THE PHOTOCHEMICAL REACTION IN PHOTOSYNTHESIS

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(Accepted for publication, July 13, 1932)

I

From the experiments of Warburg and Negelein (1923), we know that the green alga *Chlorella pyrenoidosa* can reduce one molecule of carbon dioxide for each four quanta of light absorbed, when conditions permit maximum efficiency. Chlorophyll is clearly the substance absorbing the light quanta, so we may inquire how much chlorophyll must be present for the reduction of one molecule of carbon dioxide.

In a preceding paper (1932) we have presented evidence that the mechanism involved in the photochemical reaction must undergo a slower reaction, the so called Blackman reaction, before it can again take part in the photochemical reaction. Let us consider a cell in flashing light when the dark periods between flashes are so long that each unit activated in a given flash has time to complete the Blackman reaction before the next flash. Increasing the intensity of the flashes should increase the carbon dioxide reduction per flash until each unit capable of undergoing the photochemical reaction does so once in each flash. We say then that the photochemical reaction is saturated with light. The possibility that any unit will undergo the light reaction more than once in a single flash may be neglected, because the time required for the completion of the dark reaction is about 0.02 sec. at 25°C., while the duration of a light flash is 10^{-5} sec.

We define one unit arbitrarily as the mechanism which must undergo the photochemical reaction to reduce one molecule of carbon dioxide. If we can obtain light flashes of sufficient intensity to saturate the photochemical reaction, then the number of units in a sample of cells will equal the number of carbon dioxide molecules reduced per flash. The

total chlorophyll content of the sample divided by the number of carbon dioxide molecules reduced per flash will give the number of chlorophyll molecules per unit, or per molecule of carbon dioxide. The measurement of this ratio was the objective of the work described in this paper.

II

Methods of Measurement

Photosynthesis was measured manometrically in the usual way. The cells were suspended in a mixture of 85 parts $\frac{M}{10}$ potassium bicarbonate and 15 parts $\frac{M}{10}$ potassium carbonate. The flashing light was obtained by discharging a 1 or $\frac{1}{2}$ microfarad condenser, charged to about 3000 volts, through a neon tube. The circuit is described by Emerson and Arnold (1932, p. 395). The tube was flashed twelve or twenty-one times a second.

To obtain flashes of sufficient intensity to saturate the photochemical reaction we were obliged to concentrate the light with mirrors. The voltages used on the condenser were already so high that each tube lasted only a short time. A cylindrical mirror was made by splitting a glass tube about 2 cm. in diameter and silvering the outside of one half. This mirror was hung just below the neon tube, and served to concentrate the light on the vessel containing the photosynthesizing cells. The sides and top of the vessel were also silvered, and all silvered surfaces were copper-plated to protect the silver. Using a cell suspension as a photometer, we found that the mirrors increased the light intensity three to four times.

Ordinary incandescent lamps were not adequate to saturate certain samples of cells with continuous light. We obtained very intense continuous light from 100 watt high temperature projection lamps by adjusting silvered watch-glasses of appropriate diameter and curvature so that the images of the filaments fell in the cell suspensions. Even the intensity so obtained was not wholly satisfactory.

The light intensity was varied quantitatively by attaching neutral filters of known percentage transmission to the bottoms of the vessels. These filters are sufficiently non-selective for white light, but we found denser filters could transmit more red light than their indicated values. The filters used in our experiments with neon light were calibrated for red with the spectrophotometer.

The chlorophyll content of the cells had to be determined in absolute units. We are much indebted to Dr. Hans Gaffron for a sample of chlorophyll a + b prepared from *Chlorella*, with which we standardized our measurements. 13.3 mg. of dry chlorophyll, weighed accurately to 0.1 mg., were dissolved in 1 liter of pure methanol. The extinction coefficient of this solution was measured with a König-Martens spectrophotometer, using light of 6598.95 Å from a neon tube. Prior to measurement the usual Nicol ocular mounted in the divided circle was

replaced by the Gauss ocular, which shows the lines of the neon spectrum separately when the collimator slit is small. The telescope was adjusted so that the line 6598.95 Å was about centered in the field. Then the collimator slit was opened until the neighboring lines on either side, 6532.88 and 6678.27 Å, were about to fuse with the center line. Telescope and ocular slit were then adjusted so that only the line 6598.95 Å was visible. This gives strictly monochromatic light of adequate intensity for such chlorophyll solutions as we prepared.

