[CONTRIBUTION FROM THE DEPARTMENT OF BOTANY AND THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Quantum Efficiency of Photosynthesis in Chlorella[1]

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In its energy requirements, the combination of carbon dioxide and water through the agency of sunlight in the presence of chlorophyll is unique among photochemical reactions. Thus the chemical union of one mole of carbon dioxide and water to form carbohydrate requires the absorption of at least 112,000 calories but the reaction

$$CO_2 + H_2O + Nh\nu = {}^1/_6\,(C_6H_{12}O_6) + O_2;\ \Delta H = 112{,}000 \text{ calories}$$

can be brought about in the living plant with red light of 7000 Å.,[2] which corresponds to only 38,000 calories per mole. The quantum yield in such a process could not be more than 0.34 (molecules of carbon dioxide reduced per quantum of absorbed radiation) and it is unlikely that this maximum efficiency would be achieved, since experimental evidence indicates that there is at least one exothermic step in the process of photosynthesis. Also, light may be absorbed by plant material other than chlorophyll.

Practically the only quantitative work on the absolute quantum efficiency of this photosynthetic process has been done by Warburg and Negelein.[3] These investigators used monochromatic light (except in the red, where a narrow band was used) and measured the radiation with a bolometer calibrated against a Hefner lamp. The algae (Chlorella) were suspended in a cell which was agitated while being illuminated. The gaseous exchange due to photosynthesis was followed by a manometer, which depended on the difference in solubility of oxygen and carbon dioxide. Warburg and Negelein reported a quantum efficiency of practically 0.25 molecule of carbon dioxide per quantum (after making slight corrections for absorption by carotinoid pigments) at wave lengths of 6600, 5780, 5461 and 4360 Å. This value, which is close to the theoretical maximum, has been universally accepted.

The present work was undertaken, originally, for the purpose of extending the measurements of quantum efficiency to conditions more nearly approximating those found in a natural environment. However, in experiments which were carried out even under the most favorable conditions the quantum efficiencies were found to be much less than the 0.25 reported by Warburg and Negelein. Consequently most of the experiments described here were performed in an attempt to obtain higher quantum efficiencies or to find an explanation for the lack of agreement between the two investigations.

Experimental Procedure

Except for a few experiments at 1° in a thermostat (Table VII) all of the measurements were made in a constant temperature room at 25 to 26°.

In most cases the general procedure consisted in sweeping gas through a suspension of algae in a small cell in the dark and again while illuminated with light of measured intensity. In this paper it is assumed that the respiration rate is the same in the dark as in the light; then the increase in the oxygen content or the decrease in the carbon dioxide content of the gas sample collected during illumination, as compared with the sample collected during darkness, is a measure of the amount of photosynthesis.

Photochemical Procedure.—Monochromatic light was produced with the help of a monochromator with a Wadsworth mounting. It contained a hollow glass prism, 12 cm. on an edge, which was filled with ethyl cinnamate.[4] The exit beam was rendered parallel, using a convex lens, and was only slightly smaller than the cross-sectional area of the reaction vessel. A high-pressure, mercury vapor lamp constructed of capillary quartz[5] was used to illuminate the slit. It was possible, with light of 5461 Å., to obtain total energies at the exit slit as large as 40,000 ergs per second.

The same type of mercury lamp was used also for some experiments with polychromatic light. The monochromator was removed and the beam of light, concentrated with a lens, was passed through 1 cm. of a 6% solution of copper sulfate to remove infrared radiation and through a solution of quinine sulfate to remove ultraviolet. Measurements with a rock salt spectrometer indicated that 1 cm. of this copper sulfate solution removed practically all of the infrared radiation.

In other experiments with polychromatic light the source was a tungsten filament lamp. Here also a copper sulfate solution was used to remove infrared and long wave length red radiation.

(1) The authors are glad to acknowledge the support of this investigation by the Penrose Fund of the American Philosophical Society and by the Radiation Committee of the National Research Council.

(2) Hoover, *Smithsonian Misc. Coll.*, No. 21 (1937).

(3) Warburg and Negelein, *Z. physik. Chem.*, **106**, 191 (1923).

(4) A more detailed description of this type of monochromator is given by Bauer and Daniels, THIS JOURNAL, **56**, 378 (1934).

(5) Daniels and Heidt, *ibid.*, **54**, 2381 (1932).

