THE INFLUENCE OF LIGHT AND CARBON DIOXIDE ON PHOTOSYNTHESIS*

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I

INTRODUCTION

Abundant evidence has accumulated to show that the primary reactions in the photosynthetic mechanism involve a cyclical process consisting of a photochemical reaction and a temperature-sensitive reaction ("dark" or "Blackman reaction"). This concept has been based on the studies made by Blackman (1905) on the effect of temperature and light intensity on the rate of photosynthesis and expressed by him as "the law of limiting factors." But it was the studies later made by Warburg (1919) which definitely showed the need for interpreting the properties of the system as a two-reaction process. Emerson and Arnold (1932 *a*, *b*) using intermittent illumination have made a thorough study of these cyclical reactions, and have contributed much to our knowledge of them. Thanks to the work of these investigators and others, this concept serves as one of the main bases for further progress in the understanding of the photosynthetic mechanism.

Starting with the system as a cyclical process, several investigators (Stoll, 1932, 1936; Franck, 1935; Gaffron and Wohl, 1936) have recently considered certain reactions as possibly being involved in photosynthesis. These discussions have revolved for the most part about the properties shown by chlorophyll *in vitro* and on quantum yields and the energies involved in possible reactions, and have neglected quantitative treatment of the reaction kinetics. On the other hand, many schemes have been proposed for the kinetics of the

* A preliminary account of this work has been presented (Smith, 1936).

process, particularly in relation to light and carbon dioxide (*e.g.*, Baly, 1935; Burk and Lineweaver, 1935; Arnold, 1935).

In order to evaluate the many suggestions regarding mechanism and kinetics, it is necessary to have definitive measurements of the kinetic relationships covering a range sufficient to render them critical. The existing data do not cover the necessary range or are of inadequate precision. Moreover, it has not been demonstrated that measurements made with one plant show fundamentally the same properties as with another. We have therefore made extensive measurements with one plant for the effect of CO_2 concentration and light intensity, and have compared them with the previous data for other plants under conditions which show their basic similarities and differences.

II

Apparatus and Procedure

One of the principal difficulties connected with previous studies on the effect of light intensity has been the inability to achieve a high intensity of illumination without serious temperature disturbance. Emerson (1929) records a maximum intensity of about 100,000 meter candles, which was just about sufficient to reach the maximum rate of photosynthesis under the conditions of his experiments. However, in order to be really certain of the form of the intensity-photosynthesis curves, it is necessary to have measurements which definitely indicate the maximum rate of photosynthesis. An arrangement was therefore set up whereby a maximum intensity of 282,000 meter candles (Lux) was achieved. It is shown diagrammatically in vertical section in Fig. 1.

The source was a 500 watt projection lamp. A condenser consisting of two plano-convex lenses 4½ inches in diameter and 5½ inches focal length formed an image of the filament approximately in the plane of a projection lens 18 inches from the condenser. This lens was also plano-convex of 7½ inches focal length and 4½ inches in diameter; it formed an image of the condenser in the plane of the bottom of the manometer vessel. The condenser was suitably diaphragmed in order to reduce the amount of stray light so that the illuminated area at the bottom of the vessel was just sufficient to cover it when the manometer was being shaken. The amount of light was approximately doubled by the use of a spherical mirror behind the lamp. Since the thermostat was constructed of solid opaque walls, the entire apparatus had to be mounted at an angle in a copper tray and a plane surface mirror mounted in the bath to reflect the light upward. Movement of the surface water produced by the shaking of the manometers did not affect the beam of light, since the light entered the water of the thermostat some inches below the surface. To prevent deterioration of the mirror mounted in the

water, it was necessary to place it in a brass case protected with aquarium cement on the silvered surface.

The intensity of the light was varied with neutral filters made by uniformly exposing 5 by 7 inch photographic plates. Calibrations were made by placing an opal glass plate at the level of the bottom of the manometer vessel and measuring the transmitted light directly with a Macbeth illuminometer. The absolute total brightness was determined in the same way by correcting for the transmission of the opal glass plate. In order to be certain that the filters used were neutral with regard to the visible spectrum, check calibrations were made using a filter (Corning No. 246) which transmitted only wave lengths longer than about 580 mμ. The values so obtained were identical with the white light values.

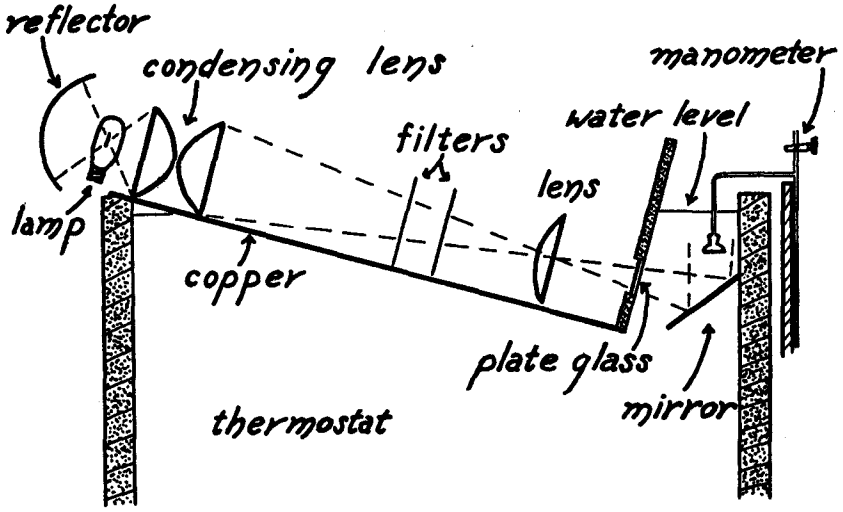

FIG. 1. A diagram in vertical section of the apparatus

Photosynthesis was determined as oxygen produced using the Warburg manometric method (Dixon, 1934). Since this method is now well known, only details of importance in this research are described. One experimental vessel was used with two thermobarometric controls containing the same solution as in the experimental vessel. The volume of the experimental vessel used in all the experiments was 9.858 cc. to the level of the Brodie's fluid, and was determined with the mercury method described by Dixon. 4 or 5 cc. of buffer solution were used and the vessel constants computed were always corrected for the volume of plant tissue in the particular experiment. In all of the experiments described below, the temperature was kept constant at 25.3° C. ± 0.005°.