We have used the definition of the extinction coefficient, ϵ, given in the Handbuch der Physik (1928, p. 189) from the equation

$$I_1 = I \times 10^{-\epsilon d}.$$

This means that ϵ is the reciprocal of that thickness of medium which will reduce the light to one-tenth its original intensity.

Our standard chlorophyll solution, 13.3 mg. per liter of methanol, gave a value of 0.634 for ϵ at 6598.95 Å. Reduced to 10 mg. chlorophyll per liter, $\epsilon = 0.476$.

For determining the chlorophyll content of cells, samples of about 10 c.mm. were used. After being washed in distilled water, boiling water was poured over them. They were allowed to stand in this for 2 minutes. The treatment does not decrease the yield of chlorophyll, and allows quicker completion of the extraction. The cells were then centrifuged out of distilled water, and extracted with methanol until they were white. Extracts were made up to 25 c.cm. in volumetric flasks. The technique of measuring the extinction coefficients was the same as described above for the standard solution.

If v is the volume in c.mm. of cells extracted, m the molecular weight of chlorophyll, ϵ the standard extinction for 10 mg. of chlorophyll per liter, and ϵ_1 the coefficient for the sample, then the number of mols of chlorophyll per c.mm. of sample equals:

$$\frac{\epsilon_1}{v\,\epsilon\,m} \times \frac{25 \times 10}{1000^2}.$$

For m we used 906.6, the value given by Willstätter and Stoll (1913, p. 128) for the average molecular weight of a mixture of chlorophyll a + b. Even large changes in the ratio a:b would not alter this value as much as 1 per cent, since the molecular weights of the two chlorophylls differ by only 14 units, according to Willstätter and Stoll.

The chlorophyll concentration per unit volume of cells was varied by growing cultures over different colors of light. It has been mentioned (Emerson and Arnold, 1932) that satisfactory changes in the chlorophyll content of *Chlorella pyrenoidosa* cannot be effected conveniently by the method of lowered iron concentration used for *C. vulgaris* (Emerson, 1929). But cultures of *C. pyrenoidosa* grown over mercury luminous tubes contain large amounts of chlorophyll per unit amount of cells, while similar cultures grown over neon tubes contain about one-fourth as much chlorophyll. Cells grown over 40 watt incandescent lamps develop an intermediate amount of chlorophyll. The chlorophyll concentration produced

appears to depend on the intensity of the light and the age of the culture, as well as on the color of the light. The neon light cultures mature faster than the incandescent light cultures, the mercury cultures much more slowly. All cultures were grown at 20°C.

III

EXPERIMENTAL

The flashing light concentrated by mirrors was barely sufficient to saturate the photochemical reaction, as shown by Fig. 1. Photosyn-

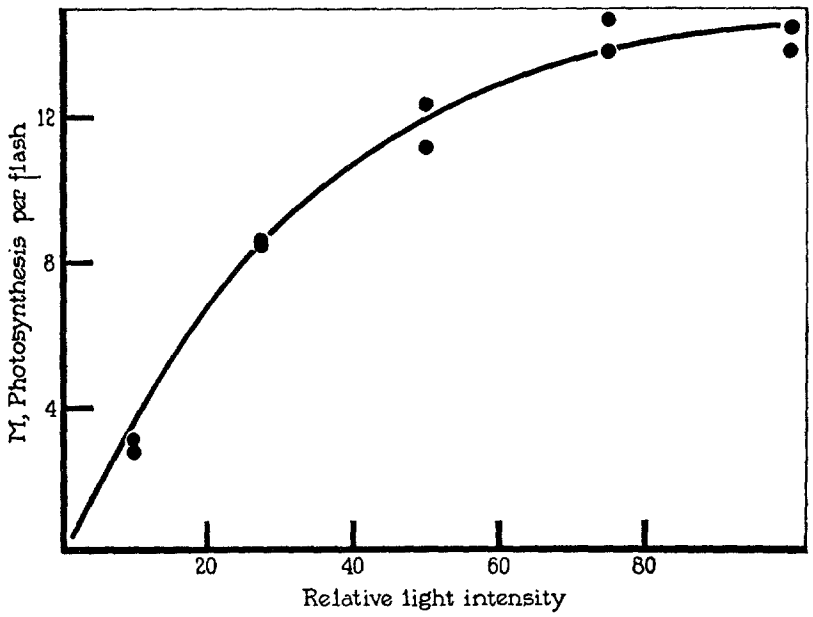

Fig. 1. Relative light intensity plotted against photosynthesis per flash, in arbitrary units. Temperature 25°C.

thesis per flash is plotted against intensity. The shape of the curve shows that higher intensities would probably increase the yield, though the maximum seems to have been nearly attained. This conclusion is in harmony with a possible theoretical explanation of the process which we shall propose in the last section. Table I gives the data for the two experiments incorporated in Fig. 1.