TABLE I

a		b		c	
Agar medium		Solution culture		Solution culture	
$NaNO_3$	0.25 g.	KNO_3	4.0 g.	$Ca(NO_3)_2$	3.28 g.
$CaCl_2$	.25	K_2HPO_4	1.7	KH_2PO_4	1.7
KH_2PO_4	.25	$MgSO_4$	1.25	$MgSO_4$	1.25
$MgSO_4$	.25	Soil extract	1 liter	Fe, Mn and Zn	0.002–0.005
Cane sugar	2				
Water	**1 liter**	Adjusted to *p*H of 6.8		Water	1 liter
Adjusted to *p*H of 6.4				Adjusted to *p*H of 5	

The reaction cell was rectangular in shape, approximately 1 cm. thick and 35 cc. in volume. In the first experiments the cell was constructed of glass plates, held together with de Khotinsky cement and mounted with a rotary glass stirrer operating through a mercury seal at the top.

Gas bubbled through the cell slowly but continuously and the bubbles served further to stir the suspension. Stirring prevented the settling of the algae and hastened the attainment of equilibrium, both between the algae and liquid phase and between the liquid and gas phases.

In later experiments the all-quartz cell shown in Fig. 1 was used. A reciprocating magnetic stirrer was mounted in the outlet tube. The quartz stirrer contained an iron core and was operated with momentary electric currents passed through an external coil of no. 30 copper wire surrounding the tube. In still another vessel glass plates were held by screws in a metal frame and rendered gas tight with petrolatum. The algal cells were placed on the inner surface of the back window as a moist film. A thin layer of fresh egg albumin served to attach the film to the glass and prevent it from slipping. A film of this type permitted more rapid exchange of oxygen and carbon dioxide with the gas stream.

Two different thermopiles were used and calibrated against a standard carbon-filament lamp obtained from the U. S. Bureau of Standards. The one used in most of the measurements had 47 junctions attached to the back of a thin, blackened receiver 1 cm. wide and 1.9 cm. high. Together with the galvanometer it had a sensitivity of 0.0111 cm. per erg per second. The other thermopile was smaller and more sensitive. The two thermopiles gave the same quantum yields and their calibrations were checked further with an actinometer of uranyl oxalate.[6] The necessary corrections were made for light reflected at the windows. Most of the light emerging from the algal suspension was scattered and it was necessary to move the thermopile over a wide area behind the reaction cell to catch all the light passing through the suspension. An average of the thermopile–galvanometer readings was taken.

In measuring the energy absorbed the thermopile reading was taken with the cell filled first with the clear suspending medium and then filled with the algal suspension. The suspensions were of such concentrations that between 10 and 50% of the light was transmitted through the cell and onto the thermopile.

Plant Material.—The unicellular green alga *Chlorella* was used in this investigation. A small-celled plant of this type is particularly suitable for quantitative experiments for several reasons. It is held closely to the temperature of the water which surrounds it. The exchange of material with its environment is rapid. The loss of light by reflection from the surface is less than for a plant surrounded by air. It is to be inferred that these algae contain a larger percentage of active chlorophyll and a correspondingly smaller percentage of photochemically inactive material than do the higher plants. Furthermore, a large number of individuals can be irradiated simultaneously either in the form of a suspension or of a moist film.

This particular alga was chosen because suitable methods for its culture are well known and because it grows rapidly in pure culture in the laboratory. Two species of this alga were used, *Chlorella pyrenoidosa* and *Chlorella vulgaris*. They were brought originally from Pringsheim's collection and were obtained from Mr. C. Barlow of Madison, Wisconsin, in 1933. They are closely related and doubtless have been confused frequently. The former species grew more rapidly under the cultural conditions used in this work.

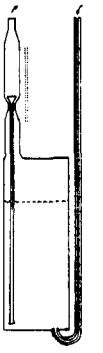

Fig. 1.

As a stock source of inoculum pure cultures of both species have been continued on solid media in test-tubes. The medium is given in Table I under (a). The agar was dissolved by autoclaving and when the medium had cooled almost to its solidification point the *p*H was adjusted with sodium hydroxide. The usual bacteriological methods were followed in preparing the test-tube slants and in inoculating them with the algae under sterile conditions. At room temperature and under fairly intense artificial light the cultures exhibited a heavy surface growth in about three weeks.

The algal cells used in preparing the suspensions and films were grown in liquid nutrient solution as shown in Table I under (b) and (c). The soil extract of (b) was used to supply the calcium, iron and other elements which might be beneficial. It was prepared by shaking approximately 500 g. of loam soil with one liter of distilled water for thirty minutes and filtering the supernatant liquid through cellite until perfectly clear. This solution may have varied slightly because of variations in the soil samples. Consequently the entirely synthetic medium given in (c) was used for all the later experiments. Manganese and zinc were added as sulfates, and the iron as ferric chloride. The (c) solution, plus 15 g. of agar, 3 g. of beef extract and 5 g. of peptone, was also used in the latter part of the work for the stock cultures.

(6) Leighton and Forbes, THIS JOURNAL, **52**, 3139 (1930).