The sources of carbon dioxide were the carbonate-bicarbonate mixtures described by Warburg (1919) using the potassium salts as recommended by Emerson and Arnold (1932*a*). The carbon dioxide concentrations were recomputed using the

more recent data of MacInnes and Belcher (1933) for the change in the dissociation constants with ionic strength at 25°C. From the law of mass action (Warburg):

$$[CO_2] = \frac{[KHCO_3]^2}{[K_2CO_3]\ K_1'/K_2'}. \tag{1}$$

In logarithmic form, this equation becomes

$$\log [CO_2] = 2 \log [KHCO_3] - \log [K_2CO_3] + pK_1' - pK_2' \tag{2}$$

where $pK_1' = -\log K_1'$, and $pK_2' = -\log K_2'$. According to MacInnes and Belcher, the following empirical expressions hold at 25°C.

$$\begin{aligned} pK_1' &= pK_1 - k_1\mu \\ pK_2' &= pK_2 - k_2\mu^{\frac{1}{2}} \end{aligned} \tag{3}$$

where μ is the ionic strength and the experimentally determined values are: $pK_1 = 6.343$, $pK_2 = 10.252$, $k_1 = 0.119$, and $k_2 = 0.382$. Although their determinations of pK_1' cover a range of ionic strengths below those used here, the change of pK_1' with μ is so small that the extrapolation to higher values is probably justified. Their determinations of pK_2' are within the range of values used here. In Table I are presented the values computed using the above equations and data. Warburg's values are presented for comparison. The CO_2 concentrations computed from the data of MacInnes and Belcher are from 0.042 to 0.073 log units lower than those found by Warburg, which is not a very serious difference considering that all of the data are displaced in the same direction.

In order to obtain a solution giving a higher CO_2 concentration than any of these buffer mixtures, tenth molar $KHCO_3$ was used. Its CO_2 concentration was computed from the following formula which gives a very close approximation (Clark, pp. 562–563, 1928):

$$\log [CO_2] = pK_1' + \log [HCO_3^-] - pH.$$

$[HCO_3^-]$ was regarded as equal to $[KHCO_3]$, and pK_1' was obtained from the formula of MacInnes and Belcher given above. A glass electrode was used to measure the pH, which is somewhat variable even with the freshly prepared solution always used in these experiments. An average value for log $[CO_2]$ equal to -3.0 was obtained; a value which is probably not in error by more than a tenth of a log unit. None of the data are seriously affected, since in this solution the rate of photosynthesis is so high that it does not change significantly with the CO_2 concentration.

In all of the experiments described, the common aquarium plant *Cabomba caroliniana* was used. Small fronds of about 100 mg. wet weight were sufficiently active to give good measurements. It was found that after an equilibration period the same piece of tissue would give constant readings for many hours as

long as the buffer mixtures were renewed often enough to prevent an effective decrease in CO_2 concentration. This enabled us to make entire runs with either CO_2 or light intensity as the variable on the same piece of tissue. Although smaller pieces of tissue (taken nearer the apex) were more active per milligram (wet weight), identical curves were obtained regardless of the amount of tissue used.

Measurements of the rate of respiration made at the beginning of a run were always lower than those made after the plant had been carrying on a high rate of photosynthesis. Since a small change in respiration rate has a large effect on measurements made at low photosynthesis rates, the respiration value used in correcting rate of photosynthesis was that obtained at the beginning of a run.

TABLE I

Carbon Dioxide Concentrations of Carbonate-Bicarbonate Mixtures

No. of mixture	Concentration in moles per liter		Ionic strength (μ)	Moles of CO_2 per liter $\times 10^6$	Log CO_2 concentration	Log CO_2 concentration (Warburg)
	K_2CO_3	$KHCO_3$				
1	0.085	0.015	0.27	0.481	−6.318	−6.276
2	0.080	0.020	0.26	0.902	−6.045	−6.000
3	0.075	0.025	0.25	1.49	−5.826	−5.770
4	0.070	0.030	0.24	2.29	−5.640	−5.585
5	0.060	0.040	0.22	4.48	−5.349	−5.276
6	0.050	0.050	0.20	8.67	−5.062	−5.009
7	0.035	0.065	0.17	20.5	−4.689	−4.638
8	0.025	0.075	0.15	37.5	−4.426	−4.366
9	0.015	0.085	0.13	78.7	−4.104	−4.041
10	0.010	0.090	0.12	131.	−3.882	−3.824
11	0.005	0.095	0.11	290.	−3.537	−3.481

The correction for respiration does not significantly change the values obtained at high rates of photosynthesis.

Plants kept in the dark for some time before the beginning of an experiment gave more reproducible respiration values than plants taken directly from the aquaria where they were kept under a moderate illumination. The plant was therefore kept in the dark in buffer for at least 1 hour before beginning an experiment. After equilibration for 15 minutes, the respiration was determined for one half hour. At low photosynthetic rates, measurements were made for 20 or 30 minutes; at high rates, duplicate 5 minute readings were taken. Before each new determination, 10 to 15 minutes were allowed for the plant to attain the new stationary state. During a light intensity run, fresh buffer mixture was used often enough to prevent an effective decrease in CO_2 concentration. With carbon dioxide concentration as the variable, two readings were made with each buffer,

the plant was then rinsed and placed in a mixture of higher CO_2 concentration. Runs were always made starting with the lowest CO_2 concentration or intensity.

