culation of the square root of the ratio of the product of the three principal moments of inertia for the two configurations. The ratios $[(I_1I_2I_3)_{cis}/(I_1I_2I_3)_{trans}]^{1/2}$ for the glyoxal and dimethylglyoxal molecules are 1.15 and 1.00, respectively. For the equilibrium *cis* $\rightleftarrows$ *trans* these values combined with the ΔE values given above correspond to equilibrium constants of 87,000 and 20 for glyoxal and dimethylglyoxal, respectively; that is, the fraction of *cis* glyoxal is negligible and that of *cis* dimethylglyoxal may be about 5%. These results agree with our electron diffraction investigations.

We express our thanks to Dr. Linus Pauling for his helpful criticism and discussion and to Dr. D. P. Stevenson for assistance with the calculations and helpful discussion.

Summary

The configurations of glyoxal and dimethylglyoxal as determined by the electron diffraction method are given by the following parameters:

Glyoxal: C—H = 1.09 Å. (assumed), C=O = 1.20 ± 0.01 Å., C—C = 1.47 ± 0.02 Å., angle C—C=O = 123° ± 2°

Dimethylglyoxal: C—H = 1.09 Å. (assumed), C=O = 1.20 ± 0.02 Å., C_2—C_3 = 1.47 ± 0.02 Å., C_1—C_2 = 1.54 ± 0.02 Å., angle CO—C=O = 123° ± 2°, angle CH_3—C=O = 122.5° ± 1°

The electron diffraction data and the dipole moment data as well as chemical information indicate uniformly that both molecules are coplanar with the *trans* configuration and that rotation around the carbon–carbon bond connecting the adjacent carbonyl groups is restricted.

PASADENA, CALIFORNIA RECEIVED OCTOBER 23, 1939

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

Quantum Efficiency of Photosynthesis in Chlorella. II

BY H. G. PETERING,[1] B. M. DUGGAR AND FARRINGTON DANIELS

Photosynthesis in algae has been the object of an extended investigation in these laboratories. The quantum efficiency was found to be of the order of 0.05 molecule per quantum,[2] much lower than the older value of 0.25 reported by Warburg and Negelein.[3] In order to study further the reasons for this large discrepancy, a new method was developed[4] for the rapid determination of dissolved oxygen making use of the dropping mercury electrode. The respiration correction seemed to be the most important source of error, and the manometric method used by Warburg and Negelein involves a considerable time lag in determining the rate of respiration and of photosynthesis. If respiration is faster under the conditions imposed by photosynthesis, then a prompt determination of respiration after the light is turned off is necessary if arbitrary assumptions are to be minimized. Results obtained with the dropping mercury electrode are given here. They agree essentially with those reported earlier[2] and are in definite disagreement with those of Warburg and Negelein.[3] Recently Rieke[5] has reported a value of 0.24 obtained with the manometric method in agreement with Warburg and Negelein.

Experimental Procedure

The material was *Chlorella pyrenoidosa*, of the same strain as used before.[2] It was grown in pure culture on agar slants and periodically transferred to fresh slants or to liquid media. The liquid culture was essentially the salt nutrient recommended by Warburg and Negelein.[3] The composition was:

Agar medium, g.		Liquid nutrient, *M*	
$NaNO_3$	0.25	$MgSO_4$	0.020
$CaCl_2$	.25	KNO_3	.005
KH_2PO_4	.25	KH_2PO_4	.018
$MgSO_4$	.25	$FeSO_4$	.00001
Cane sugar	2.00		
Water	1 liter		

Distilled water from a block tin condenser was used in most of the experiments but no differences were noted when in some of the experiments tap water from Lake Mendota was used. The algae were grown in 300-cc. flasks on a water-cooled rack illuminated from the bottom, while air, to which had been added 5% carbon dioxide, was bubbled through. Warburg's recommendations were followed in a general way, exposing algal cultures to bright light (four 200-watt filament lamps at 30 cm.) for a week and then to weaker light (six 25-watt lamps at 45 cm.).