The algae were grown in 100-cc. quantitites of these solutions in 250-cc. Erlenmeyer flasks. Each flask was provided with a rubber stopper containing two cotton-plugged glass tubes. After autoclaving and cooling, each flask was inoculated under sterile conditions with algal cells from one of the stock cultures just described. Paraffin was used to make the glass–rubber seals gas tight. The flasks (up to 36 in number) were kept at a temperature between 18 and 22° in a shallow, glass-bottomed tray and illuminated by six 60-watt frosted bulbs placed 30 cm. below the tray. Air containing 5% carbon dioxide was passed through the flask continually at a rate more than sufficient to supply the carbon dioxide necessary for growth. Ordinarily the cultures were illuminated for eight to ten hours per day.

In the first experiments the suspension of algal cells was prepared by centrifuging the algae out of the culture medium and suspending 0.4 cc. of the algal cells in 30 cc. of decolorized nutrient solution. In the later experiments, summarized in Table II, the algal cells were not centrifuged but the supernatant culture medium was pipetted off and replaced with fresh colorless nutrient solution.

Chemical Analysis.—The gas mixture of 90–92% nitrogen, 4–5% carbon dioxide and 4–5% oxygen was prepared from commercial tanks, in a 10-liter carboy. The displacing liquid of 77% water, 20% sodium sulfate, and 3% sulfuric acid dissolved the carbon dioxide only to a limited extent but, to avoid change in composition, the gas for a given experiment was stored over mercury in a half-liter glass storage vessel. The rate of gas flow through the reaction vessel was controlled by regulating the flow of mercury into the storage vessel. Tests were made which indicated that mercury vapor or other impurities present in the gas stream had no measurable effect on the observed quantum efficiencies.

After leaving the reaction vessel the gas mixture passed through a small drying tube and then to a three-way stopcock. From this point the gas stream either passed through a bubble counter and thence into the atmosphere or else it was collected for analysis. Two different methods of analysis were employed. In the first method successive portions of the gas were collected in sampling bulbs each of 100-cc. capacity, and analyzed with a Burrell–Haldane gas-analysis apparatus, Model B. Four determinations were made on each sample. In the second method, gas from the reaction vessel was drawn directly into the buret of a special, all-glass gas-analysis apparatus containing solid absorbents, ascarite for carbon dioxide, and finely divided yellow phosphorus (covered with a thin film of phosphoric acid) for oxygen. Each analysis used about 25 cc. of gas. The agreement between check analyses and between the two different analytical procedures was sufficiently close to show that any observed variations in the quantum yields were not due to the chemical analyses.

In the earlier experiments the gas from the carboy was bubbled through the reaction cell for at least three or four hours in darkness to ensure approximate equilibrium with the liquid. The mercury reservoir was then filled with gas from the carboy. This gas was bubbled through the cell at the rate of 100 cc. per hour. In the meantime, irradiation of the algae was begun. The gas was passed through the cell for an initial period of at least thirty minutes to reach equilibrium under conditions of illumination and to sweep out the drying chamber and connecting tubes leading to the sampling bulbs. The gas swept through during the next hour was collected and analyzed. Throughout these experiments the readings of the thermopile, situated at the back of the cell, were recorded at frequent intervals. The incident light was measured every fifteen minutes. At the end of an hour the light was shut off and as before the gas was swept through for thirty minutes or more. Then during the next sixty minutes a 100-cc. sample of gas was collected and analyzed to obtain the composition of gas in equilibrium with the algae in the dark. A second set of determinations was usually made directly following the first. A double series of measurements usually required a continuous period of eight to ten hours. In some of the experiments the analyses were made on the unilluminated algae first and the illuminated algae second, with the rate of flow reduced to 50 cc. per hour to obtain a greater change in composition for a given amount of photosynthesis.

In many of the experiments recorded in Table V a different procedure was followed in order to obtain a direct measure of the total change taking place during exposure to light. The gas flow during darkness was reduced to 33 cc. per hour. At the beginning of illumination the gas flow was stopped and not restarted until after the illumination was stopped (usually sixty to seventy minutes). Then the flow was restarted at a rapid rate (25 cc. in three minutes) and all of the next 25 cc. of gas was collected in the analysis buret. The flow was again stopped while the gas sample was analyzed (twenty-five to thirty-five minutes) and then another sample collected as before. In this way, it was possible to keep the over-all average rate of flow at 33 cc. per hour. Collection and analysis of samples was continued until the gas composition returned practically to its original value for darkness. Three or four samples were usually required to meet this condition. The total change in carbon dioxide and oxygen, caused by illumination, was obtained by adding the results of the successive analyses. In this method the accuracy of results does not depend on the attainment of equilibrium with the gas during illumination. No marked difference in quantum yield nor in consistency of results was noted in the two methods.

