophyll for absorption of the light. In our opinion the decline toward the infrared must be due to failure of the quanta of lower frequency to provide sufficient energy to raise chlorophyll to the excited state required for carrying out the photochemical primary process. At shorter wave lengths than 685 mμ, it is fair to expect that there will always be sufficient energy for the photochemical primary process. Therefore, it is between 685 mμ and the region where carotenoid absorption begins, that we should look for constancy of the quantum yield in order to support our assumption that the yield for light absorbed by chlorophyll is constant in regions where part of the light is absorbed by the yellow pigments.

THE YIELD FOR LIGHT ABSORBED BY CHLOROPHYLL.—The points in figure 5 leave room for doubt as to the constancy of the quantum yield in this region, although from about 580 to 685 there are only minor variations. The principal departure from constancy is the small minimum at about 660 mμ. The apparent deviation in this region appears to be outside the limits of error from instrumental sources, so there is little doubt of its physiological origin. All attempts to obtain a uniform quantum yield through the red region by modifying the method of making the respiration correction, changing the sequence in which the different wave lengths were studied, etc., were unsuccessful. Therefore, although the apparent variations in yield are admittedly no greater than might arise from such errors as small effects of light on respiration, nevertheless it seems necessary to consider the possibility that the quantum yield may vary slightly in the red region. Either the yield for light absorbed by chlorophyll may not be constant or there may be an inactive pigment present which absorbs an appreciable fraction of the light in the region around 660 mμ.

Chlorophyll *b* may be expected to absorb a sufficient proportion of the light in the neighborhood of 660 mμ, so that if it were inactive it could account for the observed dip in the quantum yield in this region. The absorption curve for live cells (fig. 4) shows a definite shoulder at 660 mμ, presumably due to the red maximum for the *b* component. The asymmetry in the curve for the extracted pigments is by contrast extremely slight, and this may be because the absorption maxima for the two components are much closer together in methanol than in the live cells. Extraction seems to cause a greater shift in the maximum of the *a* component, so that the *b* maximum becomes obscured. The same conclusion may be drawn from curves published by Katz and Wassink (1939, p. 100, fig. 1e). In recent papers on the absorption spectra of the two chlorophyll components there appears to be no general statement to the effect that the maximum for the *a* component is more subject to change than that of the *b* component as a result of extraction or transfer from one solvent to another. Egle (1939), however, gives a table showing the positions of the red bands for the two chlorophyll components for a series of solvents, and a number of pairs of solvents can be selected which show greater shift of the band for the *a* component (cf. especially alcohol and carbon bisulfide). It seems probable on the basis of these considerations that the red absorption maxima for the two chlorophyll components, though only

about 15 mμ apart in methanol, are separated by more nearly 30 mμ in the live cells. This would mean that even partial inactivity of chlorophyll *b* should produce a noticeable dip in the quantum yield curve near 660 mμ.

If the long wave limit of full photosynthetic efficiency is related to the maximum of the fluorescence band in the way that has been indicated above, then one might expect that the efficiency for light absorbed by each chlorophyll component would have its own limit, determined by the position of the fluorescence band for that component. According to this reasoning, the photosynthetic efficiency for light absorbed by chlorophyll *b* might be expected to show a sudden drop beginning near the principle fluorescence maximum for this component. The fluorescence bands for the two chlorophyll components are close to the respective absorption maxima, and like the absorption maxima they are more widely separated in the living cell than in ordinary solvents. Dhéré (1937) reports that for *Ulva* fronds the fluorescence maximum for chlorophyll *b* is at 655.5, and for the *a* component at 684.7. These values agree well with the regions where the quantum yield of photosynthesis shows a decline—the first at about 660, and the second just beyond 685 mμ. The dip in the curve at 660 mμ is about as large as might be expected on the basis of this interpretation. If *Chlorella* is typical of other green plants, it probably has two to three times as much of the *a* component as of the *b*, and the specific absorption coefficient for *a* is considerably higher. From 660 to 685 the proportion of light absorbed by the *b* component must decrease rapidly, and this would account for the rise in yield toward 685.

It has already been mentioned that the experimental evidence for the minimum drawn in the quantum yield curve at 660 mμ is not thoroughly satisfactory. The above interpretation is put forward as a suggestion which appears to us promising, and not as an established fact. But we feel that the experimental evidence justifies the hope that further attention to the behavior of the quantum yield in the red region, possibly with even narrower wave length bands, will lead to useful correlations between the absorption and fluorescence of the chlorophyll components, and their activity in assimilation.