The ratio of chlorophyll content to the maximum height of Curve 1 gives the ratio of carbon dioxide reduction per flash, to amount of

chlorophyll. It is important to know whether this ratio remains constant for different concentrations of chlorophyll, so we have measured maximum photosynthesis and chlorophyll content for a number of cell samples grown so as to have different amounts of chlorophyll. The results, shown in Columns 4 and 10 of Table II, are plotted in Fig. 2. Column 11 of Table II gives the ratio, ρ, of chlorophyll: photosynthesis per flash at saturation. A straight line is the best fit for

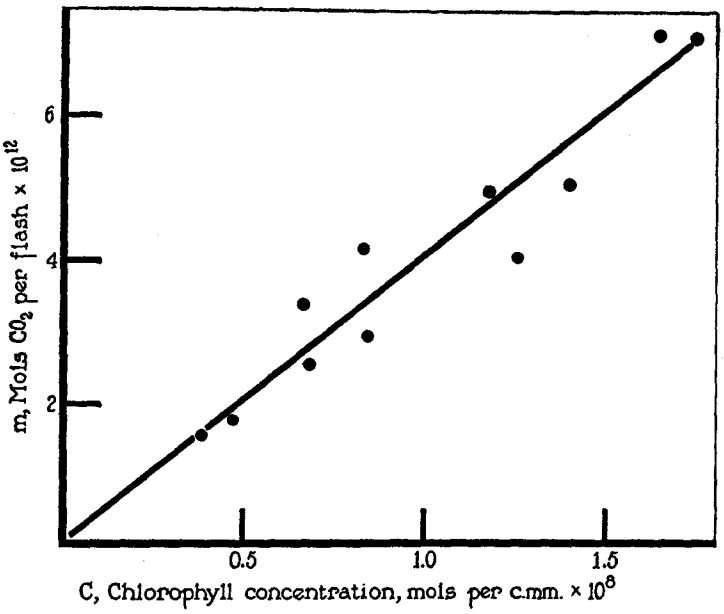

FIG. 2. Chlorophyll concentration in mols per c.mm. of cells plotted against mols of carbon dioxide reduced per flash of light at saturation. Temperature 25°C.

the points in Fig. 2. The slope of the curve, ρ, is therefore a constant for different concentrations of chlorophyll. The value of ρ obtained by averaging the last column in Table II, 2480 molecules of chlorophyll per molecule of carbon dioxide reduced per flash, agrees exactly with the slope of the line chosen as the best fit for the points in Fig. 2.

Column 8 in Table II gives the values of Q, photosynthesis in continuous light. It is to be understood that Q does not represent light saturation in all cases, since we were not able to obtain light of suffi-

cient intensity to saturate certain samples of cells. Nevertheless, it is interesting to compare the highest obtainable values of Q with those for maximum photosynthesis per flash. Q is plotted against chlorophyll in Fig. 3. The points are more scattered than in Fig. 2. We attribute the scatter to varying capacities to carry on the Blackman reaction among different samples of cells, a factor which would not

TABLE I

Photosynthesis in Flashing Light as a Function of Light Intensity. Data for Fig. 1

Capacity of condenser $\frac{1}{2}$ μfd.
Resistance of charging circuit 3340 ohms.
Twenty-one flashes per sec.
3100 volts at rectifier.
Temperature 24.9°C.

