

A SEPARATION OF THE REACTIONS IN PHOTOSYNTHESIS BY MEANS OF INTERMITTENT LIGHT

BY ROBERT EMERSON AND WILLIAM ARNOLD

(From the Kerckhoff Laboratories of Biology, California Institute of Technology,
Pasadena)

(Accepted for publication, December 14, 1931)

Experiments on photosynthesis in intermittent light have been made on two occasions. Brown and Escombe, in 1905, made use of a rotating sector to study the effect of light intensity on the photosynthesis of leaves. They found three-quarters of the light from a given source could be cut out in each revolution of the sector without decreasing the rate of photosynthesis. Willstätter (1918, p. 240) explains that this was probably due to the low concentration of carbon dioxide available for the leaves. The short periods of light would be sufficient to reduce all the carbon dioxide which could reach the cells by diffusion during the dark periods.

In 1919–20 Warburg made experiments on *Chlorella* similar to those of Brown and Escombe on leaves. Instead of stating his results as amount of photosynthesis per total elapsed time, as Brown and Escombe did, he gave photosynthesis per total time during which the cells were illuminated. Since he used sectors which cut out half the incident light in each revolution, the time during which the cells were illuminated was always half of the elapsed time of an experiment.

Working with a high intensity of light and a high concentration of carbon dioxide, Warburg found that a given amount of light reduced more carbon dioxide when allowed to fall on the cells intermittently than when allowed to fall on them continuously. The improvement in the yield of the intermittent over the yield in continuous light depended on the frequency of the flashing. With a frequency of four periods per minute the improvement was 10 per cent, and with a frequency of 8000 per minute it was 100 per cent.

Warburg proposed two alternative explanations for the improvement in the yield of the intermittent light. Either the reduction

of carbon dioxide continues in the dark, or it proceeds twice as fast during the brief light flash as during the same length of time in continuous light. He considers the latter explanation more likely, and assumes that certain steps in the photosynthetic process continue in the dark until a dark equilibrium is reached. After the dark period a short flash of light would find a higher concentration of reactive substance ready for it than is available in continuous light, and would be able to effect more decomposition than an equal amount of continuous light.

The experiments described in this paper indicate, we think, that the steps in photosynthesis which proceed in the dark involve what has hitherto been known as the Blackman reaction. Probably the reduction of carbon dioxide is not completed during the photochemical part of the process. A more correct way of representing the sequence of events in intermittent light would be as follows. Two steps are involved in the reduction of carbon dioxide: a reaction in which light is absorbed, followed by a reaction not requiring light—the so called Blackman reaction. If the light intensity is high the photochemical reaction is capable of proceeding at great speed, but in continuous light it can go no faster than the Blackman reaction. We suppose that the product formed in the photochemical reaction is converted to some other substance by the Blackman reaction, and at the same time the chlorophyll is set free to take part again in the photochemical reaction. If a green cell is illuminated, we think that the photochemical reaction proceeds rapidly until an equilibrium concentration of its product is formed. After this the photochemical reaction proceeds only as fast as the Blackman reaction removes the intermediate product. If the cell is now darkened, the photochemical reaction stops at once, but the Blackman reaction continues until its raw material, the product formed by the photochemical reaction, is exhausted. After this nothing further happens until the cell is again illuminated. Higher efficiency of the light would be obtained if each light flash lasted only long enough to build up the equilibrium concentration of the intermediate product, and each dark period were long enough to allow the Blackman reaction time to use up all the intermediate product present at the moment the light period ended. In Warburg's flicker experiments the light and dark periods were always

of equal length. He found that the amount of work done by the light could be increased by shortening both the light and the dark periods. This indicates that his light periods were too long for maximum efficiency. In the latter part of each light period the photochemical reaction must have been brought down to near the speed of the Blackman reaction.

Using 133 light flashes per second, Warburg obtained an improvement of 100 per cent over the continuous light yield. We were able to improve the continuous light yield 300 per cent to 400 per cent by using only 50 flashes per second and making the light flashes much shorter than the dark periods. This opened the possibility of determining the length of the dark period necessary for the complete removal of the intermediate product formed in a light flash of given intensity and duration. Lengthening the dark period should improve the yield until there is time enough for all the intermediate product formed in each light flash to be removed before the next light flash.

In this paper we describe experiments which show that the necessary dark time is about 0.03 to 0.4 of a second, depending on the temperature. Further experiments are described to show certain characteristics of the reactions taking place both in the light and in the dark.

I

Description of Electrical Circuits

Three types of lighting circuits were used, which we shall refer to as A, B, and C.

Circuit A, shown in Fig. 1, involved the use of a neon tube *N* about 12 inches long and $\frac{1}{4}$ inch thick, similar to those used in advertising. The tube was lighted by a transformer *T* whose primary was connected with the 110 volt 50 cycle alternating current of the laboratory. The secondary gave 15,000 volts on open circuit and 25 milliamperes on a short circuit. The neon tube was lighted about 95 per cent of the time. There was a momentary dark period each time the voltage changed sign.

In Circuit B a mercury rectifier bulb *B* was placed across the secondary of the transformer, as shown in Fig. 2. The filament of the rectifier bulb was lighted from a toy transformer, not shown in the diagram. A variable resistance *R* was put in series with the rectifier bulb. For this resistance one or more 5 watt 110 volt switchboard lamps were used, giving resistances up to 15,000 ohms.

Alternate half cycles of current were taken by the neon tube and the rectifier bulb, so that the neon tube flashed 50 times a second. The duration of each flash of the neon tube could be controlled within certain limits by varying R . With a high resistance, the tube was lighted about 45 per cent of the time, and with a low resistance about 15 per cent of the time. The per cent time of light

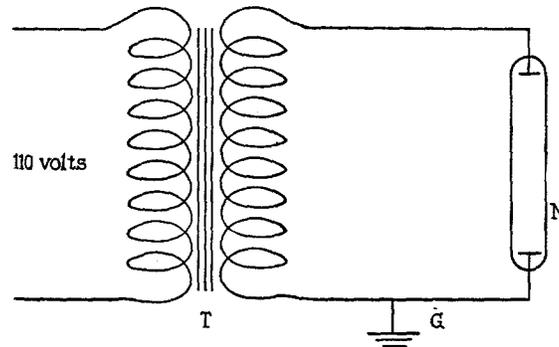


FIG. 1. Diagram of Circuit A

N = neon tube; T = transformer; G = ground

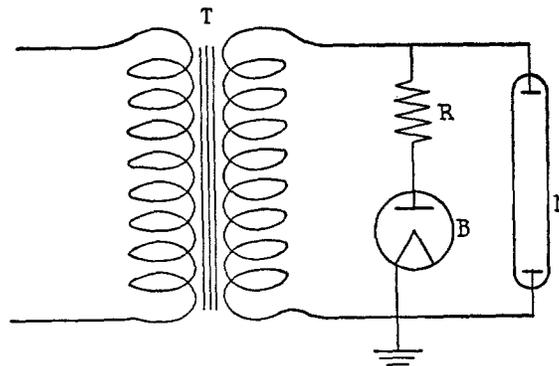


FIG. 2. Diagram of Circuit B. R = variable resistance; B = mercury rectifier bulb. Other letters are same as for Circuit A.

and dark was measured with the device shown in Fig. 3. A black disc about 4 inches in diameter, having a narrow strip of white paper along one radius, was spun on the shaft of a 4 pole synchronous motor, and the flickering light of the neon tube was thrown on the spinning disc by a mirror. Since the neon tube and the synchronous motor were operated from the same source of 50 cycle current, the light flashes kept pace exactly with the revolutions of the disc, and the

white paper radius was illuminated twice during each revolution. The spinning disc showed a stationary pattern of two dark sectors and two light sectors. By means of a protractor the angle subtended by one light sector was measured.

If this angle was x° , the per cent time of illumination was $\frac{x}{180} \times 100$.