From the evidence available, it seems reasonable to regard the yield for light absorbed by chlorophyll as approximately constant where there is no competition from other pigments. The extension of

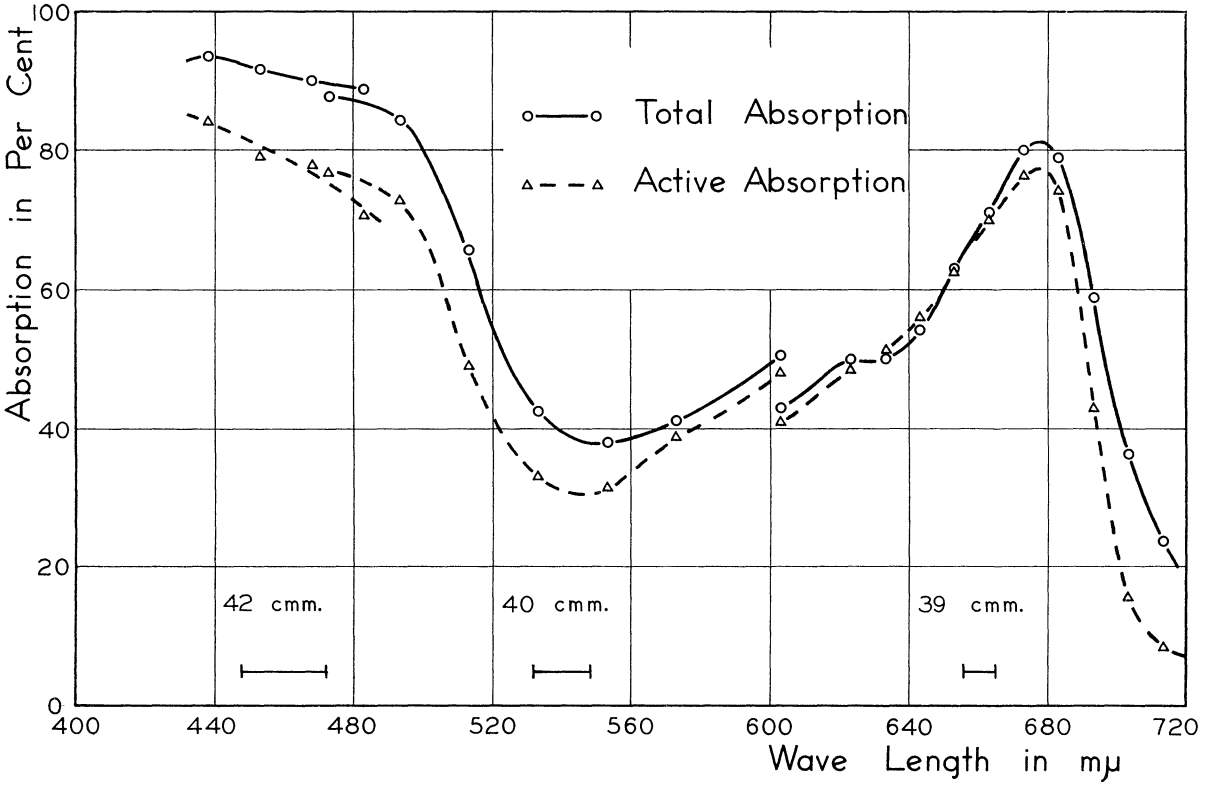

Fig. 7. Comparison of the absorption by the cells and that part of the absorption which is active in photosynthesis. These measurements are made with thin suspensions, giving incomplete absorption. The "total" absorption is measured directly. The "active" absorption is calculated from the measured photosynthesis on the assumption that all light used for photosynthesis gives a quantum yield of 0.084. This value is chosen to give agreement between the two curves in the red, where all absorption is assumed to be active. The quantity of cells used in each run, and the half widths of the bands of radiation are shown on the figure. The cells used in the run covering the red part of the spectrum were from a separate culture from the others.

this conclusion to the blue and green region, where absorption is divided between chlorophyll and the carotenoids, must be regarded as only a working hypothesis the purpose of which is to provide a logical basis for consideration of the evidence concerning photochemical activity on the part of the carotenoids.