III

Measurements

1. Light Intensity.—In Fig. 2 and Table II are presented the data for photosynthesis in relation to intensity obtained on two successive

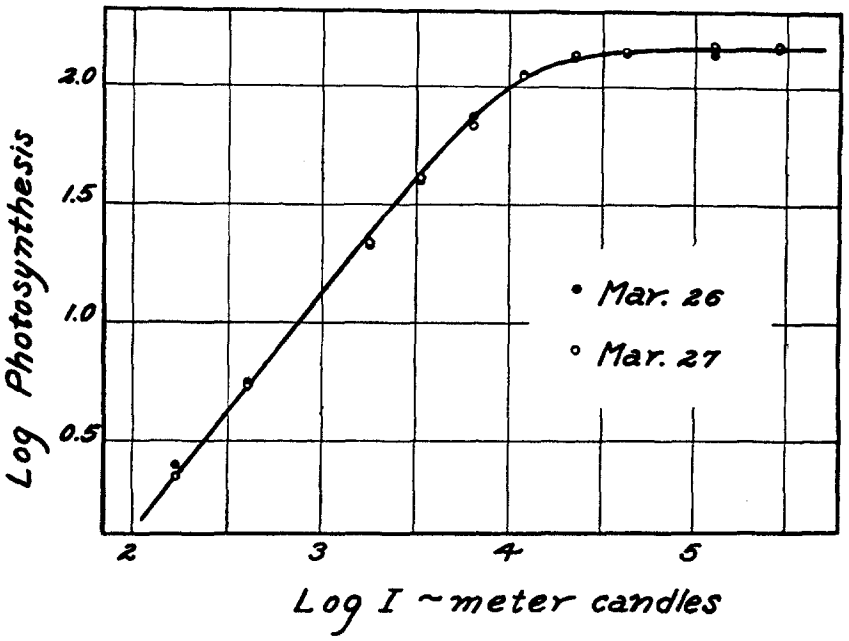

FIG. 2. Two runs made on the same frond of *Cabomba* on successive days. There is no systematic difference between the two runs. The data are given in Table II. The curve is that of equation (4).

days using the same piece of tissue for both runs. It is clear from these data that individual runs yield data of good precision and that the tissue does not change significantly over a period of 24 hours. Similar results have been obtained on many occasions. Although the data of the individual runs are sufficiently critical for the type of equation which represents them, in order to achieve greater certainty

TABLE II

Photosynthesis at Different Intensities. Two Runs on Same Tissue

Data of Fig. 2. CO_2 concentration constant at 1.31×10^{-4} moles per liter. Vessel constant = 0.535. Wet weight of tissue = 116.5 mg. Temperature = 25.3°C. Photosynthesis given as c. mm. of oxygen evolved per hour per 100 mg. wet weight of material, corrected for respiration. Respiration measured initially for 30 minutes.

Duration of each reading	Intensity	Rate of photosynthesis	
		March 26, 1936	March 27, 1936
min.	*meter candles*		
20	166	2.52	2.25
20	407	5.56	5.42
10	1,740	21.1	21.7
10	3,310	39.4	40.9
5	6,310	74.0	67.5
5	11,800	112.	109.
5	21,900	131.	135.
5	41,700	138.	142.
5	123,000	139.	150.
5	282,000	147.	149.

TABLE III

Photosynthesis and Light Intensity. Detailed Data of Fig. 3

Each set of data represents the averages of 5 similar experiments. Photosynthesis given as c. mm. of oxygen evolved per hour per 100 mg. wet weight of tissue, corrected for respiration. White light used. Temperature = 25.3°C.

Intensity	Rate of photosynthesis			
	$[CO_2] = 2.05 \times 10^{-5}$ moles per liter	$[CO_2] = 7.87 \times 10^{-5}$ moles per liter	$[CO_2] = 1.31 \times 10^{-4}$ moles per liter	$[CO_2] = 2.90 \times 10^{-4}$ moles per liter
meter candles				
166	1.42	2.99	2.48	2.44
407	4.42	5.41	4.96	5.84
1,740	16.2	27.5	22.2	27.4
3,310	23.2	43.1	42.0	47.4
6,310	31.1	74.3	72.7	91.3
11,800	37.8	104.	108.	136.
21,900	41.3	128.	135.	164.
41,700	41.9	127.	145.	186.
123,000	44.5	140.	152.	193.
282,000	45.2	136.	153.	192.

at low rates of photosynthesis five runs were made with each buffer and the data averaged.

In Table III and Fig. 3 are given the average data for rate of photosynthesis as a function of intensity for four different carbon dioxide

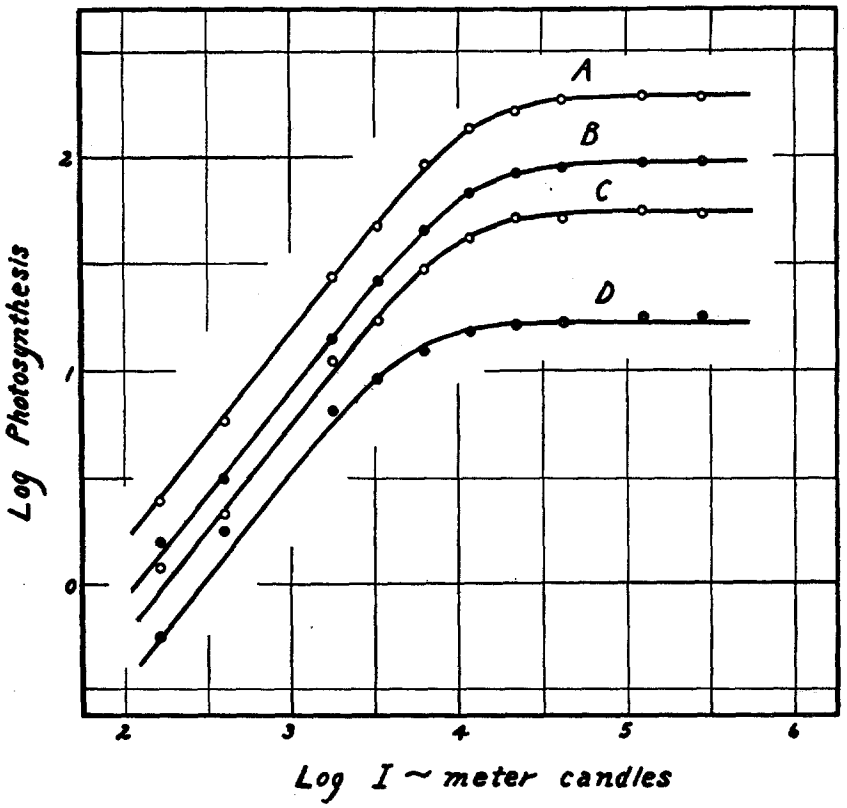

FIG. 3. Photosynthesis as a function of light intensity for *Cabomba*. The data are given in Table III. The photosynthesis scale is correct only for curve *A*. The others have been shifted downwards in order to keep the curves distinct: *B* by 0.2, *C* by 0.4, and *D* by 0.4 of a log unit. The CO_2 concentrations in moles per liter were: *A*, 2.90×10^{-4}; *B*, 1.31×10^{-4}; *C*, 7.87×10^{-5}; *D*, 2.05×10^{-5}. The same curve is drawn through all of the data and is from equation (4).