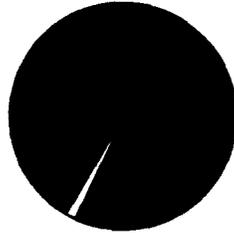


FIG. 3. The rotating disc used to measure the duration of light and dark periods

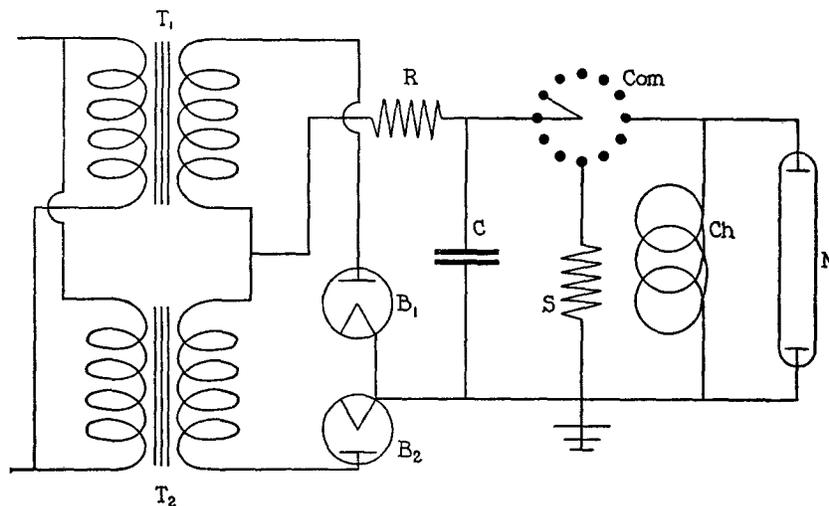


FIG. 4. Circuit C. *Com.* = commutator; *ch.* = choke coil; *S* = 600 watt electric stove; *C* = condenser; *R* = variable resistance. Other letters are same as for Circuit A.

For Circuit C a larger size of neon tube was used, about $\frac{5}{8}$ inch in diameter and again about 1 foot long. An extra large size of electrodes was used in these tubes. A diagram of the circuit is shown in Fig. 4. The half microfarad condenser *C* was charged from the full-wave rectifier system consisting of the two pole-top transformers *T*₁ and *T*₂ and two mercury rectifier bulbs *B*₁ and *B*₂ connected as

indicated. The filaments of the rectifier bulbs were lighted by a toy transformer. The transformers T_1 and T_2 were 110-2200 volt, and were rated one kilowatt each. The rate of charging of the condenser was controlled by the resistance R . When R was equal to 5000 ohms the condenser could be charged to well over the voltage necessary to strike the neon tube in half a cycle. At different times we used resistances of 2000 to 8000 ohms in the charging circuit.

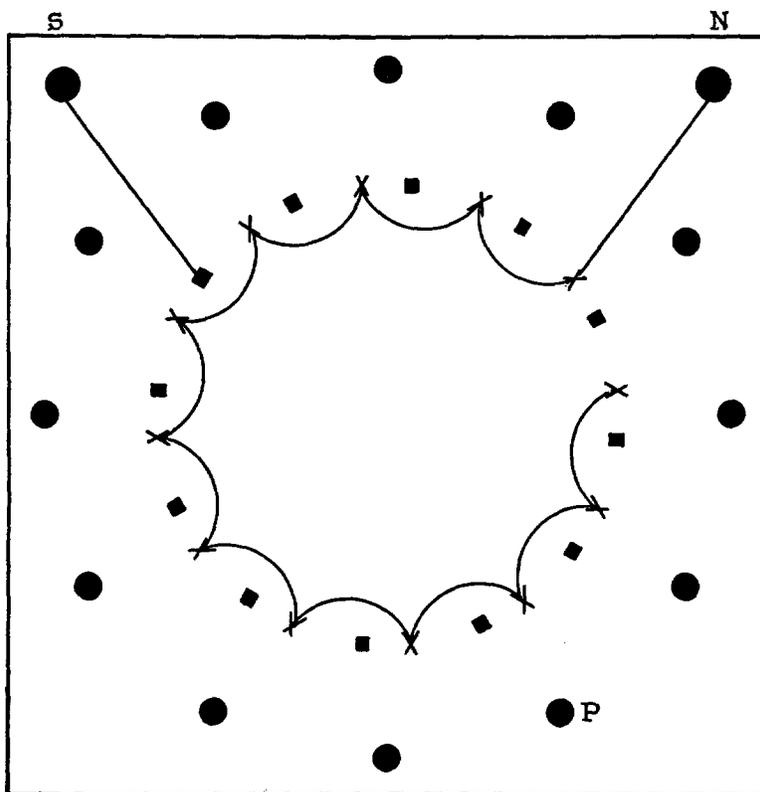


FIG. 5. Diagram of the commutator used in Circuit C. P = tungsten points; N is the binding post connected with the neon tube; S is the binding post connected with the electric stove.

Com. is the commutator which distributed the condenser discharges either through the neon tube N , or through the 600 watt electric stove S . A diagram of the commutator is shown in Fig. 5. It is a 9 inch square slab of bakelite on which twelve tungsten automobile ignition points P are mounted in a circle of about 4 inches radius. These tungsten points project about $\frac{1}{8}$ inch beyond the surface of the bakelite. By a system of switches not shown in the drawing each

point can be connected with either the neon tube or the electric stove. The crosses mark contacts connected by the wiring system shown, which leads out to the neon tube binding post. The squares are connected by a similar wiring system on the reverse side of the bakelite, leading out to the stove binding post. The rotor arm of the commutator also carries a tungsten point. This moves past each point on the commutator without touching it. The potential of 2000 volts is sufficient to break down the small air gap of about half a millimeter. The rotor arm is mounted on a reduction gear operated by the same synchronous motor used with Circuit B. In the course of the work we ran the rotor arm at speeds from 30 R.P.M. to 240 R.P.M. At 240 R.P.M. when every tungsten point on the commutator is connected with its cross contact the neon tube will flash 48 times a second. If every alternate tungsten point is connected with the square contact instead of the cross contact, the tube will light twenty-four times a second and twenty-four times the condenser will be discharged through the stove. Thus with a speed of the rotor arm of 240 R.P.M. the dark time between light flashes can be varied from 0.0208 second to 0.25 second, depending on whether twelve, six, four, three, two, or one contacts are connected with the neon tube. The purpose of connecting the points not used to light the tube with the electric stove is to allow the condenser the same charging time before each flash of light. Otherwise the longer dark periods would be followed by brighter flashes of light, since the condenser is not used at full charge.

When stating the dark time between flashes, we neglect the duration of the light flashes, which may be as short as 10^{-5} sec. We have made measurements which show that the flash lasts not longer than 2×10^{-5} sec. This was done by mounting a piece of white thread on a black wheel and rotating the wheel at 1500 R.P.M. while the flashing light was thrown on it. Each flash made the thread momentarily visible as a clear sharp image, not blurred perceptibly by motion. Even the twist of the thread could be seen. Assuming that the thread would just begin to look blurred if it moved half its diameter during the light flash, we can set an outer limit for the duration of the flash. The thread was about 0.2 mm. in diameter, and had a lineal velocity of 860 cm. per second. It would travel half its own diameter in about 2×10^{-5} sec. Since no blurring was visible, the duration of the flash was surely less than this. A calculation from the constants of the electrical circuit put the duration of the flash at about 1×10^{-5} sec. The time the light is on is therefore negligible compared to even the shortest dark period of 2.08×10^{-2} sec.

The time required for the tungsten point on the rotor arm to pass one point on the commutator is sufficient for the condenser to discharge once, and become charged again up to the striking voltage of the neon tube. This means that the first bright flash is followed closely by one or more dimmer flashes. These dim flashes stop as soon as the rotor arm moves clear of the contact in question. It was found that these secondary flashes could be prevented by the choke coil *Ch.*, a coil 5 cm. in diameter, 11 cm. long, and bearing 65 turns of No. 16 magnet wire. The steep wave-front from the well charged condenser takes the path through

the neon tube. The choke coil completely prevents the tube from lighting on any small voltage that may be set up in the system.

The discharge from a half microfarad condenser at 2000 volts subjects an ordinary neon tube to very severe strain. The tube does not last indefinitely. In time its inner walls become darkened from the copper deposited on them by the heavy current. The neon supply becomes so exhausted that the tube finally will not strike any longer. During these changes in the tube, the intensity of the flash may change considerably. This necessitates control of conditions at the close of each experiment, to make sure the tube will still produce the same photosynthesis as it did at the outset of the experiment.

II

The Technique of Measuring Photosynthesis

Photosynthesis was measured manometrically in rectangular glass vessels of about 10 cc. capacity, attached to Barcroft-Warburg manometers. Since the details of this method of measurement have already been adequately described in many different papers (Warburg, 1926) only those modifications which were necessitated by the use of the neon tubes are described here.