COMPARISON OF ACTIVE ABSORPTION AND TOTAL ABSORPTION.—The quantum yield measurements with *Chroöcoccus* (Emerson and Lewis, 1942) were supplemented with measurements on thin suspensions, where only about half of the incident light was absorbed. To find the "active absorption," or absorption spectrum of the pigments photochemically active in photosynthesis, the per cent of incident light which resulted in photosynthesis was plotted at each wave length, on the assumption that all the light utilized for photosynthesis acted with the same quantum yield. The active absorption measured in this way was compared with the absorption measured directly with the photronic cell, using the same suspension of cells and the same slit widths as for measuring the active absorption. Similar measurements have been made for *Chlorella,* and the results are plotted in figure 7. The solid curve shows the total absorption, and the broken curve shows the active absorption. These curves offer independent confirmation of the low yield between 690 and 710 mμ, where the active absorption falls below the measured absorption. The two curves run close together from 690 to 570, indicating that all the absorbed light is used for photosynthesis with the maximum yield. The strong asymmetry of the red absorption band is presumably due to chlorophyll *b,* and the action spectrum repeats this asymmetry. Evidence of reduced activity of the *b* component at 660 mμ is lacking in figure 7, but the expected effect would be small, and greater refinement of the technique, as well as more observations, might be required to bring it out in an experiment of this kind. From 560 mμ on into the violet, the action spectrum falls well below the absorption curve, presumably because part of the absorption is due to pigments photochemically inactive in photosynthesis. But again the interpretation is quantitatively unsatisfactory because, if all the yellow pigments are inactive, figure 4 indicates that there should be much more divergence at 490 mμ than is shown in figure 7, and the divergence should not start as early as it does in the green. All through the blue one would expect to find more difference between the active absorption and the total absorption, if the light absorbed by the carotenoids were inactive, and if the yield for chlorophyll were constant.

Figure 7 offers general confirmation of the conclusions drawn from the direct quantum yield measurements with totally absorbing suspensions (fig. 5). It also supports our opinion that the difference between the absorption curves for intact cells and extracted pigments (fig. 4) is not due primarily to errors arising from light scattering by the cell suspensions. If the light absorption were seriously in error due to incorrect measurement of the scattered light, one would hardly expect such good agreement between the active absorption and the directly measured absorption in the red region.

Comparison of figure 7 with the corresponding curves for *Chroöcoccus* (Emerson and Lewis, 1942, fig. 5) brings out several interesting points. The *Chlorella* curve shows no maximum at 620 mμ, where the absorption due to phycocyanin in *Chroöcoccus* showed a maximum in both the active absorption and the total absorption. The rise in the active absorption from green toward violet begins for *Chlorella* at about 540 mμ, while for *Chroöcoccus* it is not evident until 480. This difference is presumably due to the absence of chlorophyll *b* in *Chroöcoccus.* It may also be associated with differences in the absorption spectra of the carotenoid pigments of these two organisms (cf. Emerson and Lewis, 1942, p. 582). The absorption curves for the extracted pigments indicate that the yellow pigments of *Chroöcoccus* absorb further toward the red than do the carotenoids of *Chlorella.* This is in accord with the differences in the quantum yield curves for the two organisms, if we accept the evidence that light absorbed by the yellow pigments is inactive or only partially active in photosynthesis. The yield for *Chroöcoccus,* which showed stronger absorption by carotenoids, drops more steeply, going from red toward green on the wave length scale, and goes to a lower level than the yield for *Chlorella.*

The combined evidence from the quantum yield measurements with dense suspensions and the measurements of active absorption discussed above indicates the probability that part of the energy absorbed by the carotenoids must be available for photosynthesis. The net yield for the entire fraction of light absorbed by the yellow pigments must be considerably lower than the yield for the fraction absorbed by chlorophyll.

SUMMARY

Measurements of the quantum yield of *Chlorella* photosynthesis have been made at different wave lengths of light, and compared with the estimated distribution of light absorption between the yellow and green pigment components.

The estimation of light absorption by the pigments in the living cell was based on absorption curves for the extracted and separated components. The absorption characteristics of the pigments were found to be sufficiently altered by extraction so that the conclusions drawn from comparison of absorption and photosynthetic yield could lead to only tentative conclusions as to the activity of accessory pigments.

The quantum yields were measured manometrically, on the basis of oxygen exchange. Preliminary measurements established that the dependence of yield on light intensity was the same for the red,

green, and blue regions of the spectrum, at low light intensities.

Wave lengths of light in the neighborhood of 480 mμ were found under certain conditions to cause large temporary changes in rate of oxygen consumption. Measurements in this region required modification of the usual technique, in order to avoid serious errors in estimating the correction to be applied for respiration during light exposures.

