

significant differences in the moments of arrival by changing the position of the electrodes up and down the two nerve fibres. Whereas the mechanical record (isotonic and isometric) show no noticeable differences and the inhibition was complete for any position of the electrodes, the size of the action currents varied from being quite unaffected up to a reduction of more than 50%. The figure shows the relation of the size of the action currents to the position of the electrodes. We cannot discuss this very interesting result here fully, but want to point out that this is evidence of the existence of two separate inhibition mechanisms, one which works directly on the contractile elements and another influencing the action currents. It is at the same time in support of the view that action current and contraction are separate phenomena.

This latter point is further illustrated by another result obtained in the same series of experiments. The action currents of the abductor show, like those of the slow contraction of the abductor, an increase in size up to a certain maximum with continued stimulation. When the impulses are so timed as not to give a reduction of the action currents during inhibition, this growth goes on though the mechanical contraction is inhibited; when the action currents undergo a reduction, they as well as the mechanical contraction attain almost immediately the size which they would have had if no inhibition had been intercalated. From this we draw the conclusion that the facilitation processes in the muscle, caused by the repetition of the excitatory stimuli, go on notwithstanding inhibition.

¹ C. A. G. Wiersma and A. van Harreveld, *Proc. Nat. Acad. Sci.*, **22**, 190 (1936).

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PHOTOSYNTHESIS IN RELATION TO LIGHT AND CARBON DIOXIDE

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Several new proposals have recently been made for the specific chemical reactions concerned in photosynthesis,^{1,2,3} as well as for the kinetics of the process in its relation to light and carbon dioxide.^{4,5,6} In order to evaluate the various suggestions, it is necessary to have definitive measurements of these relations covering a range sufficient to render them critical. The existing data do not cover the necessary range. Moreover, it still remains to be demonstrated that measurements with one plant show fundamentally the same properties as with another. We have therefore made extensive measurements with one plant, and have compared them

with the data for other plants under conditions which show their basic similarities and differences.

The fresh water plant, *Cabomba*, was used throughout. Small branches suspended in buffer solution gave reproducible values even when the same piece of tissue was used on successive days. Carbon dioxide was supplied

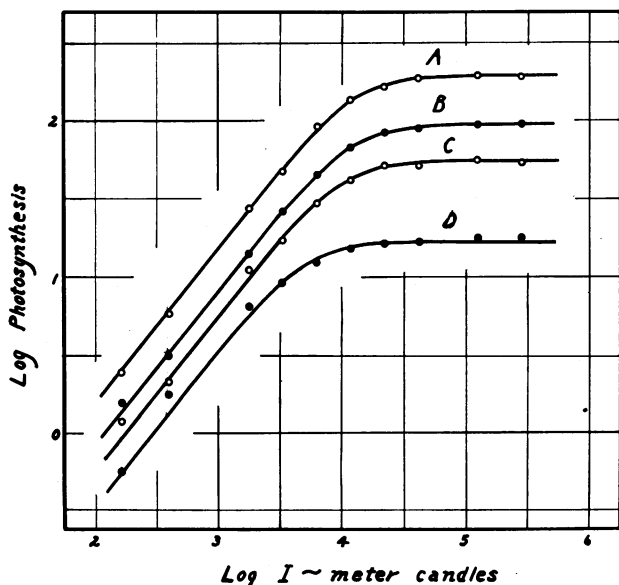


FIGURE 1

Photosynthesis as a function of light intensity for *Cabomba*. Photosynthesis is in terms of cu. mm. of oxygen per hour per 100 milligrams wet weight of tissue. Each curve is the average of five similar runs. The photosynthesis scale is correct only for curve A. The others have been shifted downward 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 carbon dioxide 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} . White light was used and the temperature was 25.3°C . The same curve is drawn through all of the data and is from equation (1).

from Warburg's carbonate mixtures using the potassium salts. Potassium bicarbonate was used to obtain the highest carbon dioxide concentration which was estimated from its pH as determined with a glass electrode. In making the measurements the buffer solution was changed as often as necessary to prevent an effective decrease in CO_2 concentration. Light was furnished by a 500-watt projection lamp in conjunction with a mirror and optical system so arranged as to give at the bottom of the manometer

vessel a maximum illumination of nearly 300,000 meter candles (Lux). The intensity was varied with neutral filters made from photographic plates which were calibrated *in situ* with a Macbeth illuminometer. The high illumination enabled us to cover an intensity range of 1 to 1700, the maximum previous range having been approximately 1 to 200. Photo-