concentrations. The data are plotted as log photosynthesis against log I with the same curve drawn through all four sets of data. It will be observed that the curves for different CO_2 concentrations differ in the intensity at which the maximum rate of photosynthesis is attained,

in accord with Blackman's idea of limiting factors. This is also shown by the measurements of Harder (1921), and of Hoover, Johnston, and Brackett (1933).

The curve drawn through the data in Figs. 2 and 3 has the equation

$$KI = \frac{p}{(p^2_{max.} - p^2)^{\frac{1}{2}}} \tag{4}$$

where p is the rate of photosynthesis at light intensity, I, K is a constant which indicates the position of the curve on the I axis and $p_{max.}$ is the asymptotic maximum rate of photosynthesis. Equation (4) solved for log p gives

$$\log p = \log p_{max.} - 1/2 \log (1 + 1/K^2I^2). \tag{5}$$

If log p is plotted against log I, the shape of the curve is independent of the constants K and $p_{max.}$. This property of the equation facilitates comparison with the data. Curves similar to those in Figs. 2 and 3, but differing in slope and in inflection, result from changing the exponents in equation (4). An equation which yields a curve very similar to that of equations (4) and (5) may be written as

$$KI = \frac{p}{(p_{max.} - p)^{\frac{1}{2}}} \tag{6}$$

Equation (6) solved for log p yields

$$\log p = \log KI + \log [(K^2I^2 + 4\, p_{max.})^{\frac{1}{2}} - KI] - \log 2. \tag{7}$$

The curves described by equations (5) and (7) differ slightly only in the rate at which they become parallel to the log I axis at high illuminations. The three upper sets of data in Fig. 3 fit equation (5) better, while the lowest set of data fit (7) with higher precision. Since no certain choice is at present possible and because a majority of the individual data decide for (5) the same curve has been drawn through all four series. Exponents other than those in (4) and (6) are definitely excluded, as for example, in the equation

$$KI = \frac{p}{p_{max.} - p} \tag{8}$$

or in logarithmic form

$$\log p = \log p_{max.} - \log (1 + 1/KI). \quad (9)$$

Equations (5), (7), and (9) have all been drawn to the same maximum in Fig. 4 for comparison. It will be observed that all three equations have the same slope at low intensities.

It is interesting to note the similarity between the above equations and those derived by Hecht (1923, 1935) for the photosensory process which have been used so successfully to describe many of the proper-

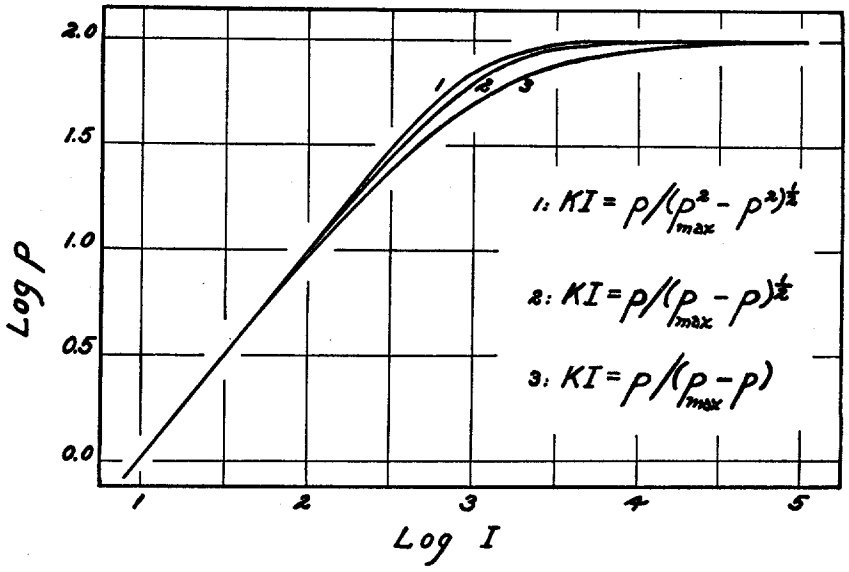

FIG. 4. The relation between photosynthesis and intensity in terms of equations (4), (6), and (8). Plotted on double logarithmic scale, the shape of these curves is independent of the constants in the equation. These equations are similar to those which describe the photostationary state for the photosensory process (Hecht, 1935).

ties of photoreception. In fact, this study began as the result of a comparison between the basic processes of photoreception and photosynthesis. Both are of a cyclical pseudo-reversible character, consisting of a photochemical reaction with a low temperature coefficient and a dark reaction with a high Q_{10} which restores the light absorbing substances to their original condition. The subsequent properties of the reactions are quite different. In one case, nerve endings are stimulated; in the other, carbohydrate is formed.