(1) Present address: Chemical Section, Michigan Experiment Station, East Lansing, Michigan.

(2) W. M. Manning, J. F. Stauffer, B. M. Duggar and F. Daniels, THIS JOURNAL, **60**, 266 (1938).

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

(4) H. G. Petering and F. Daniels, THIS JOURNAL, **60**, 2796 (1938).

(5) F. F. Rieke, *J. Chem. Phys.*, **7**, 238 (1939).

The cultures were not used after they were more than fourteen days old. A normal growth curve was obtained for the algae under these conditions, showing rapid growth from the fourth to the eighth day.

In preparing a sample for measurements the supernatant liquid was decanted and the remaining algal solution was diluted immediately with fresh nutrient solution, which had been passed through filter paper to provide sufficient organic material for proper action of the dropping mercury electrode. The *p*H of the solution was adjusted to 5.5, the value attained by the growing algae in about a week. In some earlier experiments the algae were concentrated by centrifuging and then diluting but in later experiments this was avoided since it may affect the respiration. The algae were diluted until 50–75% of the light was transmitted by a solution 1.6 cm. thick. This usually required the addition of ten volumes or more of the fresh nutrient solution.

A 500-watt projection lamp was used with a lens and filter system giving parallel light chiefly between 6100 and 7200 Å. with a sharp maximum at about 6400 Å. A Corning No. 243 signal red filter was used together with 9 cm. of an 0.05 *N* copper sulfate solution. In some of the experiments with higher light intensity the copper sulfate was 0.025 *N* and tests showed that 10 to 15% of the total radiation might have been in the longer red and short infrared, but very little of this could have been absorbed by passage through the 1.6 cm. cell.

The incident and transmitted light were measured with a large-area thermopile having thirty junctions. Calibration was effected with a U. S. Bureau of Standards radiation lamp and corrections were made for reflections at the windows. Each cm. deflection of the thermopile-galvanometer was equivalent to 72.5 ergs per second per sq. cm. The room was thermostated between 24 and 26°. All of the light falling on the reaction cell was homogeneous with respect to both color and intensity. The entire cell was illuminated and half or more of the light was transmitted. Since most of the transmitted light was scattered, the thermopile was placed as close behind the reaction cell as possible. Tests showed that the thermopile could be moved from side to side over a wide range and forward and backward for several millimeters without changing the galvanometer deflections. The total light transmitted was calculated by multiplying the light falling on the thermopile by the ratio of the area of the reaction cell to that of the thermopile.

The reaction cell, 4.5 × 3.1 cm. in cross section and 1.6 cm. in thickness,[6] was placed in front of the thermopile. The narrow neck at the top of the cell permitted the insertion of the thin capillary tip of the dropping mercury electrode and the U-tube at the bottom kept at constant level the anode pool of mercury, 1 sq. cm. in area. The algal suspension was added until it over-flowed the cell and then the cathode tip was inserted at the center without forming air bubbles. The mercury dropped at the rate of about 1 drop per second[7] and the galvanometer readings were taken at 1.00 volt and at 0.10 volt. The difference in galvanometer readings at the two voltages was a straight line function of the oxygen concentration[8] as determined by the Winkler chemical analysis for dissolved oxygen. At 25° the calibration gave 9×10^{-9} mole of oxygen per cc. for each cm. deflection. The algae were not stirred because the whole cell was illuminated and the algae did not settle appreciably during the time of the determination. Experiments in which the cell was shaken between measurements showed that the stirring was not necessary.

(6) Fig. 2B of reference 4.

(7) It is necessary to keep the dropping rate constant and the temperature of the room should not vary by more than 1 or 2°, particularly if a galvanometer shunt is used. It is essential that the drops of mercury coalesce immediately with the anode pool of mercury. If organic matter prevents this coalescence it is necessary to use an external anode connected through a salt bridge in agar.

