THE INDUCTION PERIOD IN PHOTOSYNTHESIS

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I

INTRODUCTION

When a plant is illuminated, its rate of photosynthesis is at first low and gradually increases until it becomes constant. This induction period was first observed by Osterhout and Haas (1918) for *Ulva* and independently confirmed by Warburg (1920) with *Chlorella*. It has since been found by Van der Paauw (1932) for *Hormidium*, by Briggs (1933) for *Mnium*, and by Emerson and Green for *Gigartina* (1934). It is even demonstrable in Willstätter and Stoll's (1918) measurements with *Helianthus*, *Sambucus*, and *Acer*. Though present in such a variety of plants, the induction period varies considerably, being 2 minutes in *Chlorella* and *Hormidium*, 20 minutes in *Gigartina*, 50 minutes in *Mnium*, and even longer in *Ulva*. Van der Paauw found its duration to vary with temperature.

The mere existence of the induction period demonstrates that the light process in photosynthesis must precede the dark or Blackman process (Warburg, 1920; Baly, 1934). We have therefore undertaken a quantitative description of it under various conditions in the hope that it will give further information about the processes involved in photosynthesis.

II

EXPERIMENTAL

1. Procedure.—The fresh-water plant *Cabomba caroliniana* was used with the same methods for control and measurement of photosynthesis as in a previous research (Smith, 1937).

The tissue was placed in carbonate-bicarbonate mixtures in a Warburg vessel, and after a short time in the dark its respiration determined for 30 minutes. As

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Warburg has noted, it is not possible to get accurate measurements by periodic observation of the manometer during continuous illumination; there is always a definite lag in the liberation of oxygen from the solution. The plant was therefore exposed for 1 minute to the light, and after 5 or 10 minutes in the dark the reading of the manometer was taken. The procedure was then repeated for successively longer light exposures, until a complete set of data was obtained on
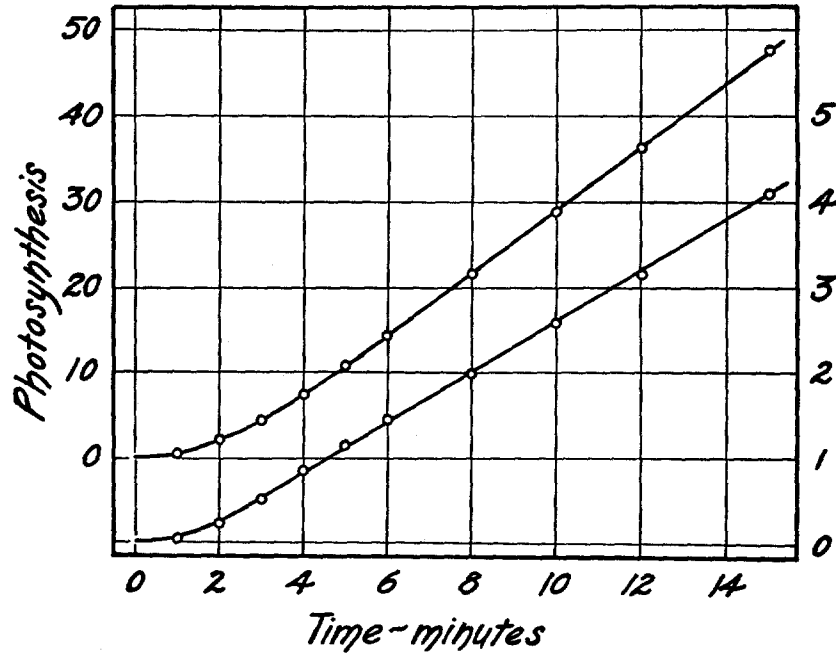

FIG. 1. Photosynthesis as a function of time of illumination for *Cabomba*. The upper curve is for an intensity of 282,000 meter candles, the lower one for 1,740 meter candles. An induction period is present at both illuminations. The data are averages, each curve representing five similar runs; they are given in Table I.

the particular tissue. Several readings were usually made for the short exposures. Where the total amount of photosynthesis was small, three or four fronds of about 300 mg. wet weight were used; otherwise a single frond of about 100 mg. was adequate. A complete run was always made with each tissue, and repeated four times with different fronds so that each point represents the average of at least five individual readings, while the points for short exposures include several more. The average data are in no way different from the single runs.