Relative light intensity	Δh, per 5 min. per c.mm. cells, corrected for respiration	K_{O_2}	Oxygen per 5 min. per c.mm. cells	Relative rate of oxygen production, M
First experiment				
	mm.		*c.mm.*	
100	1.16	0.52	0.603	14.50
75	1.18	0.52	0.614	14.75
50	0.99	0.52	0.515	12.40
28	0.68	0.52	0.354	8.50
10	0.25	0.52	0.130	3.15
Second experiment				
10	0.27	0.50	0.135	2.79
28	0.84	0.50	0.420	8.60
50	1.10	0.50	0.550	11.20
75	1.35	0.50	0.675	13.85
100	1.35	0.50	0.675	13.85

influence saturation in flashing light because the dark periods were sufficient for the completion of the Blackman reaction between flashes, but which would surely affect the balance between photochemical and Blackman reaction in continuous light. This might also explain why we could not saturate certain samples with continuous light. The cells with low chlorophyll content tended to fall short of saturation, even in our most intense continuous light. If cells with high

chlorophyll content should have their capacity for the Blackman reaction less well developed in proportion to their chlorophyll content than the cells with low chlorophyll content, then the cells rich in

TABLE II

Photosynthesis in Flashing and Continuous Light as a Function of Chlorophyll Content. Data for Figs. 2 and 3

Capacity of condenser $\frac{1}{2}$ or 1 μfd.
Resistance of charging circuit 3300 to 7500 ohms.
Twelve flashes per sec.
3100 volts at rectifier.
Temperature 25°C.

ϵ for 10 c.mm. cells, 25 cc. methanol	Source of light for culture	Chlorophyll per c.mm. cells $\times 10^2$	C Mols of chlorophyll per c.mm. cells $\times 10^9$	K_{O_2} Continuous	K_{O_2} Flashing	Continuous light: Δh, per 5 min. per c.mm. cells corrected for respiration	Continuous light: Q Mols oxygen per sec. per c.mm. cells $\times 10^{10}$	Flashing light: Δh, per 5 min. per c.mm. cells corrected for respiration	Flashing light: m Mols oxygen per flash per c.mm. cells $+ 10^{12}$	$\rho = \frac{C}{m}$
		mg.				*mm.*		*mm.*		
0.0816	Neon	0.428	0.472	0.41	0.50	3.26	1.99	0.286	1.77	2660
0.204	Mercury	1.07	1.18	0.41	0.52	5.78	3.53	0.768	4.95	2380
0.146	Neon	0.766	0.845	0.41	0.50	6.05	3.69	0.473	2.93	2380
0.242	Mercury	1.27	1.40	0.41	0.52	6.87	4.18	0.780	5.03	2780
0.0672	Neon	0.352	0.389	0.41	0.41	1.28	0.72	0.308	1.57	2480
0.144	Mercury	0.756	0.835	0.41	0.41	5.24	3.19	0.815	4.15	2010
0.0544	Neon	0.289	0.314	0.41		2.20	1.34			
0.250	40 watt lamps	1.31	1.45	0.41		6.06	3.70			
0.0423	Neon	0.222	0.245	0.41		2.73	1.67			
0.224	40 watt lamps	1.17	1.29	0.41		5.15	3.14			
0.118	Neon	0.620	0.672	0.52	0.52	2.70	2.08	0.392	2.53	2660
0.218	Mercury	1.14	1.26	0.50	0.50	4.57	3.40	0.650	4.03	3120
0.116	Neon	0.606	0.668	0.52	0.52	5.03	3.89	0.525	3.38	1980
0.284	Mercury	1.49	1.65	0.50	0.50	6.16	4.58	1.152	7.15	2310
0.303	Mercury	1.59	1.75	0.50	0.50	6.05	4.50	1.142	7.10	2460
								Average ρ...		2480

chlorophyll would be saturated with light at lower intensities than those poor in chlorophyll. This would explain the direction of curvature of the line drawn through the points in Fig. 3. It would also explain the shape of the chlorophyll-photosynthesis curves published by Emerson (1929) for *C. vulgaris*. Those curves were made with cul-

tures of the same age and nearly the same density. The maintainance of equal age and density in cultures of *C. pyrenoidosa* grown over mercury and neon lights is not as easily possible, since the cultures mature so much faster in the neon light. The capacity for the Blackman reaction may well depend on the age and rate of growth of the culture, as well as on the chlorophyll content.