A few experiments were carried out in an entirely different manner using a closed reaction vessel (a glass-stoppered bottle 37 × 37 × 90 mm. having a volume of 125 cc.). The flat sides of the bottle were silvered except for a front and back window. A second bottle identical in size was entirely covered with black paint and used to determine the correction for respiration. Both bottles were filled with algae in suspension and the bottle with windows was then illuminated with measured radiation for periods ranging from forty to two hundred minutes. Immediately after illumination both bottles were analyzed for dissolved oxygen by the Winkler method. The difference in oxygen content between the two bottles gave a measure of the amount of photosynthesis. This method is very simple and it avoids the necessity for long preliminary periods to attain equilibrium between the gaseous and liquid phases.

Results

Very early in this work it was found that despite the precautions taken in experimental procedure, the values obtained for the quantum efficiency of photosynthesis varied over a wide range, from a certain maximum to approximately zero. In some instances where the light intensity was relatively high, a low efficiency was expected, in large part because the carbon dioxide limited the process. Even at lower intensities these variations, while strictly limited in range, were actually often large compared with any possible errors introduced in the energy measurements or chemical analyses. On the other hand, we attempted to secure reasonable uniformity of the physiological condition of the plant material used, at the same time preparing the plant material according to the method which past experience had shown suited to the attainment of a high efficiency. There are undoubtedly a very large number of possible physiological combinations of the materials of the cell which cannot be regulated easily and which could be responsible for these variations. Because of these possibilities, the principal significance in the results, so far as the photochemical mechanism is concerned, must be attached to the maximum yield obtained at a light intensity where the light factor alone limits the process, or to an average of values which are near the maximum, rather than to any over-all average of the results.

The results are given in Tables II, IV, V and VII.

In the experiments recorded in Table II the green line, 5461 Å., of the mercury vapor lamp was isolated by the monochromator and the resulting radiation passed through the cell provided with a mercury-sealed stirrer. A quantity of algal cells (0.4 cc.) suspended in 30 cc. of nutrient solution (corresponding to a concentration of about 465,000 cells per cc.) was used for each experiment. The area of the parallel light beam was 25.6 sq. cm. The gas was bubbled through continuously at the rate of 100 cc. per hour. The quantum yields are recorded in molecules of carbon dioxide consumed, ϕ_{CO_2}, and oxygen liberated, ϕ_{O_2}, per quantum absorbed. Because of the small change in gas composition (see

TABLE II

QUANTUM EFFICIENCY MEASUREMENTS AT 5461 Å.

Light intensity, ergs/sq cm./sec.	ϕO_2	ϕCO_2
1618	0.043	0.046
1663	.043	.045
1747	.066	.049
1403	.010	.036
937	.010	.056
1227	.060	.078

TABLE III

SAMPLE CALCULATION

Flow method — Green line of Hg arc (5461 Å.)
Av. rate of gas flow, 100 cc. per hour. — 0.4 cc. algae in 30 cc. of nutrient soln.

Incident intensity (corrected for reflection), 1663 ergs/sq. cm./sec.
Area of illumination, 25.6 sq. cm.
Total incident energy, 1663 × 25.6 = 42,580 ergs/sec.
Total transmitted energy 8,830 ergs/sec.
Energy absorbed by nutrient soln. 1,300 ergs/sec.
Energy absorbed by algae = 32,450 ergs/sec.
Time of illumination, 3240 seconds[a]
Total quanta absorbed = $32{,}450 \times 3240/h\nu = 2.92 \times 10^{19}$

Gas analysis

Light		Dark	
$\%CO_2$	$\%O_2$	%CO	$\%O_2$
5.190	5.580	5.234	5.538
5.167	5.581	5.238	5.522
5.176	5.590	5.241	5.535
5.187		5.228	
Mean 5.180	5.584	5.235	5.532

ΔCO_2 = 0.055% (also 0.055 cc. since gas flow is 100 cc. per hr.)
≡ .049 cc. (N. T. P.) = 1.33×10^{18} molecules
ΔO_2 = .052%
≡ .046 cc. (N. T. P.) = 1.26×10^{18} molecules

$$\phi_{CO_2} = \frac{1.33 \times 10^{18}}{2.92 \times 10^{19}} = 0.045 \qquad \phi_{O_2} = \frac{1.26 \times 10^{18}}{2.92 \times 10^{19}} = 0.043$$

[a] The experiment lasted for one hour (3600 seconds), but the light was shut off from the algal suspension for a total of six minutes while intensity measurements were being made.

Table III), the experimental uncertainty of these values is large, particularly at low light intensities. In Table II, the carbon dioxide values are more reliable than the oxygen values.

Table III gives a sample calculation, showing how the quantum efficiency values were obtained for the second experiment listed in Table II.

Experiments were made also at 4358 Å. but it was difficult to obtain sufficient intensity at this wave length and the results are not very reliable. The observed yields were very low.