2. *Results.*—Warburg was unable to find an induction period with *Chlorella* at low intensities. This is not the case for *Cabomba*. Fig. 1 presents the data for a high and a low light intensity; both show a well marked induction period. These data are in Table I together with those for an intermediate intensity and for two lower CO_2 concentrations at high intensity. For *Cabomba*, photosynthesis balances respiration near 300 meter candles; reliable measurements for short exposures are thus not possible much below 1500 meter candles. Above this, the induction period is demonstrable over a range of

TABLE I

Photosynthesis As a Function of Time of Illumination

Each set of data is the average of 5 similar experiments. Photosynthesis from the beginning of the illumination given as cubic millimeters of oxygen evolved per 100 mg. wet weight of tissue, corrected for respiration. Intensities are in meter candles and CO_2 concentrations in moles per liter.

Time	Photosynthesis				
	$[CO_2] = 2.90 \times 10^{-4}$ $I = 1,740$	$[CO_2] = 2.90 \times 10^{-4}$ $I = 11,800$	$[CO_2] = 2.90 \times 10^{-4}$ $I = 282,000$	$[CO_2] = 7.87 \times 10^{-4}$ $I = 282,000$	$[CO_2] = 2.05 \times 10^{-4}$ $I = 282,000$
min.					
1	0.05	0.29	0.49	0.27	0.15
2	0.23	1.12	2.06	1.22	0.51
3	0.51	2.41	4.36	2.69	1.04
4	0.85	3.99	7.34	4.40	1.59
5	1.15	5.86	10.79	6.09	2.27
6	1.46	7.72	14.30	7.81	2.95
8	1.99	11.52	21.74	11.17	4.16
10	2.60	15.33	28.94	14.47	5.38
12	3.16	19.09	36.28	17.85	6.71
15	4.09	24.74	47.56	23.02	8.48

intensities of about 1 to 160 (from 1,740 to 282,000 meter candles) which at this high CO_2 concentration is about 85 per cent of the total photosynthesis range. Approximately the same range is covered by the three experiments at different CO_2 concentrations at high light intensity (Smith, 1937).

The data of the induction period are well described by the equation

$$\log \frac{p_m^{\frac{1}{2}} + p^{\frac{1}{2}}}{p_m^{\frac{1}{2}} - p^{\frac{1}{2}}} = Kt \tag{1}$$

where p is the photosynthesis rate at any time (t), and p_m is the maximum rate. When plotted on a double logarithmic scale, the shape
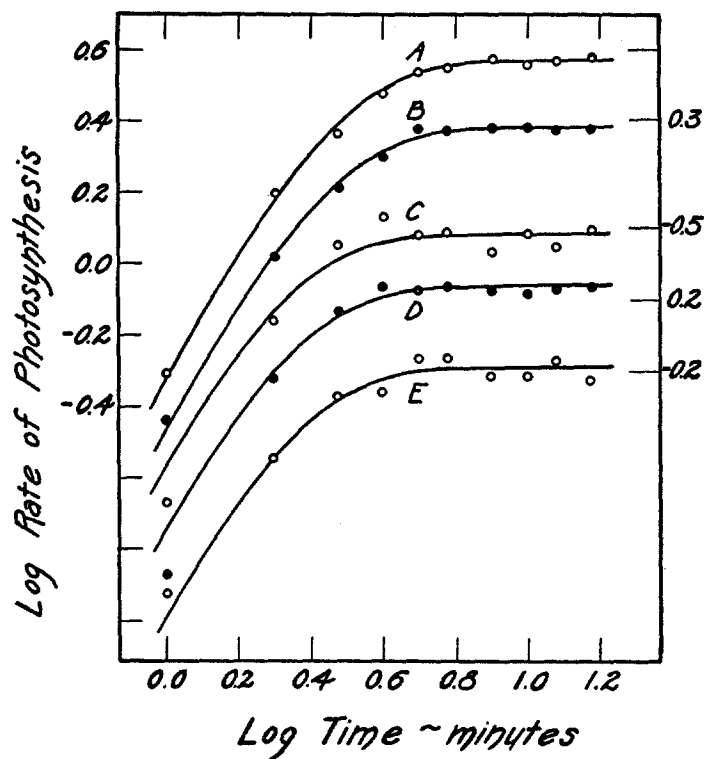