In order to obtain intense illumination of the cell suspensions, the neon tube was placed in the water of the thermostat a few millimeters below the bottoms of the vessels. The electrodes projected upwards at right angles to the main part of the tube toward the surface of the water. Fig. 6 shows the arrangement. *R, R* are pieces of rubber tubing slipped over the electrodes to prevent them from becoming wetted. *L* are the lead wires. *W* is the water level in the thermostat. *S* is the cell suspension in a rectangular glass vessel. Three such vessels could be placed over the tube at once, two with cell suspensions and one as a barometer.

When the light source is used so close to the vessels, great care must be taken that the two vessels containing the cells are the same distance from the tube, or else they will not be equally illuminated. This is very important in the flashing light, so either a given curve was made entirely with one vessel set always in the same position; or, if it was absolutely necessary to use two vessels at the same time, two were selected which would stand within a millimeter of the same distance from the tube, and which had bottom areas as nearly equal as possible. To insure that the vessels should receive light through their bottoms only, little copper jackets were made which covered the sides and tops. Without these copper jackets we found it impossible to obtain comparable sets of results.

For most of these experiments the unicellular green alga, *Chlorella pyrenoidosa* was used. The cells were produced in pure cultures as described in a paper by Emerson (1929, p. 611). The strain of *Chlorella* used for this work has now been in cultivation in our laboratory for 5 years and has shown no changes in metabolic activity.

Cells from well grown cultures were centrifuged out of the culture medium

and transferred after washing to a mixture of $M/10$ potassium carbonate and potassium bicarbonate solutions. The mixture used was 15 parts carbonate to 85 parts bicarbonate, except in experiments where low concentrations of carbon dioxide were required. Warburg (1919, p. 238) gives the properties of mixtures of $M/10$ sodium carbonate and bicarbonate. Although exact data are not available for the potassium carbonates, we have used them instead of the sodium salts because the cells seem able to withstand exposure to the potassium carbonate mixtures for a longer time without suffering a decrease in rate of photosynthesis. Although it is unlikely that the concentrations of carbon dioxide are the same as for the sodium mixtures, we have every reason to believe that they differ only

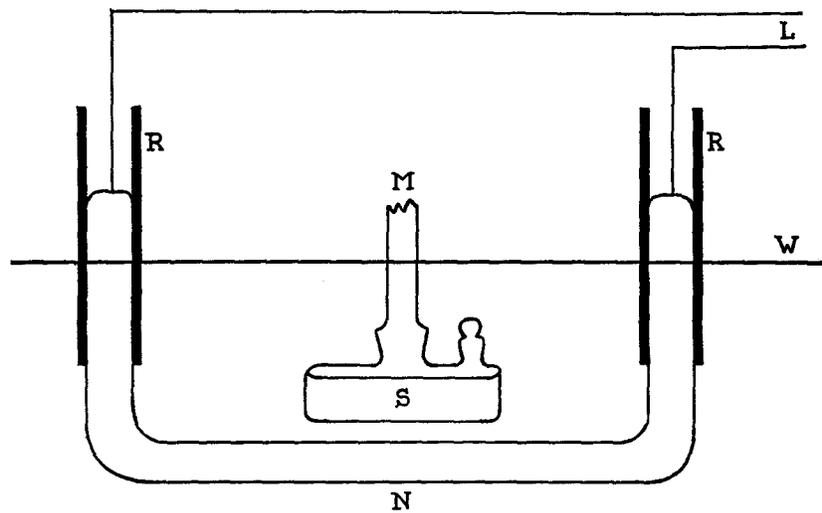


FIG. 6. Showing the positions of neon tube and vessel as used in the thermostat. L = lead-wires, one going to the commutator, the other to the condenser; R = rubber sheaths protecting electrodes; W = water level in thermostat; S = cell suspension; N = neon tube; M goes to the manometer tube.

by small amounts. Mixing the potassium solutions in the same proportions in which Warburg mixed his sodium solutions, we obtained curves closely similar to Warburg's which were made in the sodium mixtures.

Some experiments were made with the cells suspended in culture medium saturated with 5 per cent carbon dioxide in air, in order to make sure that the effects found were not connected with special properties of the carbonate mixture. In these cases it was necessary to use two vessels containing different volumes of cell suspension, as described by Warburg (1926, p. 108).

In order to ascertain whether the phenomena studied in flashing light were the same in other kinds of green cells, a few experiments were made with *Chlorella*

vulgaris, and with *Zostera marina*. The *C. vulgaris* was cultured in the same way as *C. pyrenoidosa*, and the *Zostera* was collected fresh from rock pools at low tide and used within 2 hours.

III

EXPERIMENTAL

As explained in the introduction, Warburg obtained a 100 per cent increase in photosynthesis from a unit amount of light by allowing the light to fall on the cells intermittently instead of continuously. He obtained his highest yields by using high frequencies of alternation of light and dark periods, while keeping the light and dark periods equal in length at any given frequency. To ascertain whether it was possible to still further increase the yield without further increasing the frequency, we used longer dark periods and shorter light periods. A neon tube connected as shown in Circuit B gave 50 flashes a second, and the flashes could be made to take up 45 per cent, 34 per cent, 25 per cent, or 17 per cent of the time. Readings were also taken with the tube burning only 7 per cent of the time, but the individual flashes were irregular in duration, so these readings are not included. As a standard of comparison for the photosynthesis in flashing light, we used the value of photosynthesis obtained with the neon tube connected as in Circuit A. As already stated, this does not give quite continuous light. The tube glows about 95 per cent of the time, and gives 100 flashes per second. However, photosynthesis was the same in this light as the maximum obtainable with ordinary incandescent bulbs (100 watt bulbs about 10 cm. from the cells), so we may regard the 95 per cent illumination as practically continuous as far as photosynthesis is concerned.

The results of an experiment carried out at 25°C. and at a relatively high concentration of carbon dioxide are shown graphically in Fig. 7, and numerically in Table I. The abscissae are *per cent time of illumination*, calculated from the angle measured on the rotating disc shown in Fig. 3. The ordinates are *photosynthesis per unit amount of light*. We found that for the shorter light periods the intensity was decreased, and since in flashing light photosynthesis becomes a linear function of intensity, we were obliged to make some correction for intensity differences for the different times of light and dark. We accomplished

this by comparing the readings at full intensity with others taken with a 5 per cent filter. At low intensities of light there is no improvement in photosynthesis with flashing light, and the rate is the integral of intensity \times time. The readings with no filter were divided by the corresponding readings with a 5 per cent filter to get photosynthesis per unit amount of light.

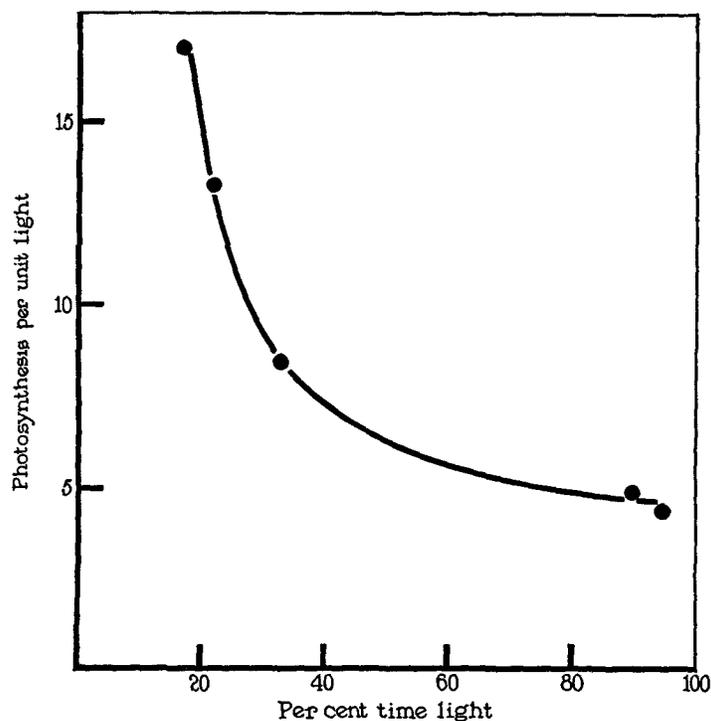


FIG. 7. Curve showing the relationship between per cent time of illumination and yield of photosynthesis per unit amount of light.