2. Carbon Dioxide.—Measurements were made of the effect of CO_2 concentration on photosynthetic rate at constant intensity. Since respiration rate was independent of CO_2 concentration, an initial measurement made in the buffer of lowest CO_2 concentration was

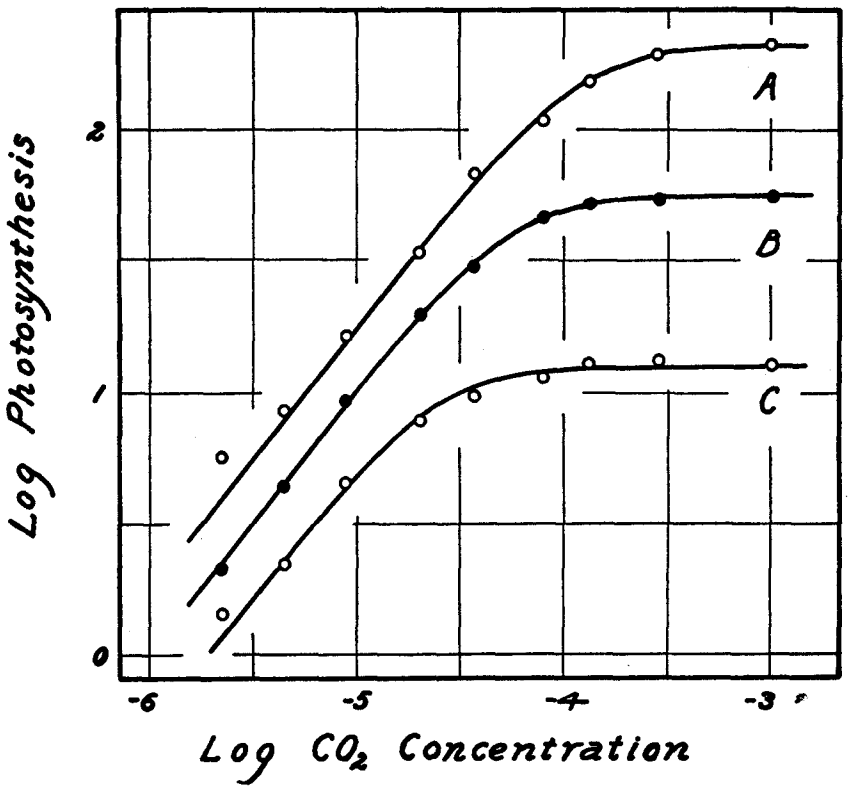

FIG. 5. Measurements on *Cabomba* with different carbon dioxide concentrations at constant light intensity. The data are given in Table IV. The scale is correct for curve *A*; curve *B* has been moved down 0.4, and curve *C*, 0.6 of a log unit. No. 246 Corning filter was used. The relative intensities were: *A*, 282,000; *B*, 21,900; and *C*, 6,310. These are the intensities in meter candles of the unfiltered light. The curve drawn through the data is from equation (4).

used in correcting all the photosynthesis rates determined for a given piece of tissue.

Because of the time necessary for changing buffers and allowing for equilibration to light and temperature with each new mixture, the

duration of a run was about 5 hours. A continuous exposure to the high light intensities used in these experiments for such a long period occasionally caused a small decrease in rate to take place after 3 or 4 hours. It was found that this decrease could be virtually eliminated by using the long wave lengths of the visible spectrum. Therefore, in all of these experiments Corning filter No. 246 was used. This filter is of the sharp cut-off type transmitting 40 per cent of the energy at 588 mμ and 5 per cent at 579 mμ. The effective energy was not decreased by more than half; which with the highest intensity avail-

TABLE IV

Photosynthesis and CO_2 Concentration. Data of Fig. 5

Each value represents the averages of 5 similar experiments. Red light used, obtained with Corning filter No. 246. Intensities are the values in meter candles as determined for the unfiltered light. Photosynthesis as c. mm. of oxygen evolved per hour per 100 mg. wet weight of tissue, corrected for respiration. Temperature = 25.3°C.

$[CO_2] \times 10^6$ moles per liter	Rate of photosynthesis		
	I = 6,310	I = 21,900	I = 282,000
2.29	5.65	5.33	5.75
4.48	8.79	11.0	8.59
8.67	17.7	23.2	16.4
20.5	31.2	49.2	33.7
37.5	38.0	75.1	68.0
78.7	44.8	115.	109.
131.	50.5	131.	152.
290.	51.9	136.	195.
1000.	50.1	138.	212.

able did not decrease the rate of photosynthesis measurably. On the other hand, those portions of the spectrum which contribute little energy for photosynthesis but which are injurious to the photosynthetic mechanism were eliminated (*cf.* Emerson, 1935). The use of this red filter changes the intensity values obtained with the white light calibrations. The intensities given are those for white light and may be regarded as only relative values.

Fig. 5 and Table IV present the rate of photosynthesis as a function of CO_2 concentration for three different illuminations. Each curve

represents the averages of five similar runs. Intensity curves cannot be derived accurately from these data since the absolute rate of photosynthesis varies somewhat with the weight of the tissue as mentioned above. For example, in the runs with $I = 21{,}900$, the average weight of the tissue was considerably lower than in the run with $I = 282{,}000$. The former therefore gave higher rates per 100 mg. than the latter at low CO_2 concentrations. However, this does not affect the shape of the curve describing photosynthesis as a function of carbon dioxide concentration.

The curve drawn through the data in Fig. 5 is the one used in Figs. 2 and 3 and is from equation (4) with carbon dioxide substituted for light intensity. Apparently the rate of photosynthesis for *Cabomba* varies in the same way with both light intensity and CO_2 concentration.

IV

Data of Other Investigators

1. Light Intensity.—What relation is there between the data presented in this paper and the data obtained by other investigators? Early experiments over a small range of intensities indicated a linear relation between photosynthesis and intensity. Reinke (1883) showed with the bubble counting method on *Elodea* that at high light intensities a maximum rate of photosynthesis is attained which is not affected by subsequent increases in the intensity of the light. Averages of his measurements as well as the later ones of Pantanelli (1903) show good agreement with equation (4) in spite of the crudity of the method used. The first modern measurements made under satisfactory conditions and with a correction for respiration are those of Willstätter and Stoll (1918). Their measurements with several different species and with both green and yellow leaves also show excellent agreement with equation (4). In Fig. 6*C* are drawn two representative curves from their data. In Fig. 6 are also presented the data of several other observers. None of these is adequately represented by equation (8), but those of Warburg fit equation (6) a little better than they do (4). Other measurements which cover a smaller range of intensities are those of Van den Honert (1930) made with *Hormidium* which are omitted as they are identical with the

later ones of Van der Paauw (1932) on the same material. The data of Emerson and Green (1934 *a*) on the marine alga *Gigartina* show good agreement with equation (4).