FIG. 2. Rate of photosynthesis as a function of time for different intensities and CO_2 concentrations for *Cabomba*. The same curve is drawn through all the data and is from equation (1). Photosynthesis is in cubic millimeters of oxygen per minute with the scale correct only for curve A; the others have been displaced by different amounts, with the correct positions indicated on the right side of the figure. The light intensities (I) in meter candles, and the CO_2 concentrations in moles per liter are as follows: (A). $I = 282,000$; $[CO_2] = 2.90 \times 10^{-4}$. (B). $I = 11,800$; $[CO_2] = 2.90 \times 10^{-4}$. (C). $I = 1,740$; $[CO_2] = 2.90 \times 10^{-4}$. (D). $I = 282,000$; $[CO_2] = 7.87 \times 10^{-5}$. (E). $I = 282,000$; $[CO_2] = 2.05 \times 10^{-5}$. These data are taken from Table I.

of the curve of this equation is independent of the constants p_m and K. In Fig. 2 this curve is drawn through all the measurements for

Cabomba given in Table I, so that a change in light intensity or CO_2 concentration affects only the position of the curve but not its character. Table II gives the photosynthesis rates as oxygen produced per minute, together with the values calculated from equation (1).

Equation (1) also describes with good precision the data of Warburg and of Briggs drawn in Fig. 3. Thus the measurements obtained on three plants, *Cabomba*, *Chlorella*, and *Mnium*, each representative of different phyla, are shown to be similar. Since the effect of light

TABLE II

Rate of Photosynthesis and Time

Observed values from Table I expressed as oxygen produced per minute. Calculated values are from equation (1) with the constants obtained by graphical fit.

Time	$[CO_2]$ = 2.90 × 10^{-4} I = 1,740 p_m = 0.303; K = 0.452		$[CO_2]$ = 2.90 × 10^{-4} I = 11,800 p_m = 1.90; K = 0.345		$[CO_2]$ = 2.90 × 10^{-4} I = 282,000 p_m = 3.68; K = 0.325		$[CO_2]$ = 7.87 × 10^{-5} I = 282,000 p_m = 1.73; K = 0.428		$[CO_2]$ = 2.05 × 10^{-5} I = 282,000 p_m = 0.646; K = 0.419	
	p_{obs}	p_{calc}	p_{obs}	p_{calc}	p_{obs}	p_{calc}	p_{obs}	p_{calc}	p_{obs}	p_{calc}
min.										
1	0.05	0.07	0.29	0.27	0.49	0.47	0.27	0.36	0.15	0.13
2	0.17	0.18	0.83	0.83	1.57	1.48	0.95	0.99	0.36	0.36
3	0.28	0.25	1.29	1.31	2.30	2.40	1.47	1.40	0.53	0.52
4	0.34	0.28	1.58	1.61	2.98	3.01	1.71	1.60	0.55	0.59
5	0.30	0.30	1.87	1.76	3.45	3.35	1.69	1.68	0.68	0.63
6	0.31	0.30	1.86	1.84	3.51	3.52	1.72	1.71	0.68	0.64
8	0.27	0.30	1.90	1.89	3.72	3.64	1.68	1.73	0.61	0.65
10	0.31	0.30	1.91	1.90	3.60	3.67	1.65	1.73	0.61	0.65
12	0.28	0.30	1.88	1.90	3.67	3.68	1.69	1.73	0.67	0.65
15	0.31	0.30	1.88	1.90	3.76	3.68	1.72	1.73	0.59	0.65