We are aware that we have published curves (1932, p. 413, Fig. 12) indicating identical capacity for the Blackman reaction relative to

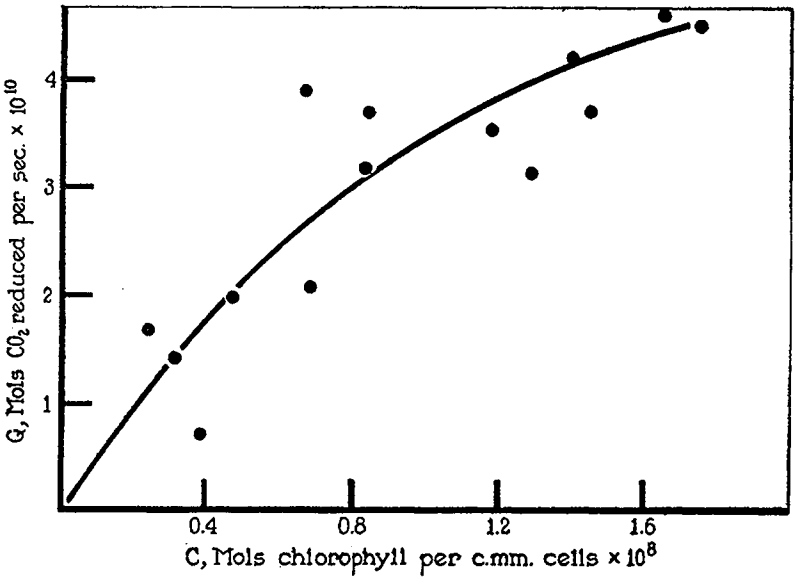

FIG. 3. Concentration of chlorophyll in mols per c.mm. of cells plotted against mols of carbon dioxide reduced per second in high intensity continuous light. Temperature 25°C.

chlorophyll content in two very different samples of cells, grown over red and blue light. We feel that we must attribute this result to chance, because the factors governing the development of the capacity for the Blackman reaction are evidently not yet under our control.

IV

Theoretical

We can give no adequate interpretation of our ratio of 2480 molecules of chlorophyll per molecule of carbon dioxide reduced per flash.

Warburg and Negelein (1923) found that under favorable conditions *Chlorella pyrenoidosa* could reduce one molecule of carbon dioxide for every four quanta absorbed. The light emitted by our neon tube is rich in red, a color strongly absorbed by chlorophyll; and we know that at high intensities the yield of photosynthesis per unit time of light is greatly improved by illuminating with short flashes separated by long dark periods. As yet we know nothing of the quantum efficiency in flashing light. We are forced to conclude that this is very low, or that most of the chlorophyll is not absorbing light. The fact that our maximum intensities nearly saturated the photochemical reaction does not mean necessarily that every chlorophyll molecule was absorbing light in each flash. We need only suppose that for every 2480 molecules of chlorophyll there is present in the cell one unit capable of reducing one molecule of carbon dioxide each time it is suitably activated by light. At our maximum intensity each flash activated nearly all these units.

It is also possible that the molecular weight of chlorophyll in the cell may be higher than that of extracted chlorophyll.

Knowing the number of units and the maximum rates of photosynthesis in continuous and flashing light, we can calculate the average time required for one unit to go through the cycle of photochemical and Blackman reactions. We suppose the Blackman reaction must be completed each time the photochemical reaction takes place, before the unit involved is again free to undergo the photochemical reaction. Therefore the mean time of one cycle, which we call S, will be longer at low temperatures than at high ones.

To calculate S, we shall let C be the chlorophyll content of the cells; m the photosynthesis per flash at saturation; and Q the photosynthesis per second at saturation with continuous light. C, m, and Q are in mols per c. mm. of cells. We have used m as a measure of the number of units capable of undergoing the photochemical reaction. If S is the mean time required for a unit to undergo photochemical and Blackman reactions, then m/S is the maximum possible rate of photosynthesis in continuous light at saturation, so we may write

$$Q = \frac{m}{S}, \text{ or } S = \frac{m}{Q}.$$

For a sample of cells whose values of m and Q fall on Curves 2 and

3, m, which is independent of temperature, is 2.52×10^{-12} mols. At 25° Q is 2.08×10^{-10} mols. From these figures S is equal to 1.2×10^{-2} sec.