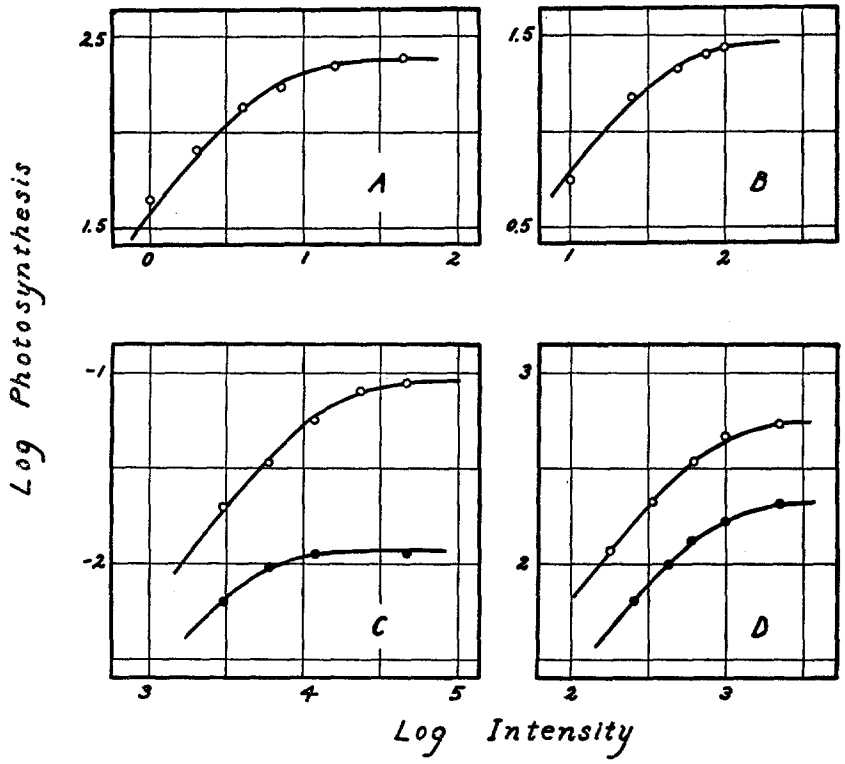

FIG. 6. Photosynthesis as a function of light intensity, the data of various investigators. *A*—Warburg on *Chlorella*; *B*—Emerson and Green on *Gigartina*; *C*—Willstätter and Stoll on *Ulmus* yellow leaves (open circles), and on *Ampelopsis* (solid circles); *D*—Van der Paauw on two varieties of *Hormidium*, Pringsheim's strain (open circles) and Van den Honert's strain (solid circles). The data are given in the original units of the various authors. The curve drawn through the data is from equation (4).

The data of Emerson (1929) on two strains of *Chlorella* with different amounts of chlorophyll are drawn in Fig. 7. It may be noted that on this double logarithmic plot these two curves are evidently of similar shape, whereas on the basis of a semilogarithmic plot Emerson stated

that these curves "are quite dissimilar, and the upper one cannot be produced by multiplying the bottom one by a constant." These data are adequately represented only by equation (4) with K having approximately the same value for both chlorophyll concentrations. The point so obviously off the lower curve is a measurement in the region where photosynthesis is smaller than respiration; the pressure

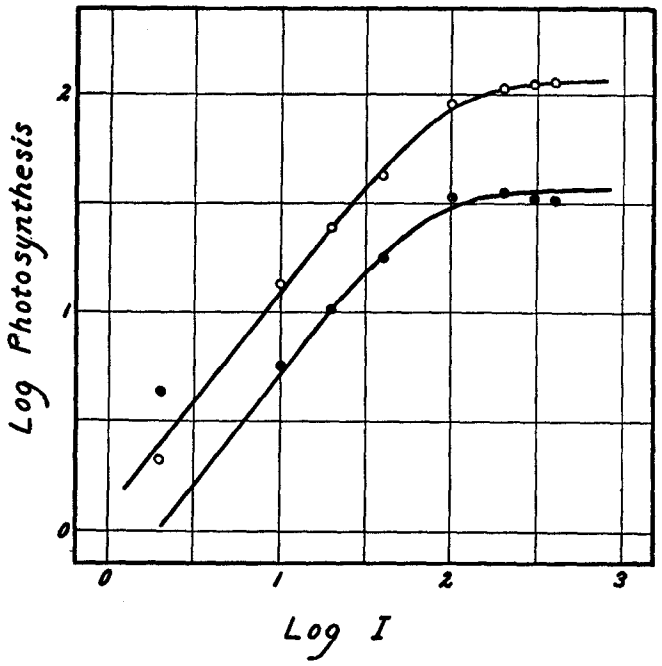

FIG. 7. Emerson's data on two strains of *Chlorella*, one of high (open circles) and the other (solid circles) of low chlorophyll concentration. The same curve has been drawn through both sets of data and is from equation (4).

change is very small and the measurements are therefore of low precision.

The intensity measurements of Hoover, Johnston, and Brackett (1933) on young wheat covering a small range of low intensities at various carbon dioxide concentrations are consistent with all the other data discussed above. The data of Harder (1921) on *Fontinalis* as well as numerous other observations in the literature mainly made

from an ecological point of view have too high an experimental error to be critical.

Considering the variety of plants, of experimental conditions, and of method, it is remarkable that all of these data give such a good fit with respect to an equation as specific in form as the one drawn through them.