The next results are for experiments carried out with an algal film. Again the gas was passed through the chamber continuously in the dark and in the light at the rate of 100 cc. per minute. Under the conditions prevailing at the time, the quantum efficiency for monochromatic light at 5461 and 4358 Å. was not significantly different from zero. The results with the polychromatic light of the mercury lamp (without the monochromator) are given in Table IV. For purposes of calculation the average wave length was taken to be 5000 Å.

TABLE IV

QUANTUM EFFICIENCY MEASUREMENTS WITH POLYCHROMATIC LIGHT FROM Hg ARC (ALGAL FILM)

Light intensi y, ergs/sq. cm./sec.	ϕO_2	ϕCO_2
1741	0.046	0.055
2845	.050	.051
2054	.021	.042
1261	.018	.041
1498	.013	.035

The number of experiments listed in Tables II and IV is not sufficient and the data are too widely scattered to justify the selection of an average maximum value for the quantum efficiency. However, it appears significant that none of the values approaches the figure 0.25, which was observed by Warburg and Negelein.[3]

In Table V are listed, in order of decreasing quantum efficiency, the results obtained with a tungsten filament lamp from which the infrared and long wave length red was filtered. For purposes of calculation the average wave length was assumed to be 6300 Å. The vessel shown in Fig. 1 was used, with the period of illumination sixty to seventy minutes. Except for two experiments, the intermittent flow method was used instead of the continuous flow method, with the average rate of gas flow 33 cc. per hour.

TABLE V

QUANTUM EFFICIENCY MEASUREMENTS WITH POLYCHROMATIC LIGHT (FILAMENT LAMP)

Algal concn., cells per cc. × 10^{-6}	Incident intensity, ergs/sq. cm./sec.	ϕO_2
89	12700	0.027
277	24000(±5%)	.015
185	25700	.014
148	24600	.014
115	23800	.013
174	7700	.013
770[a]	24000(±5%)	.013
63	19800	.012
240	23300	.012
138	24000(±5%)	.011
599[a]	24000(±5%)	.011
139	22900	.011
29	24000(±5%)	.009
201	23300	.007
76[b]	24000	.006
406	21800	.005
871	24000(±5%)	.005
131	7800	.005
321	24000(±5%)	.004
178	23300	.004
420	24000(±5%)	.003
347	24000(±5%)	.003
495	24000(±5%)	.002

[a] In these experiments the constant flow method was used. [b] Thirty-minute illumination period.

Table VI illustrates the method of calculation for experiments in which the intermittent flow method was used. The calculation is for the third experiment in Table V.

The light intensity in most of the experiments listed in Table V was considerably higher than in those in which a mercury lamp was used as a light source. The efficiencies are all based on the observed increase in oxygen, since these values were much more consistent than were those for carbon dioxide. For the experiments at intensities between 20,000 and 26,000 ergs/sq. cm. sec. there is a significant grouping of quantum efficiency values near a maximum value of approximately 0.015. For the seven experiments with values of 0.012 or higher the mean is 0.013 ±0.001, which may be considered close to the maximum yield obtainable under these experimental conditions.

A comparison with results obtained under different experimental conditions, but using the same strain of Chlorella and approximately the same total carbon dioxide concentration,[7] indicates that the quantum efficiency at 23,000

(7) Manning, Juday and Wolf, THIS JOURNAL, **60**, 274 (1938), Fig. 3.

TABLE VI

SAMPLE CALCULATION

Intermittent flow method — Tungsten fil. lamp ($CuSO_4$ filter)
Av. rate of gas flow, 33 cc. per hour — Algal concentration, 185×10^6 cells per cc.
Incident intensity (corrected for reflection), 25,700 ergs/sq. cm./sec.
Area of illumination, 27 sq. cm.
Total incident energy, $25,700 \times 27$ = 694,000 ergs/sec.
Total transmitted energy 181,000 ergs/sec.
Energy absorbed = 513,000 ergs/sec.
Time of illumination, 4170 seconds
Total quanta absorbed = $513,000 \times 4170/h\nu = 6.86 \times 10^{20}$ (av. λ = 6300 Å.)

Gas analysis

Temperature, 25° Pressure, 745 mm.

	Dark sample	1st samp. after illum.	2nd samp. after illum.	3rd samp. after illum.	4th samp. after illum.
$\%CO_2$	4.16	3.53	3.83	4.00	4.17
$\%O_2$	4.08	5.35	4.31	4.00[a]	4.04
Total gas volume, cc.	27.40	27.34	27.34	27.40	27.43
CO_2 assimilated, %		0.63	0.33	0.16	...
CO_2 assimilated, cc.		.172	.090	.044	...
O_2 evolved, %		1.27	.23	...	...
O_2 evolved, cc.		0.347	.063	...	...