We can also estimate the value of S from curves showing the duration of the dark reaction after a flash of light. The mean time of one cycle (neglecting the duration of the light reaction because it is very short compared to that of the dark reaction) will be approximately equal to the time required for the Blackman reaction, when taken by itself, to convert half the product of the photochemical reaction. This time can be read from numerous curves for the dark reaction which we have published previously (1932) for temperatures below 25°. Fig. 8 (1932, p. 403) shows that at 25° the Blackman reaction is substantially completed in less than 0.035 sec., the shortest dark time used. It would be half completed in about half this time, the exact point being determined by the order of the Blackman reaction, which we do not yet know. But we may say from Fig. 8 that S is about 0.017 sec., a figure of the same order of magnitude as the value calculated from $\frac{m}{Q}$ at 25°. The value of Q for lower temperatures can be calculated roughly from the known temperature coefficient of photosynthesis, and the resulting values of S remain of the same order of magnitude as those estimated from the dark-time curves for corresponding temperatures.

We conclude, then, that at 25° the mean time required for a unit to complete the cycle of photochemical and Blackman reactions, reducing one molecule of carbon dioxide, is somewhere between 0.01 and 0.02 sec.

Now we shall present evidence, derived from the preceding experiments, that the photochemical reaction is of the first order with respect to light intensity. We will designate the number of units ready to undergo the photochemical reaction, as they would be after a long dark period, by N. Under the influence of light some of these units are activated and become ready to undergo the Blackman reaction. We will designate the number of these photoactivated units as n. They are reconverted by the Blackman reaction. We will let K be the value of $N + n$. K is a constant for a given sample of cells, and is proportional to the chlorophyll content of the sample. We assume

also that the rate, R, at which the units undergo the photochemical reaction, is proportional to the light intensity and to the value of N. We need not make any assumptions about the reconversion of the units as long as we consider photosynthesis in flashing light with long dark periods.

Our assumptions are:

$$N + n = K, \tag{1}$$

and

$$R = AIN. \tag{2}$$

In equation (2) A is the velocity constant for the reaction $N + h\nu \rightarrow n$. Equation (2) can be expressed in differential form:

$$\frac{dN}{dt} = - AIN, \tag{3}$$

with t representing the time, neglecting the Blackman reaction because t is short.

Integrating both sides of (3),

$$N = ce^{-\int AIdt}. \tag{4}$$

When $t = 0$, equation (4) becomes

$$N = c,$$

but we know that after a long dark period, all the units are ready to undergo the light reaction, so $n = 0$, and $N = K$. Hence we may replace the integration constant c in equation (4) by K. A is a constant, and may be written before the integral sign:

$$N = K e^{-A \int Idt}. \tag{5}$$

We have good evidence that E, the total energy in a flash, is equivalent to $\int I\,d\,t$. By inserting different sized choke coils in series with the condenser and the neon tube, the light flashes can be made slower and less intense, while their energy content, E, remains proportional to the charge on the condenser, a constant. By means of our spinning thread (Emerson and Arnold, 1932, p. 397) we estimated that our

largest choke coil increased the duration of the flash about 100 times. The maximum intensity of the flash is correspondingly decreased since the total energy liberated is the same. Photosynthesis was measured in flashes of various duration while E was kept constant. Unfortunately the fact that the energy emitted by the tube remained constant does not mean that the energy absorbed by the cells was the same for the various flashes. The wave length distribution of the flashes differed with the different choke coils. The slower flashes were darker red, and may have emitted more energy as heat. For this reason the results are not quantitative. Nevertheless, we were only able to find differences of 9 per cent in the photosynthesis per flash, using the largest choke coil. These experiments indicate that it is correct to use the first power of the intensity in equation (5). A higher power of I would require that the amount of photosynthesis per flash change with changes in intensity and duration of flash produced by the different choke coils. It appears from the experiments that $\int I\,dt$, which remains equal to the charge on the condenser, a constant, produces the same photosynthesis even when the time and intensity are changed over a wide range.

We will now let N_1 denote the value of N remaining at the end of a light flash, and rewrite equation (5):

$$N_1 = K e^{-A \int I dt}. \tag{6}$$

$K - N_1$ will be the number of units activated, measured by M.

$$M = K - N_1,$$

or

$$M = K - K e^{-A \int I dt}. \tag{7}$$

Subtracting both sides of equation (7) from K, and simplifying,

$$\frac{K - M}{K} = e^{-A \int I dt},$$

or

$$\log \frac{K - M}{K} = -A \int I dt. \tag{8}$$

According to equation (8), the log of $\frac{K - M}{K}$ plotted against $\int Idt$ should give a straight line of slope $-A$, intersecting the logarithmic axis where $M = 0$. This plot can be made from Fig. 1. $\int Idt$ is proportional to the intensity scale, and M is the height of the curve at any point. K, being proportional to C, the chlorophyll content, is proportional to the maximum height to which the curve rises. It was mentioned in the discussion of Fig. 1 that our light intensities did not