The quantum yield was found to be vanishingly small in the far red beyond 730 mμ. From 730 to 685, it rose steeply to a value of about 0.09. Apart from minor variations, it maintained a constant level to about 580 mμ, then declined to a minimum of about 0.065 at 485 mμ, and rose again to nearly 0.08 at 420 mμ. The source of monochromatic light provided insufficient intensity for satisfactory measurement further toward the ultraviolet.

The significance of the quantum yield measurements was discussed. The low yield at wave lengths longer than 685 mμ was attributed to failure of the lower frequency quanta to raise chlorophyll to the excited state required for the photochemical primary process. The minor variations in yield from 685 to 590 mμ seemed to be connected with differences in the minimum energy required for excitation of the *a* and *b* components of chlorophyll. At frequencies higher than the required minimum, the results indicated that the yield for light absorbed by chlorophyll was essentially constant, at least in the region where there was no appreciable absorption by the yellow pigments. When part of the light was absorbed by the yellow pigments, the evidence suggested some photochemical activity on the part of the yellow pigments, but indicated that the net quantum yield for all the light absorbed by these pigments must be considerably smaller than the yield for light absorbed by chlorophyll.

William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology,
Pasadena, California, and
Mount Wilson Observatory,
Pasadena, California

LITERATURE CITED

Dhéré, Ch. 1937. La fluorescence en biochemie. Les problêmes biologiques. (Monograph Coll.) XXI. Les Presses Univ. de France.

Dutton, H. J., and W. M. Manning. 1941. Evidence for carotenoid-sensitized photosynthesis in the diatom *Nitzschia closterium*. Amer. Jour. Bot. 28: 516–526.

Egle, K. 1939. Ueber die Gueltigkeit der Kundt'schen Regel bei Chlorophyll. Sitzungsber. Heidelb. Akad. Wiss. 1: 19–29.

Eichhoff, H. J. 1939. Die Lichtausbeute bei der Kohlensaeureassimilation. Biochem. Zeitschr. 303: 112–131.

Emerson, Robert, and C. M. Lewis. 1939. Factors influencing the efficiency of photosynthesis. Amer. Jour. Bot. 26: 808–822.

———, and ———. 1941. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis. Amer. Jour. Bot. 28: 789–804.

———, and ———. 1942. The photosynthetic efficiency of phycocyanin in *Chroöcoccus*, and the problem of carotenoid participation in photosynthesis. Jour. Gen. Physiol. 25: 579–595.

Franck, J., C. S. French, and T. T. Puck. 1941. The fluorescence of chlorophyll and photosynthesis. Jour. Physical Chem. 45: 1268–1300.

———, and K. F. Herzfeld. 1937. An attempted theory of photosynthesis. Jour. Chem. Physics 5: 238–251.

French, C. S., 1937. The rate of CO_2 assimilation by purple bacteria at various wave lengths of light. Jour. Gen. Physiol. 21: 71–87.

Gessner, F. 1938. Die Wirkung des Lichtes und der ultravioletten Strahlung auf die Pflanzenatmung. Planta 29: 165–178.

van Gulik, D. 1915. Absorptionsspektrum des Chlorophylls. II. Ann. d. Phys. IV, 46: 147–156.

Katz, E., and E. C. Wassink. 1939. Infrared absorption spectra of chlorophyllous pigments in living cells and in extra-cellular states. Enzymologia 7: 97–112.

Manning, W. M., J. F. Stauffer, B. M. Duggar, and F. Daniels. 1939. Quantum efficiency of photosynthesis in *Chlorella*. Jour. Amer. Chem. Soc. 60: 266–274.

Mothes, K., I. Baatz, and H. Sagromsky. 1939. Die Bedeutung der Carotinoide fuer die Lichtausnuetzung bei der Photosynthese. Planta 30: 289–293.

Strain, H. H. 1938. Leaf xanthophylls. Carnegie Institution of Washington, Pub. No. 490.

Vermeulen, D., E. C. Wassink, and G. H. Reman. 1937. On the fluorescence of photosynthesizing cells. Enzymologia 4: 254–268.

Warburg, O., and E. Negelein. 1922. Ueber den Energieumsatz bei der Kohlensaeureassimilation. Zeitschr. f. phys. Chem. 102: 235–266.

———, and ———. 1923. Ueber den Einfluss der Wellenlaenge auf den Energieumsatz bei der Kohlensaeureassimilation. Zeitschr. f. phys. Chem. 106: 191–218.