For 95 per cent light we have 4.4 units of photosynthesis for a given amount of light, and when the light is on only 17 per cent of the time, the same amount of light gives 17.7 units of photosynthesis. This is an improvement in yield of about 400 per cent. Extrapolation of the curve shows that still further improvement in yield could be obtained with longer dark periods and shorter light flashes.

From this experiment it is clear that the improvement in yield

is not only a function of the frequency of flashing. It is also dependent on the relative duration of light and dark periods. No information may be obtained from this experiment on the question of whether it is the increase in dark period or the shortening of the light flash which improves the yield. It may be either or both. We suppose that certain reactions in the photosynthetic process take place in the light only, and others can proceed in the light or for a short time in the dark. At 50 cycles per second one cycle is 0.02 second. 17 per cent of this is equal to 0.0034 second, the duration of one light flash when the light is on 17 per cent of the time. The corresponding dark period is 0.0166 second. Our light period of 0.0034 second is almost

TABLE I

Photosynthesis per unit amount of light with 50 flashes per second and different lengths of light and dark periods. Measurements made at 23.75°C. in carbonate mixture No. 9.

Time light in each cycle	Oxygen per hr. of light per c.mm. cells, 5 per cent filter (A)	Oxygen per hr. of light per c.mm. cells, no filter (B)	Photosynthesis per unit amount of light, no filter $\left(\frac{B}{A}\right)$
<i>per cent</i>	<i>c.mm.</i>	<i>c.mm.</i>	
95	4.04	17.8	4.4
90	3.65	18.1	4.9
33	1.93	16.4	8.5
22	0.84	11.2	13.3
17	0.44	7.9	17.7

the same as Warburg's shortest period of 0.0038. Our improvement in yield is 400 per cent as compared to his of 100 per cent. Our dark period is about 4.5 times as long as his. We can say from this that surely the longer dark period plays a large part. Our next experiment was designed to show how long a dark period was necessary to get the best possible yield out of a light flash of given intensity and duration.

For this it was necessary to use light flashes always of the same intensity and duration, but separated by a variable dark period. Our short light flashes were obtained from Circuit C, and our dark periods were timed by the commutator shown in Fig. 5.

The amount of photosynthesis per flash of light was calculated from the number of flashes per second and from the photosynthesis

in 5 minute periods, properly corrected for respiration. The photosynthesis per flash was plotted against the dark time between flashes. Fig. 8 shows three series of results plotted in this way. All were made with cells from the same culture. The points plotted as open circles were made first, at a temperature of 25°C. The ordinates

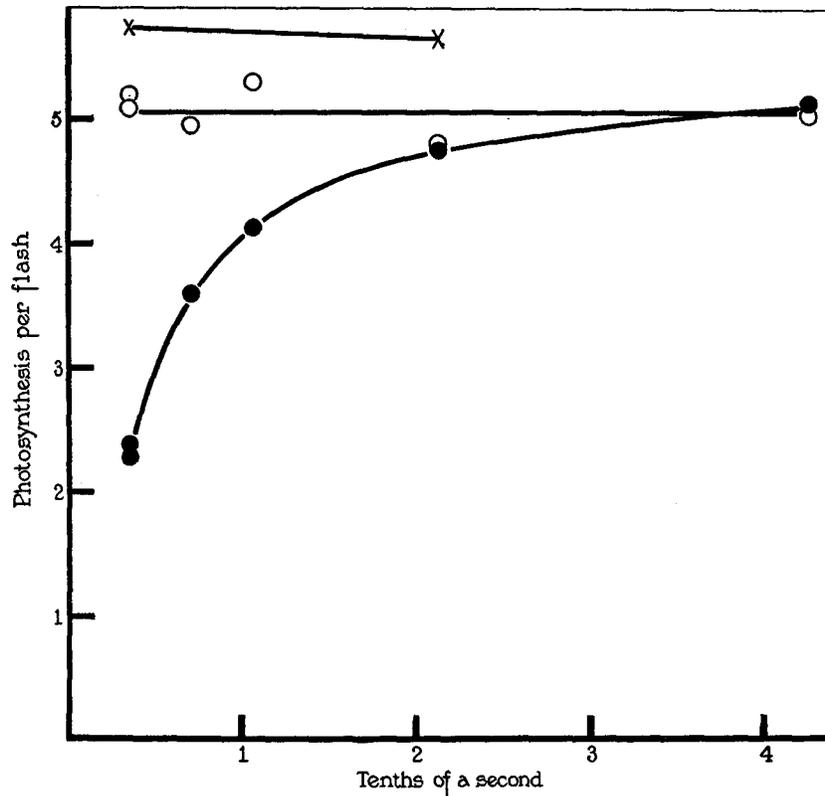


FIG. 8. The effect of dark time on yield of photosynthesis per flash of light. Open circles are points made at 25°C., solid circles at 1.1°C. The crosses are a check made at 25°C.

are photosynthesis per flash, and the abscissae the dark periods in tenths of a second. The shortest dark period used, about 0.04 second, is adequate for the complete removal of the material remaining at the end of each light flash. The longest dark period shown on this figure is over 0.4 second, and the yield per flash after this dark period is no

greater than the yield per flash after 0.04 second. We have tried lengthening the dark period to over 2 seconds, without finding any increase in photosynthesis per flash.

The points indicated by closed circles, plotted on the same scale as the open circles, were made at 1.1°C. Here the yield per flash is much lower with the short dark periods, but rises with increasing dark time until, after 0.4 second it is equal to the yield at 25°.

The crosses are measurements made at 25°C., after the completion of the curve at 1.1°C., to make sure that the original results obtained at 25°C. could be duplicated.

In considering these curves, and others like them to be described in this paper, it must be remembered that the light is "on" for a relatively small proportion of the time which elapses between readings. Consequently the dark reading used to correct for respiration plays a more important part than in continuous light. The longer the dark periods the smaller is the reading obtained for photosynthesis, and the more does the accuracy of the photosynthesis determination depend on the accuracy of the respiration reading. Respiration is never very accurately determined in the thin cell suspensions used for studying photosynthesis, and the respiration during light periods, either continuous or intermittent, may differ slightly from the respiration in an unbroken dark period, the only time when we can measure respiration. A small error in respiration will displace the points at the left hand ends of the curves only slightly, but will displace the right hand points much more, because there are only one-twelfth as many light flashes per unit time for the right hand readings as for the left hand readings. It is most favorable to work at low temperatures, where respiration is almost zero.

The numerical values of the points plotted in Fig. 8 are given in Table II. Protocol 1 at the end of the paper gives all experimental data. This experiment shows that the dark process is temperature-sensitive, and that the light process is not. If the light process were also temperature-sensitive, Curve B would not attain the same level as Curve A. Since that part of the photosynthetic process which is temperature-sensitive is usually called the Blackman reaction, we feel justified in saying that it is the Blackman reaction which goes on in our dark period, without implying whether or not other reactions are involved as well.

These curves would be the same in shape whether the Blackman reaction preceded or followed the photochemical reaction. In the first case the long dark period would improve the yield because *preparation* for the light reaction had time for completion; in the second case because changes *following* the light reaction had time for completion. Our experiments offer no decision on this point, but since Warburg (1920, p. 188, Section VI) has presented evidence that the Blackman reaction follows rather than precedes the photochemical reaction, we shall speak of the dark process as following the light process.

TABLE II

Photosynthesis per flash of light after different lengths of dark period. The light flashes were kept constant and only the dark periods varied. Complete data in Protocol 1.

Dark time	Oxygen per flash per c.mm. cells $\times 10^6$		
	At 24.85°C.	At 1.1°C.	At 24.87°C.
<i>sec.</i>	<i>c.mm.</i>	<i>c.mm.</i>	<i>c.mm.</i>
0.035	5.20	2.28	5.75
0.425	5.04	5.12	
0.212	4.80	4.75	5.64
0.106	5.30	4.14	
0.071	4.97	3.60	
0.035	5.10	2.39	

The light reaction, we suppose, produces an intermediate product. This product might either be converted to other intermediate or final products of photosynthesis through the operation of the Blackman reaction, or it might spontaneously revert to its original state or component parts. Since the curve plotted with solid circles in Fig. 8, made at 1.1°C., attains after 0.4 second the same level as that shown by the open circles after 0.03 second, made at 25°C., it is clear that the slower rate of the Blackman reaction at 1.1°C. has resulted in no loss of intermediate product. There has merely been a delay in the utilization of the product of the photochemical reaction. Therefore if any spontaneous decomposition of the intermediate product formed by the photochemical reaction takes place, the loss must be the same after 0.03 second at 25°C. as after 0.4 second at 1.1°C. In other words, the temperature coefficient of the spontaneous decom-

position must be the same as the temperature coefficient of the Blackman reaction. This is an unlikely possibility, and we consider it more probable that no spontaneous decomposition takes place. This is supported by experiments in which the Blackman reaction was inhibited by prussic acid. It is most improbable that the prussic acid would have the same specific effect on both the Blackman reaction and any spontaneous decomposition or deactivation.