Total volume change (25°, 745 mm.): $\Delta CO_2 = 0.172 + 0.090 + 0.044 = 0.306$ cc. $= 7.41 \times 10^{18}$ molecules
$\Delta O_2 = 0.347 + 0.063 = 0.410$ cc. $= 9.93 \times 10^{18}$ molecules

$$\phi_{CO_2} = \frac{7.41 \times 10^{18}}{6.86 \times 10^{20}} = 0.011 \qquad \phi_{O_2} = \frac{9.93 \times 10^{18}}{6.86 \times 10^{20}} = 0.014$$

[a] In most of the experiments with the intermittent flow method, the oxygen concentration dropped to slightly below the original dark value before finally rising to approximately the original figure. This is most easily interpreted as indicating that respiration was more rapid for a short time after illumination than it was before illumination. The carbon dioxide values did not oscillate in this manner, probably because of the greater slowness with which the carbon dioxide equilibrium was attained.

ergs/ sq. cm./sec. should be roughly two-thirds of the maximum low-intensity value. Actually the maximum efficiency at 23,000 ergs/sq. cm./sec. for determinations shown in Table V is less than two-thirds of the highest efficiency values shown in Tables II and IV. The probable explanation for the discrepancy is that for all of the experiments in Table V the algal concentration was great enough so that at fairly high light intensities the carbon dioxide concentration may have been reduced below the equilibrium value, whereas the other experiments referred to[7] were made with low algal concentrations and in a closed system with the carbon dioxide supplied by a buffer mixture.

For the seven experiments in Table V giving a mean quantum efficiency of 0.013 on the basis of the oxygen evolved, the average quantum efficiency calculated from the amount of carbon dioxide absorbed was approximately 0.009 with an average deviation three times as large as for the oxygen determinations. The lower quantum efficiency calculated on the basis of carbon dioxide suggests that there are complications in the physiological behavior of the algae under the conditions of the experiment. Possibly in the long period of darkness some of the products of respiration are only partly oxidized, giving for example plant acids, which may then undergo photochemical change during illumination. In the results of Tables II and IV, where there is no definite tendency for the carbon dioxide change to be less than the oxygen change, the products of reaction during most of the first thirty minutes of illumination were not collected for analysis and the preliminary dark period was three or four hours instead of about fifteen hours.

In Table VII are given the results of experiments carried out by Dr. R. O. Sutherland in closed bottles using the Winkler method to de-

TABLE VII

Temp., °C.	Algal concn., cells/cc. $\times 10^{-6}$	Wave length, Å.	Illumination period, min.	Incident intensity, ergs/sq. cm./sec.	ϕ_{O_2}
25.0	38	5461–5780	73	4020	0.043
25.0	20	5461	135	1760	.050
25.0	6.9	4358	142	830	.065
1.0	..	4358	75	3600	.027
1.0	..	4358	40	4770	.019

termine the dissolved oxygen. In the experiments at 1° an entirely different monochromator, thermopile, and galvanometer were used.

The quantum efficiency values fall in the same range as the values given in Tables II and IV in which light of similar intensity was used. In the experiments at 1°, particularly with the higher light intensity, it is probable that the rate of intermediate thermal reaction (or reactions) was slow enough to reduce the quantum yield by a perceptible amount.

The results of experiments using closed reaction vessels vary much less between themselves than do those in the other type of experiments, perhaps a further indication that part of the physiological variability may be introduced during long preliminary treatment of the algae before irradiation. Further experiments using this type of vessel and the Winkler method are being carried out in this Laboratory by Mr. H. G. Petering. These results, to be published in detail in a later paper, give yields approximately the same as recorded here for low intensities. Possible stimulation of respiration by light is also being studied.

Discussion

The differences in experimental procedure between the work of Warburg and Negelein[3] and the present work do not provide any obvious explanation for the wide discrepancy between their results and ours.

Warburg and Negelein sometimes referred to the species of alga used in their work as *Chlorella vulgaris*, but it probably was the same species as the *C. pyrenoidosa*[8] used in our experiments. All of the results reported in this paper were obtained from measurements with *C. pyrenoidosa*, but a few preliminary experiments were made using a slower-growing species of Chlorella which was identified as *C. vulgaris*. The quantum efficiencies observed for this second species were not significantly different from those with *C. pyrenoidosa*. Despite some physiological differences,[8] it would be surprising if any fundamental difference were to exist in the photochemical part of photosynthesis between two such similar species. Nevertheless, one of us (J. F. S.) is now conducting a series of experiments with *Chlorella vulgaris* and *C. pyrenoidosa* in which the culture conditions and experimental procedure are being patterned as closely as possible after the methods described by Warburg and Negelein.

The carbon dioxide concentrations and many of the light intensities in our experiments were approximately the same as those used by Warburg and Negelein.