intensity and CO_2 concentration is the same for all plants which have been investigated (Smith, 1936; 1937), the induction period provides an additional aspect of the similarity of the photosynthetic mechanism in different plants.

In Fig. 4 are plotted the data for *Hormidium* for the three temperatures studied by Van der Paauw. Although of lower precision than the others, these measurements are consistent with equation (1). The large shift of the curves on the time axis with an increase in temperature suggests that it is not a photochemical or a diffusion process

which is rate limiting. Both the time shift and the increase in the final maximum involve only a change in the constants which describe the data.

Emerson and Green's measurements made with *Gigartina* immersed in sea water are well described by equation (1). However, equation (1) does not fit the data obtained when the plant was immersed in sea
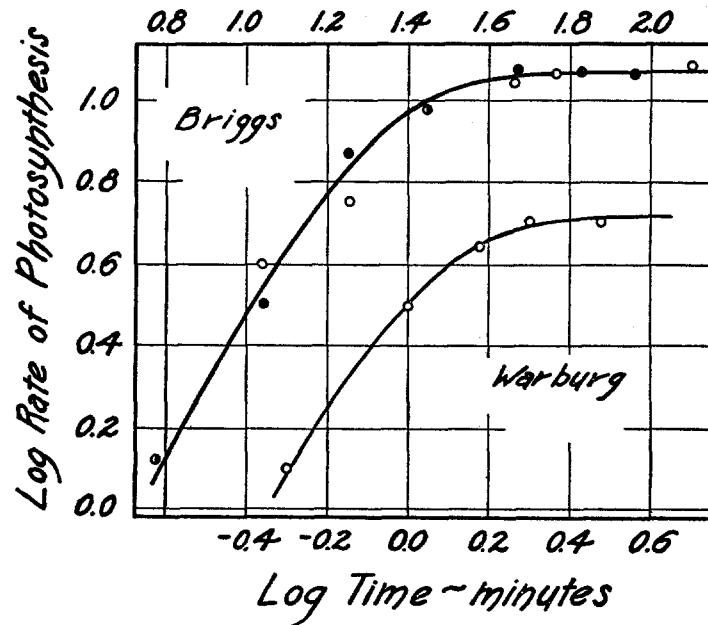

FIG. 3. Rate of photosynthesis as a function of time. The upper set of data are for two runs, *D.I.* (open circles) and *E.V.* (solid circles) on *Mnium* by Briggs (1933) with the correct time scale indicated at the top of the figure. The lower set of data are those of Warburg (1920) on *Chlorella.* The photosynthesis scale is arbitrary. The same curve is drawn through the data for both plants and is from equation (1).

water saturated with 5 per cent carbon dioxide in air. The latter data show a longer induction time but reach the same maximum as that attained in sea water. The different curves probably represent some special effect, since the observations on other species are similar in spite of different experimental conditions. The measurements of Osterhout and Haas who first observed this induction phenomenon are