2. Carbon Dioxide.—Comparison of previous results with ours is difficult because the method of supplying CO_2 influences the results. Warburg supplied CO_2 from buffer mixtures similar to those used here;

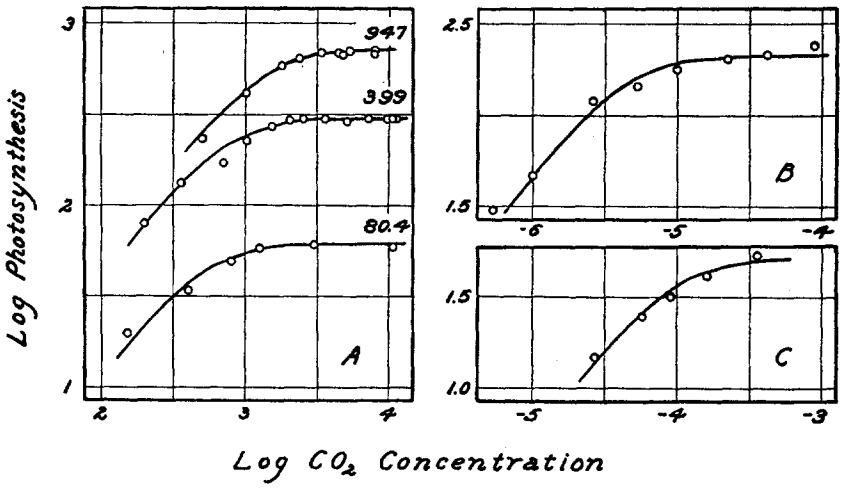

FIG. 8. *A.* The data of Hoover, Johnston, and Brackett on young wheat. The numbers on the curves give the light intensity in foot candles. *B.* Warburg's data on *Chlorella*. *C.* Those of Emerson and Green on *Gigartina*. The same curve as in the preceding figure has been drawn through these data.

his data can therefore be compared directly with ours. This is done in Fig. 8*B*. The agreement with equation (4) is not so good as desired; this may be because the data represent only single experiments. The data fit equation (6) better but do not exclude (8). The work of Emerson and Green on *Gigartina* (Fig. 8*C*) is complicated by the use of buffers with a high salt content and a different piece of tissue for each determination. The small range of concentrations makes impossible a choice between the various equations although the data

are not inconsistent with equation (4). The data of Harder on *Fontinalis* are omitted as we cannot be certain that a constant CO_2 supply is provided at low CO_2 concentrations by proportionate dilutions of a bicarbonate solution. Moreover, there are too few points available for testing these data.

Fig. 8*A* gives some of the data obtained with young wheat by Hoover, Johnston, and Brackett. These three curves as well as their others give a good fit with equation (4) and cannot be adequately described by equation (6) or (8). In these experiments, CO_2 was supplied in gas mixtures circulated rapidly through an enclosed chamber. However, the data of Van den Honert and Van der Paauw on *Hormidium* using gas mixtures do not resemble the other measurements cited above. External diffusion rate is probably limiting in these experiments since at low CO_2 tensions Q_{10} is unity, whereas in the experiments of Warburg and of Emerson (1936) with *Chlorella* using buffer mixtures Q_{10} is high.

V

General Considerations

It has been suggested (Hoover, Johnston, and Brackett, 1933; Brackett, 1935) that shading by the plastids may produce a gradation of light intensities at different plastids and thus affect the shape of the curve relating intensity and photosynthesis. While the light intensity is certainly not the same at all the different chlorophyll centers in the plant, it does not seem likely that the intensity-photosynthesis relation is determined by such an effect, particularly since the curve is the same for many different species, and the size and number of chloroplasts must be very different for unicellular algae such as *Chlorella* and *Hormidium* and higher plants like wheat and *Cabomba*. The fact that Emerson's data for two widely different chlorophyll concentrations in *Chlorella* give the same curve, lends support to the idea that these curves represent some other mechanism than shading.

The argument has also been advanced that the CO_2-photosynthesis curves may be affected by unequal CO_2 concentrations at different photosynthetic centers. When diffusion rate limits photosynthesis, this is certainly true, but when CO_2 is supplied at a rapid rate this

situation probably does not occur. In those cases where diffusion is non-limiting, the curves relating photosynthesis with both CO_2 and intensity are identical. It does not seem likely that two such effects on different variables should produce identical equations.

The effect of both CO_2 and intensity may be expressed in an equation of the type used by Baly (1934, 1935) and by Emerson and Green (1934 *b*), where p is the rate of photosynthesis and

$$p = k_1 I(a - x)^{\frac{1}{2}} = k_2[CO_2]x^{\frac{1}{2}} \tag{10}$$

$$p = k_1 I(a^2 - x^2)^{\frac{1}{2}} = k_2[CO_2]x. \tag{11}$$

a may be regarded as representing the total concentration of chlorophyll, and x the amount of chlorophyll activated by light. If x is eliminated and equation (10) or (11) is solved for p, equations are obtained relating p and either I or $[CO_2]$, which describe curves identical with that of equation (5).

Similarly, the equation

$$p = k_1 I(a - x)^{\frac{1}{2}} = k_2[CO_2]x \tag{12}$$

with x eliminated and solved for p, yields curves identical with (7). It is assumed that CO_2 cannot enter in the same term as the light intensity, since this would result in low temperature coefficients at low CO_2 concentrations, which is not true when the external diffusion rate is non-limiting (Emerson and Green, 1934 *b*; Emerson, 1936).

The CO_2 does not appear to be bound by the unilluminated chlorophyll. If it were, the concentration of the CO_2-chlorophyll compound would be at a maximum after a period of darkness. The maximum rate of photosynthesis would then be obtained at the beginning of illumination. Actually the measurements of Warburg, 1920) (also see Baly, 1934) show that after a period of darkness the rate of photosynthesis slowly rises to a maximum indicating that the dark reaction follows the photochemical reaction.

The above equations (10, 11, and 12) may be derived on the assumption that two reactions are involved in the cycle; a photochemical reaction during which light is absorbed, and a dark process which accomplishes a transfer of energy for the reduction of CO_2. The rate of photosynthesis (p) is equal to the rate of the dark reaction because this appears to be the reaction during which CO_2 is reduced and

oxygen is liberated. But there is apparently a third reaction which is involved in the cycle since CO_2 appears to be taken up in the dark by some protoplasmic constituent, as shown by Willstätter and Stoll. It is not the purpose of the present paper to develop a kinetic scheme including this third reaction. This has already been considered by Briggs (1935) and others. We merely wish to indicate that equation (10) or (11) will give a quantitative description of the data relating rate of photosynthesis with CO_2 concentration and light intensity. Including the third reaction will not change the properties of these equations but the interpretation. The velocity of the dark reaction will depend not on the CO_2 concentration directly but on the concentration of the CO_2-containing compound.