The algal concentrations in our experiments were such that from 10 to 50% of the incident light was transmitted. Some light was lost by scattering, but measurements made with various shapes of absorption vessels indicated that this lost light always amounted to less than 5%. Warburg and Negelein worked with cell suspensions heavy enough to absorb practically all of the incident light, eliminating the necessity of making transmission measurements. This introduces the disadvantage of having, at any instant, a large proportion of the algal cells receiving little or no light. The total amount of respiration, for which correction must be made, then becomes very large. Warburg and Negelein partially compensated for this by making their measurements at a temperature of 10°, where respiration is much slower than at 25°.

There is a possibility that at sufficiently low light intensities the process of photosynthesis may have a temperature coefficient less than unity, which would tend to produce a higher efficiency at 10 than at 25°. A possible reason for a coefficient of less than unity may be found in the probability that a number of intermediate products may be formed during the successive light absorptions which occur in the course of the reduction of carbon dioxide to formaldehyde.[9] If the time between the absorption of successive quanta is long enough for a perceptible fraction of intermediate compounds to decompose thermally, then either an increase in temperature or a decrease in light intensity probably would result in a decrease in quantum efficiency. An increased temperature should increase the rate of decomposition of the intermediate compounds while a decreased light intensity would allow more time for decomposition to occur. On this basis, at intensities no lower than those used by Warburg and Negelein, and by us, the temperature coefficient could be only slightly, if any, less than unity, since in neither investigation is there any definite indication of a decrease in efficiency with decreasing light intensity.

(8) Emerson and Green, *Plant Physiol.*, **12**, 538 (1937).

(9) (a) Emerson, *Ergebnisse Enzymforschung*, **5**, 305 (1936); (b) Franck and Herzfeld, *J. Chem. Phys.*, **5**, 239 (1937).

At intensities near 1000 ergs/sq. cm./sec., thirty seconds or more must elapse, on the average, between the absorption of successive quanta by a single chlorophyll molecule. Recent work on wheat plants by McAlister,[10] in which high intensity intermittent light (equal periods of light and darkness) was used, indicates that at room temperature the rate of photosynthesis passes through a minimum for light and dark periods of about sixty seconds. McAlister holds that this minimum is caused by the plant spending much of its time in an induction period. If the dark period is long enough for many of the intermediate compounds to decompose, then the quanta which were used to build up those intermediate compounds will have been wasted, and the efficiency correspondingly decreased. The induction period would be the time required to again build up the concentration of intermediates to the equilibrium value.[11] Thus the situation is similar to that which may exist with very low intensity continuous light.

If the trend in McAlister's data is actually due to decomposition of intermediate compounds, the rate of decomposition is probably fast enough to cause a measurable falling off of quantum efficiency with continuous light at intensities not far below 1000 ergs/sq. cm./second. If such a falling off is not found, another argument would be created for the existence of a photosynthetic unit, with a large number of chlorophyll molecules coöperating in the reduction of one carbon dioxide molecule.[12] A study of this problem is now being carried on in our Laboratories.

In conclusion, it should be noted that all of the procedures described in this report are in agreement, in so far as they give quantum efficiencies much lower than the value found by Warburg and Negelein. The measurements have extended over a period of five years, making it very improbable that a temporary physiological condition of the algae was responsible for the low results. During the course of these measurements two different monochromators and three different thermopile–galvanometer combinations, each independently calibrated, have been employed. Experiments using monochromatic and polychromatic light, and using constant and intermittent methods of gas flow, as well as experiments in closed reaction vessels, all have given results in approximate agreement. Moreover, experiments carried out under conditions approximating a natural environment, in Trout Lake, Wisconsin,[7] in which the same strain of Chlorella was used, gave results in agreement with those reported here.

A calorimetric investigation of the efficiency of photosynthesis is now being carried out in these Laboratories by Mr. T. W. DeWitt. Complete results will be published later, but in measurements made thus far, between 80 and 90% of the visible radiation absorbed by the algae has appeared as heat, even under conditions supposedly favorable for a high efficiency of photosynthesis. With a quantum efficiency of 0.05, at 5461 Å., 89% of the absorbed energy should appear as heat. Therefore, preliminary results with the calorimetric method are in agreement with the results presented in this report.

It is possible that physiological differences in plant material are responsible for the difference between the efficiencies found by Warburg and Negelein and by us. However, the algae used in our experiments have been cultured under a wide variety of experimental conditions, without producing any marked increase in efficiency.

If further work confirms our results, rather than those of Warburg and Negelein, the present ideas of the nature of the light process in photosynthesis must be greatly modified. On the basis of the quantum efficiency of 0.25 reported by Warburg and Negelein, the available energy must be used with nearly 100% efficiency, a fact which would sharply limit the number of possible mechanisms. On this basis, attempts to formulate a specific mechanism, with identifiable intermediate compounds, are justifiable and profitable.[9b] But if the maximum efficiency is only 0.04 or 0.06, then the possibilities for energy dissipation by side reactions, deactivation of chlorophyll, and inefficient forward steps in the process become very numerous, and the number of energetically possible mechanisms becomes large.