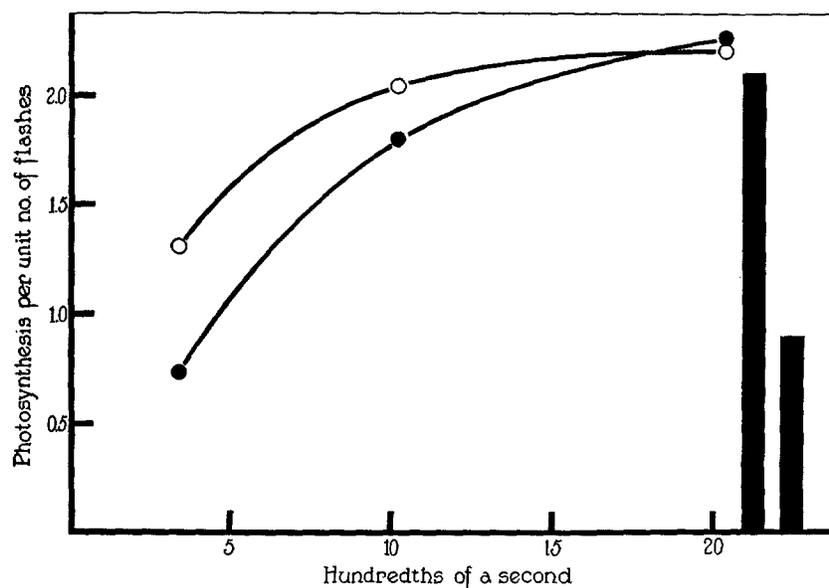


FIG. 9. The effect of prussic acid on the course of the dark reaction. Open circles are points made without prussic acid, solid circles points made with 1.14×10^{-5} mols prussic acid per liter. The black columns show the effect of the same concentration of prussic acid on photosynthesis in continuous light. The high column is the rate without prussic acid, and the short column is the rate when inhibited by prussic acid.

We used a free prussic acid concentration of 1.14×10^{-4} mols per liter in Warburg's carbonate mixture No. 9. (For the calculation of free prussic acid in carbonate mixture containing potassium cyanide see: Emerson, 1929, p. 635.) This concentration of 1.14×10^{-4} mols per liter is sufficient to inhibit photosynthesis in continuous light 60 per cent. In flashing light the longer the dark periods the

less the inhibition, and if the dark periods are long enough the cells in cyanide will give the same value as the cells without cyanide. This is shown graphically in Fig. 9. The cyanide curve plotted with solid circles slopes upward more steeply than the normal curve, which is plotted with open circles. After 0.2 second the cyanide curve has reached the level of the normal, and there is no inhibition. Changing to continuous light there appears immediately a 60 per cent inhibition of photosynthesis, shown graphically by the black columns. The numerical values for Fig. 9 are given in Table III. Photosynthesis has not been reduced to the value per single flash of light, but only

TABLE III

The effect of cyanide on the course of the dark reaction, and on photosynthesis in continuous light. Measurements made in carbonate mixture No. 9, at a temperature of 13°C., using 9.6 c. mm. cells suspended in 7 cc. of fluid.

Dark time ,	Oxygen per unit No. of flashes		Inhibition by HCN
	Without cyanide	Free HCN 1.14×10^{-4} mols per liter	
<i>sec.</i>	<i>c.mm.</i>	<i>c.mm.</i>	<i>per cent</i>
0.035	1.31	0.74	50
0.106	2.04	1.80	12
0.212	2.20	2.26	0
5 min. continuous light.....	8.43	3.60	57.

to the value per unit number of flashes. For convenience the unit used was the number of light flashes in 5 minutes when the neon tube was flashed once (or in some cases twice) in each revolution of the commutator. It was not thought necessary to reduce the figures to photosynthesis *per* single flash except in the case of Table II and Fig. 8.

Fig. 9 shows that the Blackman reaction requires a longer time to complete its work in the presence of cyanide, but the total amount of photosynthesis done is no less in the cyanide if the dark periods are long enough. We can say then that whatever is produced in the light reaction is stable for at least 0.4 second, and is removed only through the operation of the Blackman reaction.

It might be that chlorophyll alone is involved in the light reaction, and the absorbed energy transferred later to a carbon dioxide compound, or it might be that both chlorophyll and carbon dioxide are required for the light reaction. In the first case we would expect that lower carbon dioxide concentrations would not decrease the yield per flash but would necessitate longer dark periods for full utilization of the light. In the second case we would expect the yield per flash to be cut down by low carbon dioxide concentrations, but the course of the dark reaction should remain unchanged.

TABLE IV

A comparison of the effect of carbon dioxide concentration on photosynthesis in continuous and intermittent light. Each measurement made with 8.2 c. mm. cells, at a temperature of 24.27°C. Carbon dioxide concentrations are for sodium mixtures.

Carbon dioxide concentration in mols per liter $\times 10^{-4}$ (from Warburg)	Oxygen produced in 5 min. continuous light	Oxygen produced in 5 min. flashing light, 24.05 flashes per sec.
	<i>c. mm.</i>	<i>c. mm.</i>
2.6	4.46	2.80
9.8	9.84	3.62
23.0	15.55	4.36
91.0	20.35	5.44
91.0	20.97	5.92
23.0	16.05	4.85
9.8	9.20	3.80
2.6	3.00	2.46

Before making this experiment it was necessary to test whether the process of photosynthesis is the same function of carbon dioxide concentration in flashing light and in continuous light, in order to select appropriate concentrations. This was done at a high temperature and with dark periods of about 0.1 second to allow for completion of the dark reaction between flashes. Table IV shows the rates of photosynthesis in four different carbonate mixtures in both flashing and continuous light. The values are plotted against carbon dioxide concentration in Fig. 10. The points shown by open circles were made in continuous light, and those shown by solid circles in intermittent. The upper curve is similar in shape to the lower curve.

Carbon dioxide saturation is approached in the same way in continuous and in flashing light. In carbonate mixture No. 9 the rate is changing only very slowly with carbon dioxide concentration, and in mixture No. 5 the rate is cut down about half, and varies proportionately to concentration. Complete data for this experiment are given in Protocol II.

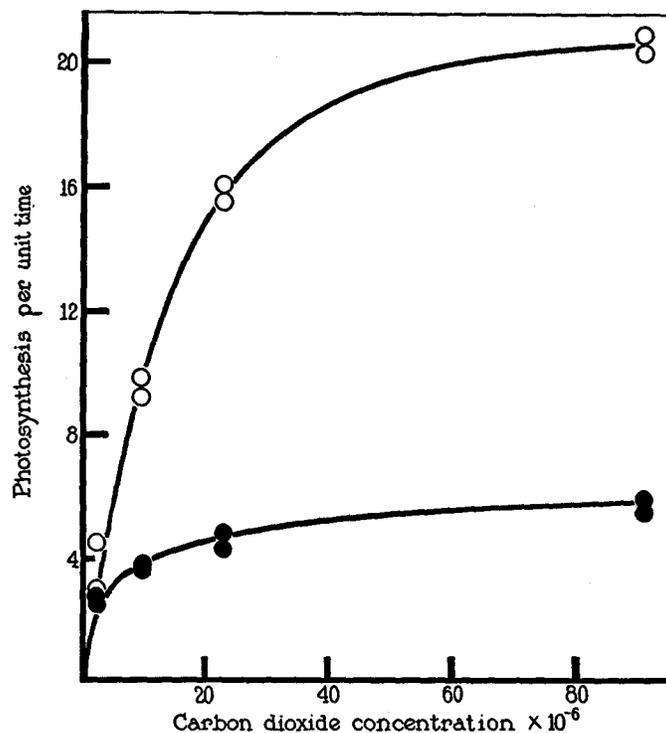


FIG. 10. The effect of carbon dioxide concentration on photosynthesis in continuous light (open circles) and in flashing light (solid circles), twenty-four flashes per second.