not sufficiently reliable for comparison with other data, because their data are vitiated by lack of suitable control of the CO_2 concentration, a variable which produces large changes in photosynthesis rate.
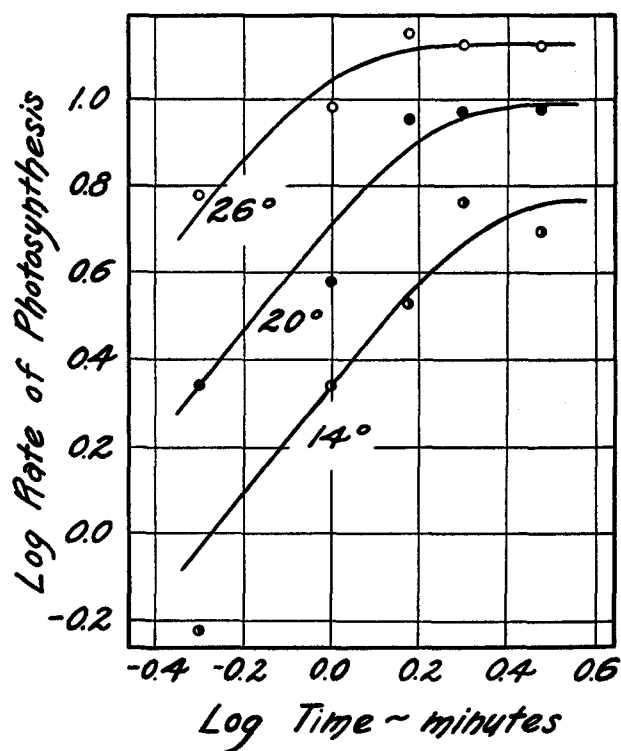

FIG. 4. The data of Van der Paauw (1932) on *Hormidium* for the relation between photosynthesis rate and time at three temperatures. Photosynthesis is given as rate per minute in Van der Paauw's units. For the 26° data a value at 2.0 minutes has been interpolated in place of an experimental point that is obviously out of line with the rest of the data. The curve from equation (1) has been drawn through the three sets of data. A change of temperature does not alter the shape but only the position of the curve with respect to the ordinates.

III

Theoretical

Equation (1) which describes the data of the induction period may be derived by considering the rôle of chlorophyll in the cycle of ligh

and dark reactions.[1]   Chlorophyll is apparently involved in the photo-chemical reactions by the absorption of light quanta, and in the Black-man reaction by transfer of energy affecting the reduction of carbon dioxide.   The cycle may be pictured:

Chlorophyll + light → activated chlorophyll
S + oxygen + chlorophyll ← activated chlorophyll + CO_2 + H_2O
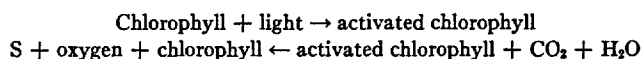
This scheme involves no assumptions regarding the intimate nature of the reactions concerned, and represents merely a minimum picture of the changes which take place.   The position of the substances other than chlorophyll in this scheme will not affect the equations to be derived since these are constant during a study of the induction period, although both the CO_2 (Emerson and Green, 1934) and the water (Pratt, Craig, and Trelease, 1937) are probably involved in the dark stage. S represents the carbohydrate formed.

The existence of the induction period indicates that the light process precedes; therefore it is the dark reaction which determines photo-synthesis, and we may write

$$p = f_2(x) \qquad (2)$$

where p is the rate of photosynthesis, and x is the concentration of activated chlorophyll.   The concentration of activated chlorophyll depends on the difference in rates of light and dark reactions, since activated chlorophyll is formed in the light and used up in the dark. Thus

$$dx/dt = f_1(I, \text{unactivated chlorophyll}) - f_2(x). \qquad (3)$$

The relation between x and t is the integral of equation (3).

[1] Gaffron (1935) has suggested that the diminished oxygen production during the induction period is caused in part by the photo-oxidation of metabolites which accumulate while the plant is in the dark.   Such a photo-oxidation would require a longer induction time at low intensities.   As Warburg has pointed out, this would be similar to the induction period present in the hydrogen-chlorine reaction, which is distinctly not the case for photosynthesis.   We therefore as-sume that the induction period is a real property of the photosynthetic mechanism as such.