The equations of Ghosh (1928), Emerson and Green (1934 *b*), Baly (1935), Burk and Lineweaver (1935), and Arnold (1935) describing photosynthesis as a function of intensity may all be put into the same form as equation (8).[1] Ghosh, and Burk and Lineweaver used Harder's data, which have so high an experimental error that they are not critical. Baly used only the intensity data of Warburg and did not obtain a satisfactory agreement with them. Emerson and Green, and Arnold (1935) have not published any tests of their equations with the data of intensity and CO_2 concentration. The fact that the data presented in this paper, both original and from others, do not fit equations derived by the above investigators provides a specific criticism of their equations.[2]

Arnold's kinetic scheme is based on studies made with intermittent illumination, which indicate that both the Blackman reaction (Arnold, 1933) and the photochemical reaction (Emerson and Arnold, 1932 *b*) are first order.[3]

[1] Since it is not the purpose of this paper to present a critique of the various kinetic schemes which have been suggested, the equations of these authors are considered together. It is realized that the various formulations differ in many important respects, but we are concerned here only with the quantitative treatment of the variables studied in this research.

[2] Briggs has pointed out that equations similar to (8) are inadequate, but does not give any quantitative test of his own scheme for photosynthesis rate as a function of I and $[CO_2]$.

[3] We are not entirely satisfied with the assumptions inherent in both of these

It may be that the Blackman reaction is first order, as in equation (11) or (12); but the data relating intensity and photosynthesis are such that the photochemical reaction must be half order. However, by squaring the stationary state equation (10) we obtain

$$k_1^2 I^2(a - x) = k_2^2[CO_2]^2 x \tag{13}$$

which will describe the data if p remains proportional to $x^{\frac{1}{2}}$, as in equation (10). Such a mechanism might be correct as it would yield first order photochemical and Blackman reactions, but I and $[CO_2]$ would now enter as the square. Emerson and Arnold also state that the yield per flash of light is independent of the intensity if the total energy per flash of light is constant; *i.e.*, the product of intensity and time is constant. From this it is concluded that I must enter as the first power. But the product of intensity and time could still be equal to a constant if both intensity and time were squared. It is difficult to understand why p should be proportional to $x^{\frac{1}{2}}$ in such a system, but it may be necessary if the findings of Arnold and of Emerson and Arnold are correct.

The fact that photosynthetic rate is the same function of both CO_2 concentration and intensity is a simplifying feature of the kinetic scheme. Still, the presence of a fractional exponent or of intensity as the square indicates a complex system. There is no difficulty in accepting an equation in which CO_2 enters as the square, but in simple photochemical systems intensity enters as the first power, or in some reactions, such as those involving halogens, as the square root (Griffith and McKeown, p. 407, 1929). We are not aware of any photochemical reactions for which there has been accepted an equation in which I enters as a power above one. Nevertheless, such may

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proofs, and Emerson (1936) is likewise inclined to be skeptical of Arnold's proof of the first order character of the Blackman reaction.

The evidence indicating that the light reaction is first order depends upon measurements made by varying the light intensity of short flashes of red light. The total intensity range used was 1 to 10 or 1 log unit. Over this small range, the data are just as easily satisfied by assuming a half-order reaction. To be certain of the proof, it would be necessary to reinvestigate this problem over a satisfactory range of light intensities making certain that a condition of light saturation had been reached.

be the case for photosynthesis and would perhaps indicate a chain process with more than one light reaction. This would be in keeping with the discovery of Warburg and Negelein (1923) that 4 quanta are necessary for the reduction of a single CO_2 molecule.

Recent attempts to formulate a chemical mechanism for photosynthesis involve the postulation of several light reactions (Stoll, 1932, 1936; Willstätter, 1933; Franck, 1935). Gaffron and Wohl (1936) have reviewed these efforts, and have shown that these 4 light reactions would have to be concurrent rather than consecutive. Support for this idea has come from Kohn (1936) who has pointed out that 4 quanta could not be absorbed by the same chlorophyll molecule during a short light flash and still yield the amount of oxygen actually produced per flash.

Such considerations indicate the necessity for a revision of our ideas concerning the cyclical process involved in photosynthesis. A scheme would have to be developed in which several light reactions take place concurrently with the absorption of quanta by different chlorophyll molecules. Subsequent dark reactions would restore the chlorophyll to its original condition, and the energy released used for the reduction of CO_2 and water. However, for a description of the data of CO_2 and intensity, the simple two-part cycle appears to be adequate, provided that the equation has the exponents given above. The accumulation of more kinetic data will determine the further utility of the two-reaction cycle.

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SUMMARY

1. An optical system is described which furnishes an intensity of 282,000 meter candles at the bottom of a Warburg manometric vessel. With such a high intensity available it was possible to measure the rate of photosynthesis of single fronds of *Cabomba caroliniana* over a large range of intensities and CO_2 concentrations.

2. The data obtained are described with high precision by the equation $KI = p/(p^2_{max.} - p^2)^{\frac{1}{2}}$ where p is the rate of photosynthesis at light intensity I, K is a constant which locates the curve on the I axis, and $p_{max.}$ is the asymptotic maximum rate of photosynthesis. With CO_2 concentration substituted for I, this equation describes the data of photosynthesis for *Cabomba* as a function of CO_2 concentration.

3. The above equation also describes the data obtained by other investigators for photosynthesis as a function of intensity, and of CO_2 concentration where external diffusion rate is not the limiting factor. This shows that for different species of green plants there is a fundamental similarity in kinetic properties and therefore probably in chemical mechanism.

4. A derivation of the above equation can be made in terms of half-order photochemical and Blackman reactions, with intensity and CO_2 concentration entering as the first power, or if both sides of the equation are squared, the photochemical and Blackman reactions are first order and intensity and CO_2 enter as the square. The presence of fractional exponents or intensity as the square suggests a complex reaction mechanism involving more than one photochemical reaction. This is consistent with the requirement of 4 quanta for the reduction of a CO_2 molecule.

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