The course of the dark reaction was studied in these two mixtures at 6°C . Table V gives the relative values of photosynthesis per flash for the various dark periods, and the results are plotted in Fig. 11. The upper curve is for the higher carbon dioxide concentration, 71×10^{-6} mols per liter, and the lower curve for the lower concentration, 4.1×10^{-6} mols per liter. Both curves bend parallel to the abscissa

after a dark time of 0.08 second. There is no indication that the lower curve will rise to the level of the upper one. The lower concentration of carbon dioxide prevents the light flashes from producing as much photosynthesis as at high concentrations, regardless of dark time. The dark reaction apparently runs its course in about the same length of time, regardless of the amount of intermediate product produced by the light flash.

This experiment shows that the carbon dioxide enters the process of photosynthesis either before or coincident with the photochemical reaction. It seems unlikely that the carbon dioxide molecules could

TABLE V

The course of the dark reaction at two concentrations of carbon dioxide. For each measurement 9.0 c. mm. cells were used. Temp. = 5.9°C.

Dark time	Oxygen produced per unit No. of flashes	
	CO ₂ conc. 4.1×10^{-8} mols per liter	CO ₂ conc. 71×10^{-8} mols per liter
<i>sec.</i>	<i>c.mm.</i>	<i>c.mm.</i>
0.02	0.91	2.34
0.04	2.11	3.89
0.08	2.89	4.92
0.12	2.78	5.13
0.02	1.14	2.36

move into position and react during the extremely short light flash of about a hundred-thousandth of a second. We think it more reasonable to suppose that the carbon dioxide reacts first, possibly combining with chlorophyll in the dark before the light flash. The concentration of chlorophyll-CO₂ formed would depend on the concentrations of chlorophyll and of carbon dioxide. The light flash would activate the chlorophyll-CO₂ molecules and leave them ready to undergo the Blackman reaction. The possibility that carbon dioxide combines with chlorophyll in photosynthesis has already been suggested by Willstätter (1918, p. 172). If such a compound is formed we would expect that low chlorophyll concentrations would affect the course of the dark reaction like low concentrations of carbon dioxide.

The chlorophyll content of *Chlorella pyrenoidosa* cells cannot be

varied conveniently with the method used by Emerson (1929) for *Chlorella vulgaris*, but it was found possible to change the chlorophyll concentration per unit volume of *C. pyrenoidosa* cells by another method. Details and quantitative data will probably be given in a later paper. When cultures are grown over red (neon) and blue (mercury) luminous tubes, the cells produced in red light contain roughly one-fourth the chlorophyll of those produced in the blue light.

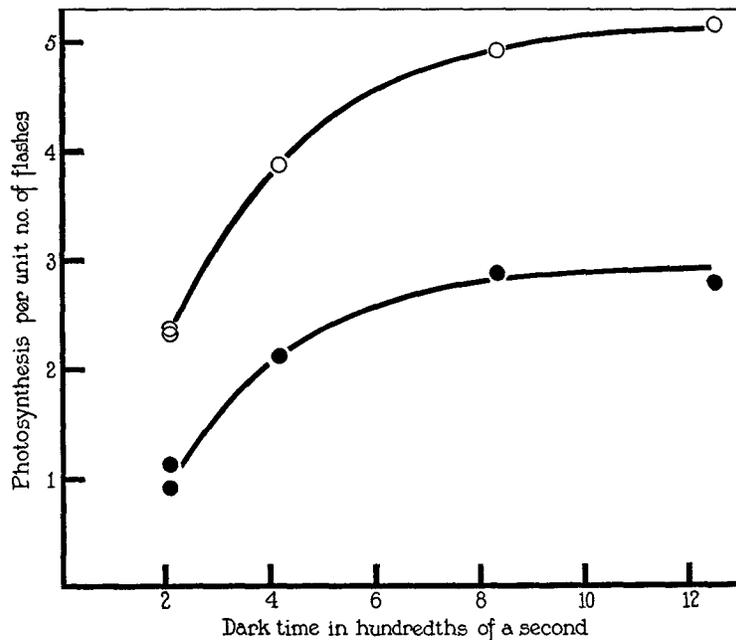


FIG. 11. The course of the dark reaction at two different concentrations of carbon dioxide. Open circles, CO₂ concentration 71×10^{-6} mols per liter. Solid circles, CO₂ concentration 4.1×10^{-6} mols per liter.

A unit volume of cells grown in blue light will photosynthesize almost four times as fast as the same volume of cells grown over red light, when both kinds are used over continuous incandescent light.

Table VI gives data for the course of the dark reaction with these two types of cells. Photosynthesis per unit number of flashes is plotted against dark time in Fig. 12. Owing to the much smaller values for the cells grown in red light these points, plotted as solid

circles, have been multiplied by a constant to make the curves readily comparable. The open circles represent points made with cells grown over red light, solid circles with cells grown over blue light. The experiment shows that the course of the dark reaction is not affected by the chlorophyll content of the cells. Only the amount of intermediate product formed in the light reaction is affected by this factor.

So far, then, we know that the total amount of photosynthesis derivable from a given flash of light is determined by the chlorophyll concentration per unit volume of cells, and the concentration of carbon dioxide in the medium. Neither of these factors affect the length of dark period required for maximum yield. This is determined by the

TABLE VI

The course of the dark reaction at two concentrations of chlorophyll. Measurements made at 6.92°C. in carbonate mixture No. 9.

Dark time	Oxygen produced per unit No. of flashes	
	11.6 c.mm. cells with high concentration of chlorophyll	22.0 c.mm. cells with low concentration of chlorophyll
<i>sec.</i>	<i>c.mm.</i>	<i>c.mm.</i>
0.02	2.06	1.20
0.04	3.35	1.67
0.08	4.02	2.28
0.12	4.04	2.22
0.02	1.97	1.24

temperature, and can also be affected by prussic acid. Because Warburg (1925, p. 399) found that indifferent narcotics would inhibit photosynthesis at both high and low intensities of light, he concluded that they inhibited both photochemical reaction and dark reaction. We should expect that narcotics would cut down the yield per flash, and also prolong the time for completion of the dark reaction. We performed experiments with thymol and phenylurethane. A complete series of urethanes was not obtainable. Since two such different compounds as thymol and phenylurethane produced the same effect we may consider their action to be non-specific, and due only to adsorption.

The experiments were carried out in carbonate mixture No. 9, and a concentration of narcotic was chosen which inhibited photosynthesis

in continuous light about 50 per cent. The phenylurethane could be weighed out and dissolved conveniently, but the thymol was so insoluble that we were obliged to saturate a solution at a given temperature by adding excess thymol, warming, and filtering after the solution had cooled to the temperature desired. The filtered solution was then diluted until we found a concentration giving 50 per cent inhibition.

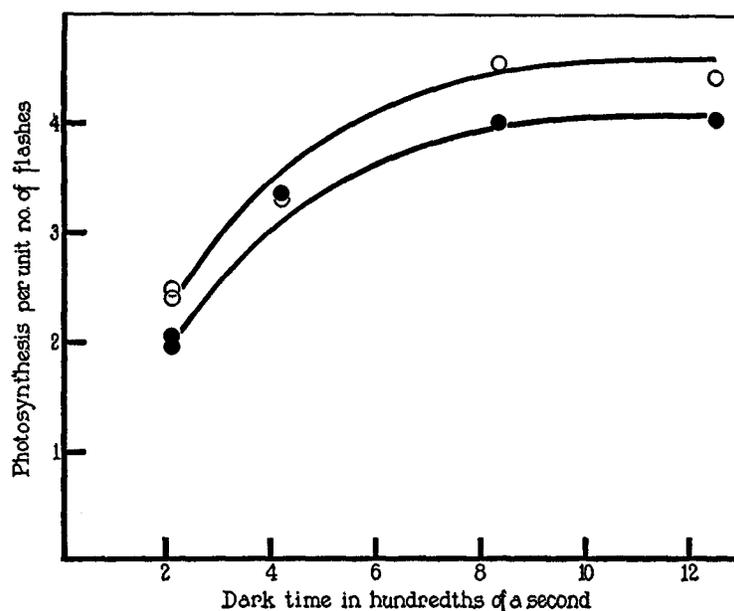


FIG. 12. The course of the dark reaction at a low concentration of chlorophyll (open circles) and at a high concentration of chlorophyll (solid circles). The observations for the open circles have been multiplied by a constant to bring the curves close together and make them easily comparable.