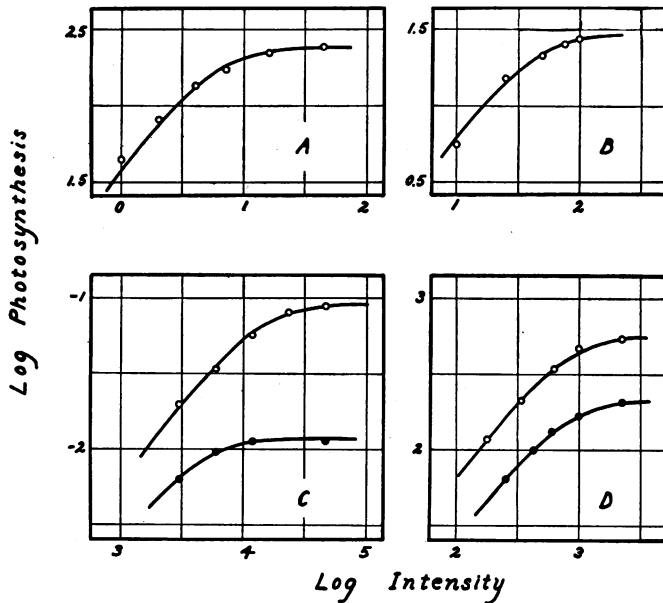


FIGURE 2

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 (1).

synthesis was measured as oxygen production with the Warburg manometric technique.⁷

Figure 1 presents the rate of photosynthesis as a function of light intensity for four different carbon dioxide concentrations. Though each point is the average of five separate experiments, the data of the individual experiments are uniform with the averages, except for the two lowest intensities where the photosynthesis is near the compensation point.

The curve drawn through the data has the equation

$$KI = p/(p^2_{\max} - p^2)^{1/2} \quad (1)$$

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. When $\log p$ is plotted against $\log I$, the shape of the curve is independent of the constants K and p_{\max} , a fact which facilitates comparison with the data. Curves similar to that in figure 1 but

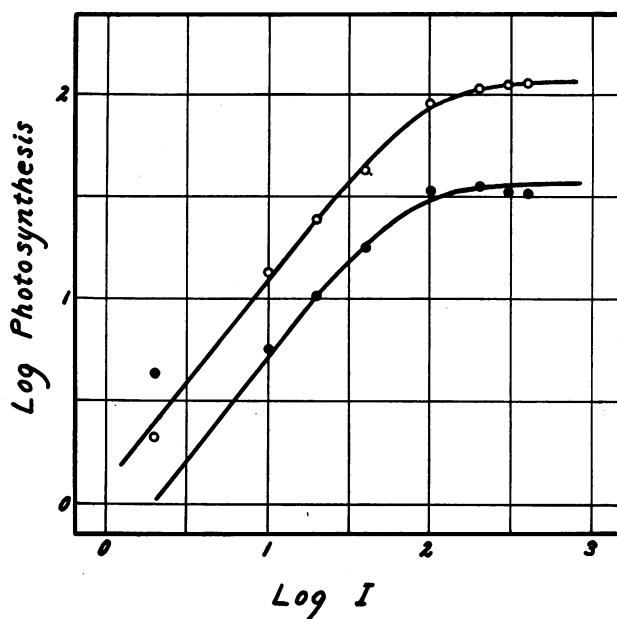


FIGURE 3

Emerson's data on two strains of *Chlorella*, one of high (open circles) and the other of low chlorophyll concentration. The lowest points represent readings below the compensation point and are of low precision. The same curve has been drawn through both sets of data and is from equation (1).

differing in slope and inflection result from changing the exponents in equation (1). For example,

$$KI = p/(p_{\max} - p)^{1/2} \quad (2)$$

yields a curve which is almost the same as in figure 1 but differs slightly in the rate at which it becomes parallel to the $\log I$ axis at high illuminations. The three upper sets of data in figure 1 fit equation (1) with better precision than (2), while the lowest set of data fit (2) somewhat better. Since no certain choice is at present possible, the curve for equation (1) has been drawn in all four series. Exponents other than those in (1) and (2) are definitely excluded, as for example, in equation

$$KI = p/(p_{\max} - p) \quad (3)$$

used by other observers.