If for (2) we write

$$p = k_2 x^2 \qquad (4)$$

and for the integral of (3)

$$\int dx/dt = \int k_1(a^2 - x^2) - k_2 x^2 \qquad (5)$$

we can derive equation (1). Light intensity, water, and carbon dioxide are constant in any experiment, and are included in the velocity constants k_1 and k_2; a is a constant which may be related to the total available chlorophyll.

Carrying out the integration required by (5) and the substitution from (4), we obtain

$$\ln \frac{\left[\dfrac{k_1^{\frac{1}{2}} k_2^{\frac{1}{2}} a}{(k_1 + k_2)^{\frac{1}{2}}}\right] + p^{\frac{1}{2}}}{\left[\dfrac{k_1^{\frac{1}{2}} k_2^{\frac{1}{2}} a}{(k_1 + k_2)^{\frac{1}{2}}}\right] - p^{\frac{1}{2}}} = 2k_1^{\frac{1}{2}} a(k_1 + k_2)^{\frac{1}{2}} t \qquad (6)$$

Calling

$$\frac{k_1^{\frac{1}{2}} k_2^{\frac{1}{2}} a}{(k_1 + k_2)^{\frac{1}{2}}} = p_m^{\frac{1}{2}} \quad \text{and} \quad \frac{2k_1^{\frac{1}{2}} a(k_1 + k_2)^{\frac{1}{2}}}{2.303} = K^{*}$$

we obtain equation (1)

$$\log \frac{p_m^{\frac{1}{2}} + p^{\frac{1}{2}}}{p_m^{\frac{1}{2}} - p^{\frac{1}{2}}} = Kt.$$

* If k_1 and k_2, the constants for the light and dark processes include the light intensity and CO_2 concentration respectively, then an increase in either of these factors indicates an increase in p_m, the stationary state rate, which is actually the case (Table II). However, the K values, which are also expected to increase, seem to show a trend in the opposite direction. The values are too uncertain for any definite conclusion to be drawn since each set of measurements was made on different material.

Many curves have been derived from equations similar to (4) and (5) but having different exponents. These curves are invariant in form when plotted on a double logarithmic scale, and may readily be compared with the data. They all show a steeply rising portion, a rapidly curving transition region, and a horizontal section at the

stationary state. The slope of the steep portion depends very largely on the exponent in the term of the dark reaction. The approximate slopes are 0.5, 1.0, and 1.5 where x is respectively raised to the powers 0.5 and 1 and 2. The principal effect of the different exponents in the light reaction is to alter the curvature of the transition region.

The slope of the steep portion of the curve is 1.5 (Figs. 2, 3, and 4), indicating that the dark reaction is of the second order. When the equations are of simple bimolecular form as in

$$\int dx/dt = \int k_1(a - x)^2 - k_2 x^2 \tag{7}$$

with

$$p = k_2 x^2, \tag{8}$$

the integral obtained, with the substitution required by (8), gives a slope for the steep portion similar to that of the curve derived from the data and of equation (6), but differs markedly in the curvature at the transition region.

Where the light and dark processes are first order as in

$$\int dx/dt = \int k_1(a - x) - k_2 x \tag{9}$$

with

$$p = k_2 x \tag{10}$$

the resultant equation

$$\ln \left[1 - \frac{k_1 k_2 a}{(k_2 + k_2)p} \right] = (k_1 + k_2)t \tag{11}$$

has a slope of 1.0 for the steep portion, and is wholly inadequate for a description of the data. In Fig. 5 are drawn for comparison the curves for equations (6) and (11), and the integral of (7) with the substitution required by (8).