Results

A series of twenty-nine determinations was first obtained with the Winkler titration method and the quantum yields were between 0.02 and 0.07 in agreement with the earlier work.[2] However, it was found that the rate of oxygen consumption after illumination changed with time and the method was abandoned in favor of the rapid electrical method described here which permits a prompt determination of the rates of

TABLE I

QUANTUM YIELDS IN PHOTOSYNTHESIS

Expt.	Age culture in days, light intense + weak	Incident intensity (ergs/sec. sq. cm.)	% Absorption	Molecules per quantum
1	11 + 5	2210	26.7	0.069
2	3 + 0	2080	23.3	.069
3[a]	3 + 0	2080	23.3	.065
4	9 + 7	1820	27.2	.070
5[a]	9 + 7	1820	27.2	.068
6	3 + 0	1490	42.0	.070
7	3 + 0	1490	42.0	.085
8	4 + 0	1860	48.0	.080
9	4 + 0	1860	48.0	.063
10A	7 + 5	1460	34.1	.100
10B	7 + 5	1460	34.1	.086
10C	7 + 5	1460	34.1	.067
11A	5 + 5	1100	36.9	(.096)[b]
11B	5 + 5	1100	36.9	.069
11C	5 + 5	1100	36.9	(.064)[b]
12A[a]	5 + 5	1100	36.9	(.135)[b]
12B[a]	5 + 5	1100	36.9	.059
19	5 + 5	1770	40.0	.068
13A	7 + 2	6080	40.0	.059
13B	7 + 2	6080	40.0	.054
14[a]	7 + 2	6080	40.0	.066
15	6 + 5	4530	31.0	.059
16A	8 + 5	4690	41.0	.045
16B	8 + 5	2410	41.0	.048
17A[a]	8 + 5	4630	44.0	.063
17B[a]	8 + 5	2380	44.0	.061
18A[a]	8 + 5	4660	42.5	.073
18B[a]	8 + 5	4660	42.5	.079
19	8 + 5	1773	33.8	.068

[a] Glucose or sodium glycolate added. [b] Calculations by the method of Warburg and Negelein are indicated by parentheses.

(8) Curve D, Fig. 4, ref. 4.

respiration and photosynthesis. An example of these measurements has been given.[9] The mercury was shown not to affect the living algae under the conditions of the experiment.

The results are shown in Figs. 1, 2, and 3 and in Table I. The temperature was always 24–26°.

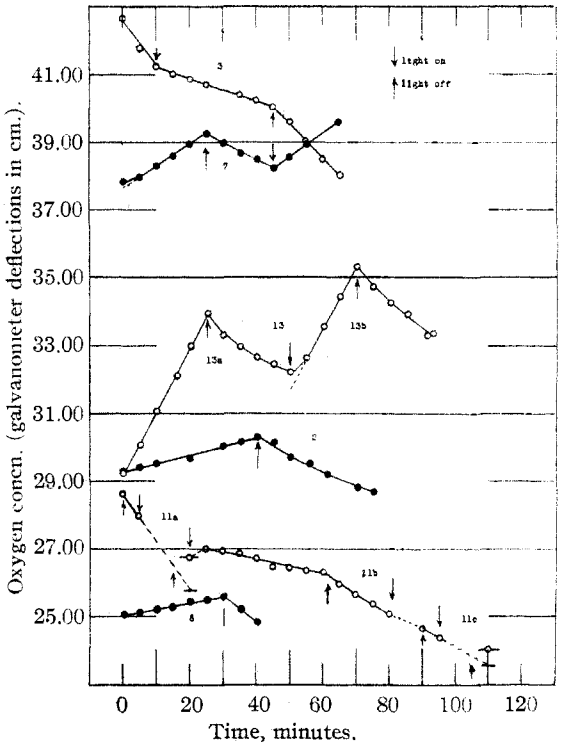

Fig. 1.—Oxygen concentration during photosynthesis and respiration.