A narcotic concentration which inhibits photosynthesis 50 per cent in continuous light will give about 50 per cent inhibition in flashing light if the flashes are far enough apart to allow for completion of the Blackman reaction. Neither thymol nor phenylurethane had a marked effect on the Blackman reaction. In order to make the effect clearer, the cells inhibited by narcotic were compared with others whose photosynthesis was cut down by a 50 per cent filter, so that in each

case the Blackman reaction would have about the same amount of intermediate product on which to work. It should be mentioned that the rate of photosynthesis in flashing light is almost a linear function of intensity. The work on intensity is not yet completed, and will be published later.

Figs. 13 and 14 show the course of the dark reaction in narcotic and with a 50 per cent transmission filter. The narcotic curves rise

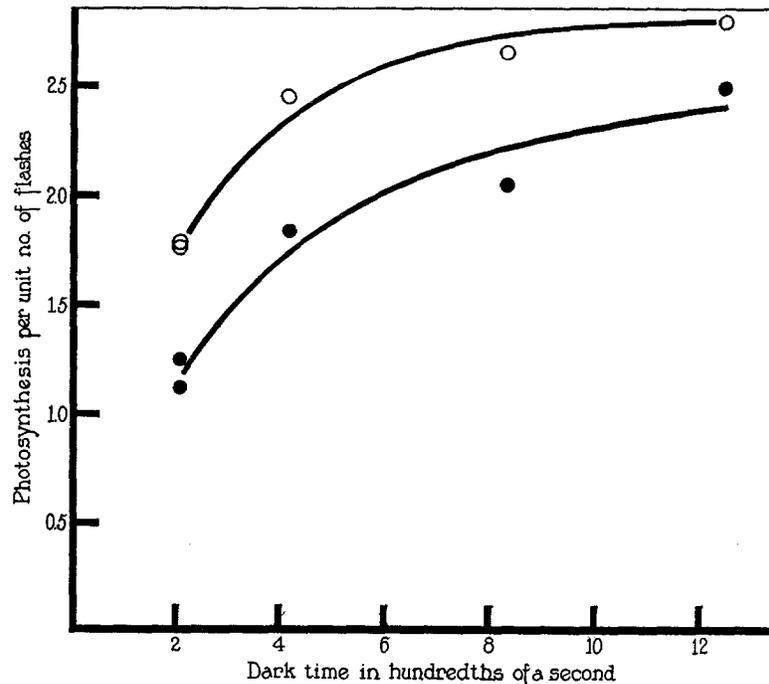


FIG. 13. The effect of phenylurethane on the course of the dark reaction. Open circles are points made without urethane, solid circles points made with urethane 0.0034 per cent.

slightly more gradually and for a longer time than the curves for the 50 per cent filter, indicating that the Blackman reaction may be slightly inhibited by narcotics. However, the chief effect of narcotics is an inhibition of the formation of intermediate product, possibly by preventing some of the chlorophyll from combining with carbon dioxide, or possibly by a direct inhibition of the photochemical

reaction. This would also explain why photosynthesis at both high and low intensities is inhibited by narcotics, even though the Blackman reaction is not much affected.

To make sure that the phenomena described in this paper were not special characteristics of *Chlorella pyrenoidosa*, some experiments were made with *Chlorella vulgaris* and with *Zostera marina*. Increase of yield with increasing dark time could be demonstrated in both organ-

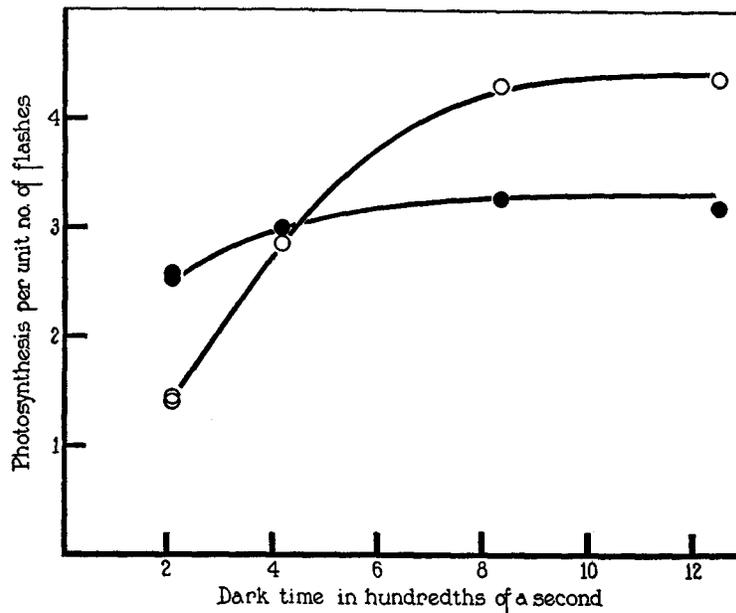


FIG. 14. The effect of thymol on the course of the dark reaction. Solid circles are points made without thymol, open circles points made with 1/70 saturated thymol.

isms, using the same intensity of light, temperature, and dark periods as for *Chlorella pyrenoidosa*. The course of the dark reaction for *C. vulgaris* (solid circles) is plotted with a curve for *C. pyrenoidosa* (open circles) in Fig. 15. The curves were made at the same time under identical conditions. The cells of *C. vulgaris* are much larger than *C. pyrenoidosa*, the chloroplasts are different, the chlorophyll content smaller, the rate of growth slower, and the relative values of

TABLE VII

Effect of narcotics on the course of the dark reaction. Temperature 7°C., cells suspended in carbonate mixture No. 9.

Dark time <i>sec.</i>	Oxygen produced per unit number of flashes		
	With 50 per cent filter, no narcotic <i>c.mm.</i>	With 100 per cent filter, and narcotic <i>c.mm.</i>	
0.02	1.77	1.25	0.0034 per cent phenyl- urethane, 13.5 c.mm. cells
0.04	2.45	1.84	
0.08	2.64	2.05	
0.12	2.75	2.48	
0.02	1.78	1.12	
0.02	2.54	1.42	1/70 saturated thymol, saturated at 21°C., 16.5 c.mm. cells
0.12	3.18	4.37	
0.08	3.26	4.30	
0.04	3.00	2.81	
0.02	2.58	1.45	

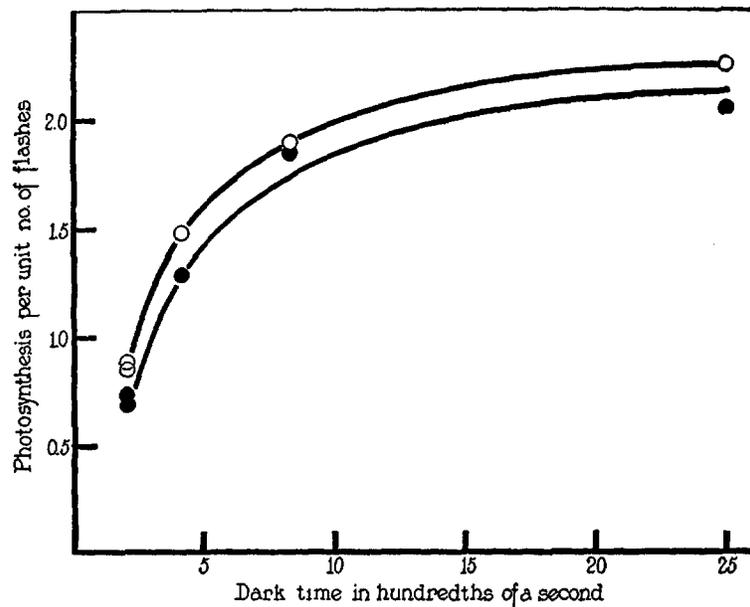


FIG. 15. A comparison of the course of the dark reaction in *Chlorella pyrenoidosa* (open circles) and *Chlorella vulgaris* (solid circles).

photosynthesis and respiration are different. The similarity of the curves is striking, and we believe this relationship between light and dark reactions is probably a general property of chlorophyll photosynthesis.