That the simple assumptions of light and dark processes are not completely adequate is shown by the fact that the equations which describe the data for the induction period are not in harmony with those that describe the relation between intensity and photosynthesis

at the stationary state. The data for intensity and photosynthesis
at the stationary state can be described by the equation[2]

$$dx/dt = k_1 I^2(a^2 - x^2) - k_2 x^3 = 0 \qquad (12)$$

with

$$p = k_2 x \qquad (13)$$

whereas, for the induction period, p must be proportional to x^2 in
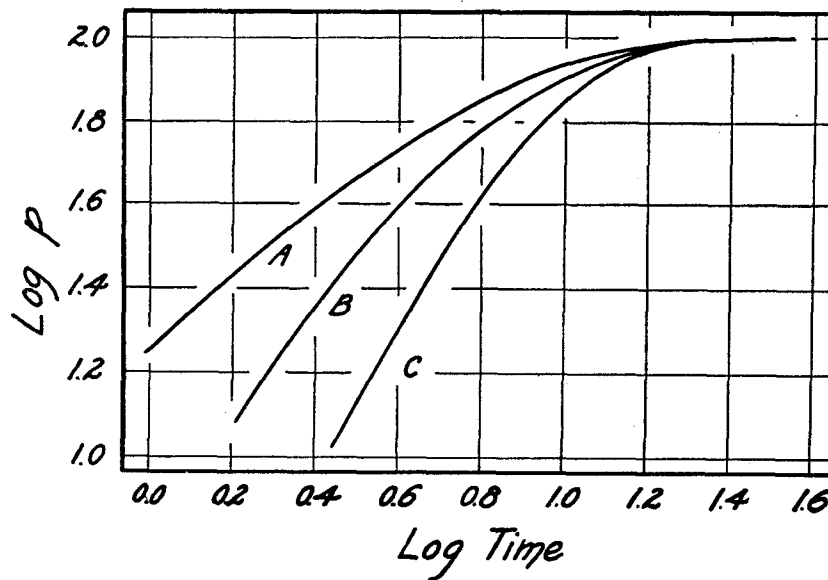equation (13). This might indicate that the dark reaction which

FIG. 5. Theoretical curves for the course of photosynthesis rate with time.
A is from equation (11); B is from the integral of (7) with the substitution of
(8); C is from equation (6). The units are arbitrary, and the curves have been
made to coincide at the stationary state for comparison. Drawn on a logarithmic
scale, the curves have a shape which is independent of the constants in the equa-
tions.

limits the rate at the stationary state is not the same reaction which
determines the rate of increase of photosynthesis during the induction
period. The term for the light process $(a^2 - x^2)$ is the same in both

[2] In an earlier research (Smith, 1937) it was thought that the light reaction
might be half-order, which would have made intensity enter as the first power.
Squaring the stationary state equation brings the intensity and CO_2 data in line
with the evidence supplied by the induction period.

cases, with intensity entering as the square, and indicates that the same light reactions are involved in both phenomena.

For the first part of the induction period, *i.e.* until the stationary state is approached, the amount of photosynthesis is proportional to the square of the time. This can be related to the results of Emerson and Arnold (1932) who found that the amount of photosynthesis was independent of light intensity when the product of intensity and time was constant. They assumed that this indicated a photochemical process directly proportional to the intensity. With the short light exposures used by these investigators, well within the time of the induction period of *Chlorella*, photosynthesis is proportional to the square of the time, and their results indicate that the product of the squares of intensity and time is constant. This would be in keeping with equation (12) which indicates that intensity enters as the square in the photochemical reactions of photosynthesis.

The author is grateful to Professor Selig Hecht for much friendly advice and criticism.

SUMMARY

1. Measurements on the photosynthesis of *Cabomba caroliniana* show an induction period at low and high light intensities and CO_2 concentrations.

2. The equation which describes the data for *Cabomba* also describes the data obtained by other investigators on different species. The phenomenon is thus shown to be similar in plants representative of three phyla.

3. A derivation of the induction period equation is made from a consideration of the cycle of light and dark processes known to occur in photosynthesis. The equation indicates that light intensity enters as the square, and that the same light reactions are involved as those which affect the stationary state rates. However, a different dark reaction appears to limit photosynthesis during the induction period.

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