Experiments with lower light intensities ranging from 1100 to 2200 ergs per second per sq. cm. are given first. The quantum yields range from 0.059 to 0.10. In expts. 13 to 18 the intensity was higher, 4500 to 6000, except that in expts. 16B and 17B the light intensity was reduced to about half the value in 16A and 17A by the introduction of a wire screen.

In expts. 3, 5 and 14, about 1% of glucose was added. In expt. 12, 1.0% of sodium glycolate was added. Experiments 16, 17 and 18 were practically the same except that in expt. 17 the solution was 0.05 *M* in sodium glycolate and in 18 it was 0.025 *M* in sodium glycolate and 0.025 *M* in glucose.

The method of calculation is illustrated with the data of experiment 13A.

Rate of oxygen change in light

$$\frac{(33.95 - 32.95)\text{ cm.}}{(25.0 - 20.0)\text{ min.}} = 0.20\text{ cm./min.}$$

Rate of oxygen change in dark

$$\frac{(33.30 - 33.95)\text{ cm.}}{(25.0 - 30.0)\text{ min.}} = -0.13\text{ cm./min.}$$

Rate of photosynthesis (corrected for respiration) = 0.20 − (−0.13) = 0.33 cm./min. In some experiments the calculations were made from tangents to the curves rather than from individual data.

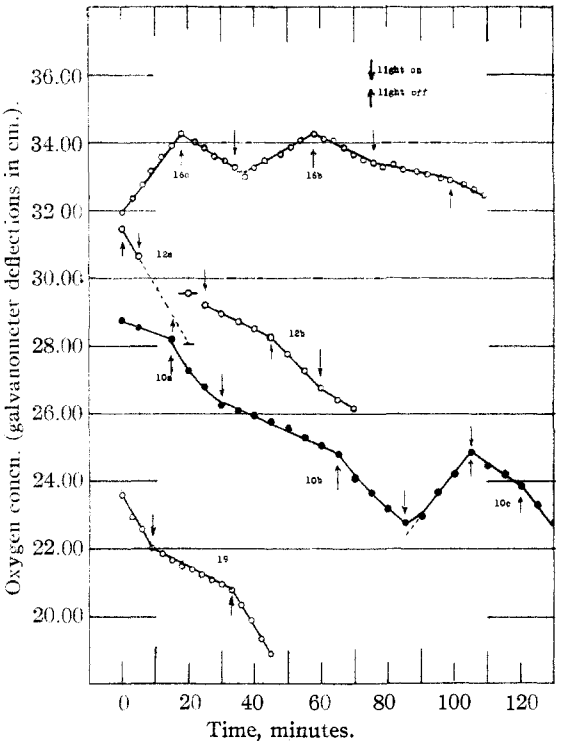

Fig. 2.—Oxygen concentration during photosynthesis and respiration.

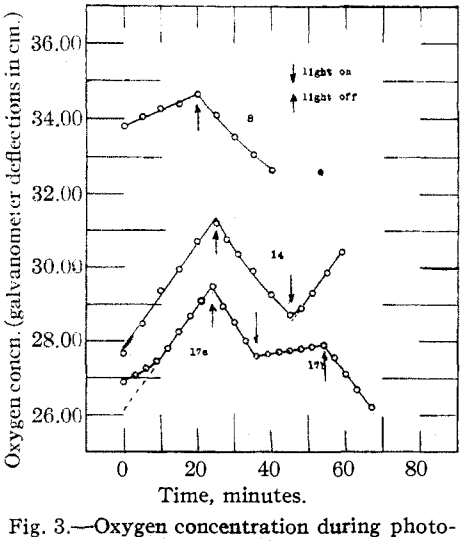

Fig. 3.—Oxygen concentration during photosynthesis and respiration.

(9) Reference 4, Fig. 5.