Figures 2 and 3 give some of the data of previous workers.^{7,8,9,10,11} The curve for equation (1) has been drawn through them all. In view of the variety of plants, of experimental conditions, and of method, it is remark-

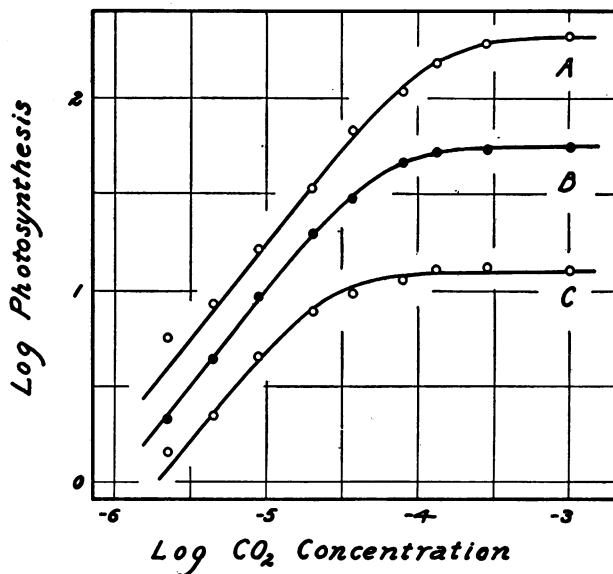


FIGURE 4

Measurements on Cabomba with different carbon dioxide concentrations at constant light intensity. Photosynthesis in cu. mm. of oxygen per hour per 100 mg. wet weight of tissue. The scale is correct for curve A; curve B has been moved down 0.4 and curve C, 0.6 of a log unit. The temperature was 25.3°C. and red light beyond 580 m μ obtained with No. 246 Corning filter was used. The relative intensities were: A, 282,000; B, 27,200; and C, 6300, which are the intensities in meter candles of the unfiltered light. Each curve represents the averages of five similar runs. As in the preceding figures, the curve from equation (1) has been drawn through the data.

able that the data fit so well an equation as specific as the one drawn through them. While some of the data presented (e.g., those of Warburg) fit equation (2) a little better, none of them are adequately represented by equation (3). Of the measurements not shown in figures 2 and 3, those of Van den Honert¹² are identical with Van der Paauw's on the same material. The experiments of Harder on Fontinalis¹³ have too high an experimental error to be critical. The data of Hoover, Johnston and

Brackett¹⁴ do not go to high intensities but are consistent with the others shown. The remaining data in the literature are too sparse and inaccurate for critical use.

Figure 4 presents the rate of photosynthesis as a function of carbon dioxide concentration for several different illuminations with Cabomba. The curve drawn through the data is the one used in figures 1, 2 and 3, and is from equation (1) with carbon dioxide concentration substituted for light intensity. Apparently the rate of photosynthesis varies in the same way with both light intensity and CO₂ concentration.

Comparison of previous results among themselves and with ours is

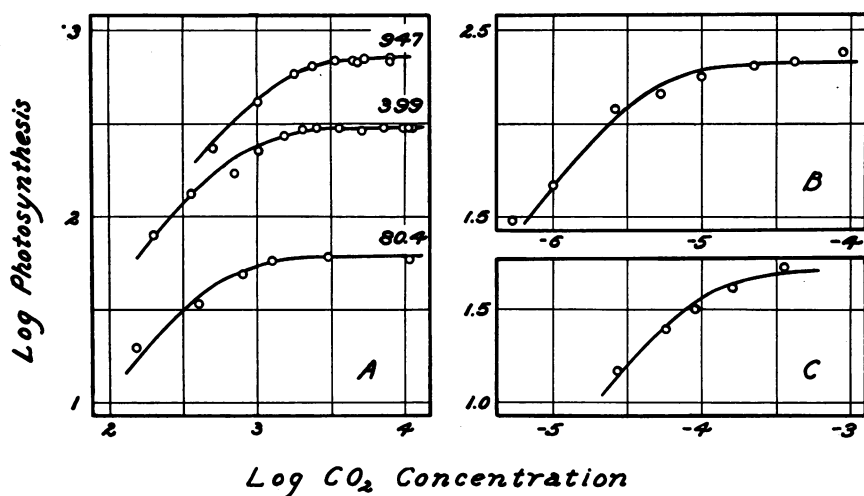


FIGURE 5

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 figures has been drawn through these data.