We have also measured photosynthesis in flashing light with cells suspended in phosphate solution saturated with 5 per cent carbon dioxide in air, to make sure that the carbonate mixture was not responsible for our curves. The photosynthetic quotient was found to be unity, and the effect of lengthening the dark time was the same as in carbonate mixture No. 9.

TABLE VIII

A comparison of the dark reactions of *Chlorella pyrenoidosa* and *Chlorella vulgaris*. Cells suspended in carbonate mixture No. 9 at a temperature of 6.5°C.

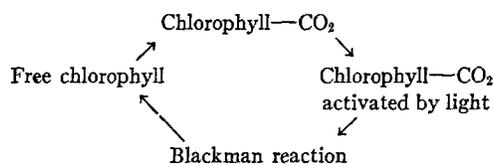
Dark time	Oxygen produced per unit No. of flashes	
	8.75 c.mm. <i>C. pyrenoidosa</i>	20.5 c.mm. <i>C. vulgaris</i>
<i>sec.</i>	<i>c.mm.</i>	<i>c.mm.</i>
0.02	0.86	0.69
0.04	1.48	1.29
1.08	1.90	1.85
0.12	1.78	1.83
0.25	2.25	2.05
0.02	0.88	0.74

VI

RECAPITULATION

The experiments described in this paper show that photosynthesis involves a light reaction not affected by temperature, and capable of proceeding at great speed, and a dark reaction dependent on temperature, which requires a relatively long time to run its course. The light reaction can take place in about a hundred-thousandth of a second. The dark reaction requires less than 0.04 second for completion at 25°C., and about 0.4 second at 1.1°C. The light reaction is dependent on carbon dioxide concentration and is inhibited by narcotics. The dark reaction is not noticeably inhibited by narcotics, is independent of carbon dioxide concentration, and is strongly inhibited by cyanide.

Throughout the paper the results have been treated on the supposition that the Blackman reaction follows the photochemical reaction. This is true in the sense that a given carbon dioxide molecule undergoes first the photochemical reaction and then the Blackman reaction. But it is likely that the Blackman reaction also sets free the chlorophyll, enabling the latter to react again with carbon dioxide. Emerson (1929) has presented evidence that the chlorophyll is involved in the Blackman reaction. To this extent, then, the question of whether the photochemical reaction precedes the Blackman reaction is only an academic one. From the point of view of the carbon dioxide the photochemical reaction comes first, but the process may equally well be regarded as a cycle in which free chlorophyll combines with carbon dioxide, becomes activated by light, undergoes the Blackman reaction, and is again set free to combine with more carbon dioxide:



Our thanks are due to various members of the electrical engineering and physics staff of the California Institute, and especially to Professor Mackeown and Professor Bowen, for much helpful advice on the electrical circuits. We are also indebted to Mr. Erickson of the Electrical Products Corporation for his advice on the type of tubes and electrodes best suited to our work, and to the Electrical Products Corporation for supplying the tubes.

Protocol I

Complete Data for Fig. 8 and Table II

All measurements made in same vessel. *C. pyrenoidosa* suspended in 7 cc. carbonate mixture No. 9.

Rotor arm making 141 R.P.M.

Resistance in charging circuit 7500 ohms.

Half-wave rectifier, 2200 volts, used to charge condenser.

No. of flashes per revolution of rotor arm	Dark time	Date...	Aug. 9		Aug. 10		Aug. 11	
		Temp..	24.85°C.		1.1°C.		24.87°C.	
		K ₂ O ₂	0.41		0.464		0.41	
		C.mm. cells..	10.4		9.0		10.3	
		Change in pressure in 5 min.	Oxygen per flash per c.mm. cells × 10 ⁵	Change in pressure in 5 min.	Oxygen per flash per c.mm. cells × 10 ⁵	Change in pressure in 5 min.	Oxygen per flash per c.mm. cells × 10 ⁵	
	<i>sec.</i>	<i>mm.</i>	<i>c.mm.</i>	<i>mm.</i>	<i>c.mm.</i>	<i>c.mm.</i>	<i>mm.</i>	
12	0.035	+9.33	5.20	+3.50	2.28	+10.27	5.75	
1	0.425	-0.93	5.04	+0.43	5.12			
2	0.212	-0.10	4.80	+1.04	4.75	+0.03	5.64	
4	0.106	+1.97	5.30	+2.00	4.14			
6	0.071	+3.50	4.97	+2.70	3.60			
12	0.035	+9.03	5.10	+3.67	2.39			
Dark respiration		-1.83		-0.27		-1.97		

Protocol II

Complete Data for Fig. 10 and Table IV

The effect of carbon dioxide concentration on photosynthesis in continuous and flashing light. Temperature 24.27°C. 8.2 c. mm. cells suspended in 7 cc. carbonate mixture used for each determination.

K₂O₂ = 0.41. Resistance in charging circuit 5000 ohms.

No. of mixture	Composition of mixture		Concentration of CO ₂ in mols per liter*	Continuous light			24 flashes per sec.		
	$\frac{M}{10}$ K ₂ CO ₃	$\frac{M}{10}$ KHCO ₃		Change of pressure, 5 min.		O ₂ per hr. per c.mm. cells	Change of pressure, 5 min.		O ₂ per hr. per c.mm. cells
				Light	Dark (respiration)		Light	Dark (respiration)	
	<i>cc.</i>	<i>cc.</i>		<i>mm.</i>	<i>mm.</i>	<i>c.mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>c.mm.</i>
4	70.	30.	2.6	+5.47	-1.97	4.46	+2.70	-1.97	2.80
6	50.	50.	9.8	+14.50	-1.93	9.84	+4.10	-1.93	3.62
7	35.	65.	23.0	+23.75	-2.17	15.55	+5.10	-2.17	4.36
9	15.	85.	91.0	+31.80	-1.57	20.35	+7.50	-1.57	5.44
9	15.	85.	91.0	+33.37	-1.57	20.97	+8.30	-1.57	5.92
7	35.	65.	23.0	+24.60	-2.17	16.05	+5.93	-2.17	4.85
6	50.	50.	9.8	+13.45	-1.93	9.20	+4.40	-1.93	3.80
4	70.	30.	2.6	+3.05	-1.97	3.00	+2.15	-1.97	2.46

* Figures from Warburg for sodium carbonate mixtures.

Protocol III

Complete Data for Fig. 11 and Table V

The course of the dark reaction at two concentrations of carbon dioxide.

9.0 c. mm. cells suspended in 7 cc. of carbonate mixture used for each measurement. $K_{O_2} = 0.453$. Temperature = 5.9°C.

Resistance in charging circuit 5000 ohms.

Rotor arm turning 240 R.P.M.

No. of flashes per revolution of rotor arm	Dark time	Carbonate mixture 5, 60 cc. $\frac{M}{10}$ K_2CO_3 , 40 cc. $\frac{M}{10}$ $KHCO_3$, 4.1×10^{-6} mols CO_2 per liter		Carbonate mixture 9, 15 cc. $\frac{M}{10}$ K_2CO_3 , 85 cc. $\frac{M}{10}$ $KHCO_3$, 71×10^{-6} mols CO_2 per liter	
		Change of pressure, 5 min.	O_2 per unit No. of flashes	Change of pressure, 5 min.	O_2 per unit No. of flashes
		mm.	c.mm.	mm.	c.mm.
12	0.02	+4.45	0.91	+13.25	2.34
6	0.04	+5.35	2.11	+10.85	3.89
3	0.08	+3.30	2.89	+6.55	4.92
2	0.12	+1.75	2.78	+4.30	5.13
12	0.02	+5.80	1.14	+13.30	2.36
Dark reading, respiration...		-1.03		-0.83	

CITATIONS

- Brown, H. T., and Escombe, F., *Proc. Roy. Soc. London, Series B*, 1905, **76**, 29.
 Emerson, R., *J. Gen. Physiol.*, 1929, **12**, 609.
 Emerson, R., *J. Gen. Physiol.*, 1929, **12**, 623.
 Warburg, O., *Biochem. Z.*, 1919, **100**, 230.
 Warburg, O., *Biochem. Z.*, 1920, **103**, 188.
 Warburg, O., *Biochem. Z.*, 1925, **166**, 386.
 Warburg, O., *Über den Stoffwechsel der Tumoren*, Berlin, Julius Springer, 1926.
 Willstätter, R., and Stoll, A., *Untersuchungen über die Assimilation der Kohlensäure*, Berlin, Julius Springer, 1918.