One unit cell volume is taken as 1 sq. cm. surface multiplied by the thickness of the cell, or 1.60 cc. One cm. deflection (difference between galvanometer reading with 1.00 volt and 0.100 volt) is equivalent to 9×10^{-9} mole of oxygen per cc. or 8.68×10^{15} molecules of oxygen per unit cell volume. The deflection of the thermopile back of the reaction cell was 45.0 cm. and 80.3 cm. when the cell was removed. With corrections for reflections, the incident light was 6080 ergs per second and the transmitted light 3620, giving an absorption per unit cell volume of 2460 ergs per second. At 6500 Å. one quantum contains 3.03×10^{-12} erg. Then the quantum yield is

$$\Phi = \frac{0.33 \times 8.68 \times 10^{15}}{2460 \times 60/3.03 \times 10^{-12}} = 0.059 \text{ molecule per quantum}$$

An hour later with the same light intensity, at 13B the slope was 0.18 cm. per minute in the light and −0.12 cm. in the dark, leading to a photosynthesis value of 0.30 cm. per minute and a quantum yield of 0.054.

The steady rate of respiration attained between the thirty-fifth and fiftieth minutes might be considered the significant rate, leading to a still lower quantum yield. In this period the slope is −0.05 cm. per minute. Combining this with the rate of oxygen uptake for the first exposure to a light a quantum yield of 0.045 is obtained; and combining it with the slope during the second exposure a value of 0.041. However, since there is disagreement regarding the value of quantum yields in photosynthesis, only the highest values are recorded in Table I. When the respiration rate decreased with time, the rate immediately after illumination was used in the calculations. The biologically significant and continuing quantum yields may be lower.

Discussion

A wide variety of conditions are covered by the data of Table I and Figs. 1, 2, 3, and yet the quantum yields are always low, *i. e.*, less than 0.10,[10] except in the case of 12A which was calculated in a different way. No correlation could be found between the quantum yield and the age or concentration of the algae or other factors. Many factors seem to influence the rate of respiration, such as the concentration of algae, the addition of glucose or glycolate and exposure to light, but the effect is compensated for in photosynthesis. When the slope for the oxygen change in the light is added to the slope for the oxygen change in the dark and combined with the amount of light absorbed, the quantum yields are almost always between 0.04 and 0.09. When the concentration of cells is low and the intensity of light is high, oxygen is evolved rapidly giving a positive slope but when the concentration is high and the light low, the respiration may be so great that illumination simply lessens the rate at which the oxygen is consumed and the slope of the oxygen-concentration–time curve remains negative but less steep.

Although the rate of respiration in the dark may change, it is seen that the amount of oxygen evolved in the light is a linear function of the time at constant light intensity. Apparently in the light a steady state of intermediate products may be reached which assures a uniform rate of respiration, at least over moderate time intervals.

In expts. 16B and 17B the light intensity was reduced by half, giving a slower rate of assimilation. However, the respiration was slower also with the result that the quantum yields are the same as in experiments 16A and 17A, respectively. Under these conditions the light must be the limiting factor because the rate of photosynthesis is directly proportional to the intensity.

It is well known that the addition of glucose accelerates respiration and in expts. 3, 5, 14, 17 and 18 this tendency is shown by the steeper curves. At low light intensities the quantum yields are not affected by this increased respiration but in expts. 14, 17 and 18 at higher light intensities there is evidence of an increase.

The discrepancy between the results obtained in this Laboratory and those obtained by Warburg and Negelein[2] and by Rieke[4] may be explained in part by the different methods of measurements. These investigators measured the oxygen change with a manometer and it is difficult under these conditions to obtain separately the actual rates of oxygen change in the light and in the dark on account of the time lag involved during the attainment of equilibrium between the gas phase and solution. Accordingly, a method of extrapolation was used[11] based on a few measurements and on the assumption that the rate of

(10) In new experiments, by Mr. W. E. Moore, to be reported later, algae from a different source have been used, the water has been specially purified and various inorganic and organic substances have been added. The same low quantum yields have been obtained consistently.