difficult because the method of supplying carbon dioxide influences the results. Warburg, and Emerson and Green, supplied carbon dioxide from buffer mixtures similar to those used here; their data can therefore be compared directly with ours. This is done in figure 5 B, C. The agreement with equation (2) is not so good as desired; this may be because the data represent only single experiments. In Emerson and Green's work on Gigartina this is further complicated by the use of a different piece of tissue for each point. Figure 5 A gives the data of Hoover, Johnston and Brackett for three light intensities obtained with young wheat. Carbon dioxide was supplied in gas mixtures circulated rapidly through an enclosed chamber. Their data are easily fitted with equation

(1). However, the data of Van den Honert and Van der Paauw on Hormidium using gas mixtures do not resemble the other measurements. This may be due to the fact pointed out by Emerson¹⁵ that external diffusion rate is probably the limiting factor in these experiments since at low carbon dioxide tensions Q_{10} is unity, whereas in the experiments of Warburg and of Emerson with *Chlorella* with buffer mixtures Q_{10} is high.

The fact that photosynthetic rate is the same function of both carbon dioxide concentration and light intensity argues for simplicity of the kinetic mechanism. Still, the presence of a fractional exponent indicates a complex system. Squaring both sides of equations (1) and (2) eliminates the square root, but intensity and CO_2 concentration now enter as the square. This may indicate a chain process with perhaps more than one light reaction, and would be in keeping with Warburg and Negelein's discovery¹⁶ that four quanta are necessary for the reduction of a single CO_2 molecule.

The kinetic equations of Arnold, of Baly and of Burk and Lineweaver, all reduce to equations identical in form with (3), which we know does not fit the measurements. Arnold has not attempted to fit his equations to the data of intensity and carbon dioxide concentration, while Baly used only the intensity data of Warburg and did not obtain a satisfactory agreement with them. Burk and Lineweaver used Harder's data, which have so high an experimental error that they are not critical. The fact that the data here presented, both original and from others, do not fit the equations derived by the above investigators provides a specific criticism of their equations. Our own equations (1) and (2) are to be considered as purely empirical descriptions of the data; actually they are variants of the general photostationary state equation used by Hecht¹⁷ for the visual process, and may represent basically the same ideas. However, no matter what their derivations are, the equations primarily describe the measurements, and can be used as a criterion for the validity of any theoretical description of photosynthesis.

A more complete discussion and the details of this work are expected to be published in the *Journal of General Physiology*.

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¹⁵ Emerson, R., *Ergebn. Enzymforsch.*, **5**, 305 (1936).
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ON THE PHYSIOLOGY OF THE FORMATION OF NODULES ON LEGUME ROOTS

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It is now about 50 years since Hellriegel and Wilfarth finally elucidated the nature and importance of the nodules on the roots of *Leguminosae*, and Beijerinck obtained the nodule bacteria in pure culture. Nevertheless on the three essential points in regard to the nodules we are just as much in the dark as ever. These are:

1. How does the nodule fix nitrogen? The bacteria alone, in culture, fix no nitrogen, neither does the plant without them.
2. How do the bacteria enter the root? They pass through the cell-walls with apparent ease, yet in culture they do not attack cellulose.
3. How does the nodule develop? Morphologically, of course, its development has been described many times, but how do the bacteria bring about the proliferation and enlargement which constitute the nodule?

The present note is an attempt to answer the third of these questions, and to put forward a physiological theory of nodule formation, which is strongly supported, if not proved, by the facts.

Studies upon the plant growth hormones, or auxins, especially in relation to roots, have established the following facts (for literature up to Dec., 1934, see the author's review¹):

a. A large number of substances, most, if not all, of which are unsaturated organic acids of various types, or their esters, have plant-growth-promoting activity. Not only the actual activity, by assay depending on cell-elongation, but also the ability of the substances to be transported in plant tissues, varies widely.² Indole-3-acetic acid is one of the most active.³

b. These auxins act not only on cell elongation, but they also promote, and are necessary for, the formation of roots.¹

c. They also inhibit the elongation of roots very strongly.^{1,4}