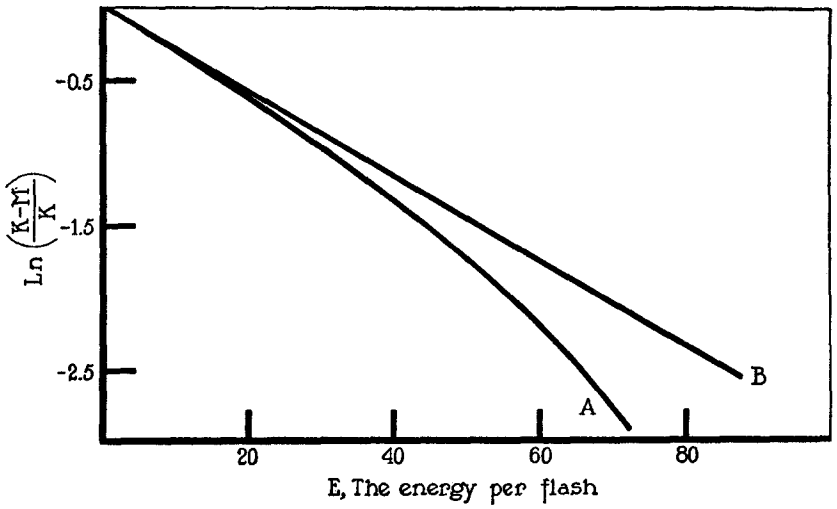

FIG. 4. See text for explanation. Curve A is made from Fig. 1, taking K as the maximum height, 14.5, attained by the curve drawn in Fig. 1. Line B is plotted from Fig. 1 on the assumption that K would finally reach the value 15.5, if we could obtain flashes of sufficient intensity.

permit the experimental determination of this maximum height. We may assign to K either the highest value attained by Curve 1, or a slightly higher value, the probable maximum. Curve A in Fig. 4 is a plot of I against $\log \frac{(K - M)}{K}$ when K is given the value 14.5, the highest level actually attained by Curve 1. Curve A deviates most from a straight line in the region where too low a value of K would make the largest error. If we assign to K the value 15.5 instead of 14.5, we obtain the line B, in Fig. 4, as required by equation (8). Since 15.5 is probably nearer the maximum height attainable by the

curve in Fig. 1 than 14.5, we think our interpretation comes close to fitting the experimental results.

Any interpretation in which light intensity enters this mechanism at a power higher than the first, fails to explain these results as satisfactorily. If we retain the concept that there are units in the photosynthetic mechanism which go through the photochemical and Blackman reactions in a cycle, our explanation is a good fit. Future work may show that we are mistaken in interpreting the behavior of photosynthesis in flashing light to mean that a cyclical type of reaction is governing the process. In this case our scheme will have to be abandoned. It covers the photochemical reaction only, but gives promise that it may be extended to include the Blackman reaction as well. Qualitatively it will explain the shape of the ordinary intensity curves published by Warburg (1925), van den Honert (1930), van der Paauw (1932), and others. We suppose that saturation in continuous light is reached when the photochemical reaction produces its product as fast as the Blackman reaction can use it. But an exact interpretation of the balance between the photochemical and Blackman reactions must await a better understanding of the Blackman reaction.

SUMMARY

Measurements of photosynthesis were made in continuous and flashing light of high intensity, using cells varying in chlorophyll content. The amount of chlorophyll present per molecule of carbon dioxide reduced per single flash of light was found to be about 2480 molecules. The length of time required for one unit in the photosynthetic mechanism to complete the cycle of photochemical and Blackman reactions was found to be about 0.02 sec. at 25°C. The equation $R = AIN$ was shown to give a good description of the rate of the photochemical reaction, when A is a velocity constant, I the intensity of light, and N the number of units in the photosynthetic mechanism.

We are greatly indebted to Mr. Erickson and to the Electrical Products Corporation for the large number of tubes which they furnished us. Our thanks are due especially to Professor R. C. Tolman for helpful criticism.

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