(11) This method is illustrated by Rieke in Fig. 1 of ref. 3.

respiration does not change. Periods of illumination and dark were chosen which were found experimentally to be favorable for the development of high apparent quantum yields.

Determinations 11A, 11C and 12A have been calculated by this extrapolation method as shown by the dotted lines in Fig. 1. After a period of illumination the algal suspension was kept in the dark for a period of five minutes. Oxygen concentrations were determined at the beginning of this dark period and at the end of the fifth minute. The light was turned on from the fifth to the fifteenth minute, and after five minutes in the dark the final reading was taken at the twentieth minute. The difference between this oxygen concentration and the value extrapolated from the fifth to the twentieth minute (assuming constant respiration), was used in calculating the amount of oxygen evolved during the ten-minute exposure to light. In 11A and 12A high values were obtained by this method of calculation. In 11C where the extrapolation sequence was started only after the algae had come to equilibrium with the light, as defined by a uniform rate of oxygen change (straight line), the calculations by the extrapolation method and the differential rate method are the same, and both are low.

The authors are glad to acknowledge the support of the Wisconsin Alumni Research Foundation in this investigation.

Summary

Quantum yields in photosynthesis by algae have been measured under a variety of conditions, making use of the dropping mercury electrode for the rapid measurement of dissolved oxygen. The results show that at 25°, 0.04 to 0.1 molecule of oxygen is evolved in photosynthesis by chlorella per quantum of red light absorbed. This efficiency is much lower than the efficiency 0.25 which has been hitherto accepted.

MADISON, WISCONSIN RECEIVED OCTOBER 13, 1939

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

A Photocalorimeter. The Quantum Efficiency of Photosynthesis in Algae

BY JOHN L. MAGEE, THOMAS W. DEWITT, ELIZABETH COOLIDGE SMITH AND FARRINGTON DANIELS

The calorimeter described in this paper is designed to measure heat changes accompanying photochemical reactions. It was developed originally for determining thermally the quantum efficiency of photosynthesis by *Chlorella*. The hitherto accepted value of 0.25 molecule of carbon dioxide converted per quantum absorbed was obtained by Warburg and Negelein[1] with a differential manometer. Work originally undertaken in this Laboratory to extend the investigation of Warburg led to a much lower quantum yield, of the order of 0.05 molecule per quantum.[2] It was thus desirable to measure the quantum yield by other methods. Warburg's high efficiency demands that a large fraction of the absorbed radiation be converted into chemical energy while the lower efficiencies of Manning, Stauffer, Duggar and Daniels require that most of the radiation be dissipated as heat. A calorimetric method was suggested as an independent check on the work. The calorimeter consists of a small thin-walled quartz cell mounted in a cylindrical aluminum container. A multijunction thermocouple measures the temperature difference between the cell and container. A double thermostat keeps the latter at constant temperature. A thermopile placed behind the cell measures the amount of radiation transmitted. The calorimeter is calibrated at different light intensities by filling it with a solution of india ink or other chemically-inert, opaque liquid, and plotting galvanometer deflections against time until a steady state is reached. The heat evolved or absorbed in a chemical reaction can then be obtained by comparing the galvanometer–time curve with the curve for a chemically inert material under similar conditions.[2a]

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

(2) W. M. Manning, J. F. Stauffer, B. M. Duggar, and F. Daniels, THIS JOURNAL, **60**, 266 (1938).

Experimental Procedure

Apparatus.—The construction of the photocalorimeter is shown in Fig. 1. The cylindrical quartz cell with pol-

(2a) Dr. William A. Arnold of the Hopkins Marine Station of Stanford University at Pacific Grove, Calif., has developed independently a microcalorimeter which in August, 1938, was giving quantum yields with algae slightly greater than those obtained in this investigation.