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(10) McAlister, *Smithsonian Misc. Coll.*, **95**, No. 24 (1937).

(11) However, Gregory and Pearse, *Ann. Bot.*, **1**, 3 (1937), believe that results such as McAlister's may be at least partially explained by stomatal behavior.

(12) Arnold, *J. Gen. Physiol.*, **17**, 135 (1933).

Summary

Using monochromatic and polychromatic light of relatively low intensity, measurements have been made of the quantum efficiency of photosynthesis in *Chlorella pyrenoidosa*. These measurements indicate a probable value of approximately 0.05 molecule per quantum, which is smaller than the value which is now generally accepted.

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Photosynthesis in Chlorella. Quantum Efficiency and Rate Measurements in Sunlight

BY WINSTON M. MANNING, C. JUDAY AND MICHAEL WOLF

Warburg and Negelein,[1] using monochromatic light at intensities of about 10^3 ergs/sq. cm./second, found a value of 0.25 (molecules of carbon dioxide assimilated per quantum absorbed) for the quantum efficiency of photosynthesis in Chlorella. Recent experiments with Chlorella at the University of Wisconsin, in which low intensity monochromatic light was used, have yielded very much lower values for the quantum efficiency, not higher than 0.06.[2] Because of the large discrepancy between these values, it seemed desirable to make additional quantum efficiency determinations using an entirely different technique. Accordingly, the experiments described in this paper were carried out at Trout Lake, Wisconsin, using the sun as a light source and varying the intensity of the light by placing samples of Chlorella at various depths in the lake.

These experiments were carried out in July and August, 1936, and constitute one phase of the photosynthesis investigations which are a part of the general limnological program of the Wisconsin Geological and Natural History Survey.[3]

Experimental

The *Chlorella pyrenoidosa* used in this work was the same strain as used in the experiments with monochromatic light.[2] The average cell diameter was about four microns. For each experiment, material was taken from a liquid culture suspension,[4] centrifuged, and then suspended in filtered Trout Lake water. This suspension was then transferred to round or square calibrated bottles (average volume approximately 140 cc.). The sides of the square bottles, while not optically perfect, were plane enough to permit a fairly accurate measurement of the fraction of light absorbed by suspensions of Chlorella. The concentration of the suspension, in cells per cc., was determined by counting under a microscope the number of cells per unit volume. For each depth during a run, four of the bottles were placed in a small wire basket, the two lower bottles in each basket being painted black and covered with black cloth bags to keep out light. The baskets were suspended simultaneously in the lake for a definite time (usually one to four hours), at depths varying from the surface to fourteen meters, and then analyzed immediately for dissolved oxygen, using a modified form of the Winkler method. In addition, from two to six bottles of the cell suspension had been analyzed for oxygen at the beginning of each run. The change in oxygen content of the black bottles gave a measure of the respiratory rate, while the difference in final oxygen content of the black and clear bottles gave a measure of photosynthesis. This of course involves the assumption that respiration is the same in light and darkness.

Oxygen analyses on duplicate bottles usually agreed to better than 0.2 mg. of oxygen per liter except when the amount of photosynthesis was sufficient to raise the concentration of dissolved oxygen far above the usual saturation value.

The carbon dioxide concentration of Trout Lake water is approximately 38 mg./liter. This figure includes free, half-bound and bound carbon dioxide. The carbonate–bicarbonate concentration is high enough to give a moderate buffering action.

The water temperature at the surface of Trout Lake varied from 24 to 21° during the period of this work. Down to about eight meters (top of the thermocline) the temperature dropped only slightly, usually one degree or less, and the rate of photosynthesis at eight meters in Trout Lake is in all cases so far below the maximum rate at high light intensities that the sharp temperature drop in the theromocline probably has little effect on observed rates.

The amount of solar radiation reaching the surface of the lake during the experiments was measured with a self-recording solarimeter; the percentage of transmission and the spectral composition of the radiation at various depths

(1) Warburg and Negelein, *Z. physik. Chem.*, **106**, 191 (1923).

(2) Manning, Stauffer, Duggar and Daniels, THIS JOURNAL, **60**, 266 (1938).

(3) (a) Schomer and Juday, *Trans. Wis. Acad. Sci.*, **29**, 173 (1935); (b) Curtis and Juday, *Intern. Rev. ges. Hydrobiol. Hydrog.*, **35**, 122 (1937); (c) Manning, Juday and Wolf, submitted to *Trans. Wis. Acad. Sci.*

(4) Details concerning the culture methods and other parts of the procedure may be found in refs. 2 and 3.