

Experimental Researches on Vegetable Assimilation and Respiration. IX.—On Assimilation in Submerged Water-Plants, and its Relation to the Concentration of Carbon Dioxide and other Factors.

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INTRODUCTION.

The fourth paper of this series* contained an account of quantitative investigations on the magnitude of assimilation in the leaves of land-plants under different conditions of illumination and temperature.

The general conclusion reached was that the actual magnitude of assimilation in a leaf at any moment is determined by one or other of the main controlling conditions, light, temperature, or CO₂-supply, acting as a limiting factor.†

Since then it has been thought desirable to attempt a quantitative study of the assimilation in submerged water-plants on similar lines, and in particular to study the relation of assimilation to CO₂-supply, a subject which had been left on one side in the work on land-plants.

A preliminary difficulty was the absence of any satisfactory method of measuring, through a wide range of conditions, the assimilation of plants submerged in water. This difficulty has been removed by a new procedure which we have described in the previous paper of this series.‡ We may now proceed to consider some of the results obtained in this way.

The first section will deal with the factor of CO₂-supply, and the succeeding

* Blackman and Matthaei, 'Roy. Soc. Proc.,' B, 1905, vol. 76, p. 402.

† Blackman, "Optima and Limiting Factors," 'Annals of Botany,' 1905, vol. 19, p. 281.

‡ Blackman and Smith, "Assim. and Resp., VIII," 'Roy. Soc. Proc.,' B, 1911, p. 374.

ones with the more general problem of how to disentangle the action of the different factors of the environment in any given case. In Section IV an attempt will be made to clear up and interpret the results of previous workers from our own point of view.

Section I.—THE RELATION OF ASSIMILATION AND CO₂-SUPPLY.

In this section we give the results of our experiments on the effect of varying the factor of CO₂-content upon the assimilation of submerged water-plants. The greater part of this work has been done upon *Elodea*; in addition a series of experiments was made with the "water moss" *Fontinalis antipyretica* and a few isolated ones with *Ceratophyllum* and *Potamogeton*.

Our expectation in approaching this subject was that there would be only one *direct primary relation* between CO₂-supply and assimilation, namely that the assimilation should be directly proportional to the amount of CO₂ available. In some sets of conditions, however, this relation will not be realised, owing to the limit imposed upon functional activity by some other factor. Light, temperature, or the "time factor" may each act thus as a limiting factor. Working over a wide range of CO₂-content, it will therefore make all the difference to the results obtained what temperature and what illumination are selected for the series of experiments.

Our first series consists of 10 evaluations of the assimilation by *Fontinalis antipyretica* in different strengths of dissolved CO₂.

In Table I these experiments are arranged in order of increasing CO₂-content of the water flowing through the assimilation chamber. Each experiment proceeded exactly like the "typical experiment" described in "Assim. and Resp., VIII," Section II (a). For most of them medium temperatures and a medium illumination were employed. The number expressing the *real assimilation* in each experiment is the final value calculated in the way set out in "Assim. and Resp., VIII," Section II (c), and is fully corrected for CO₂ lost in gas-bubbles, and for CO₂ supplied by respiration.

The unit of tissue to which it applies is the standard area of 137 sq. cm. of illuminated leaf.*

In the lower part of the diagram—fig. 1—the values of the real assimilation in the series of experiments with *Fontinalis* are plotted against the CO₂-content of the surrounding water. This diagram presents several features of interest, and the broken line which represents the idealised relation of the successive values shows in a typical manner the anticipated effect of some limiting factor.

* "Assim. and Resp., VIII," p. 384 *supra*.

Table I.

Plant.	CO ₂ -supply.		Light in arbitrary units.	Temp. of chamber, ° C.	Real assimilation, grm. CO ₂ per hour, per standard area.	Date.
	Grms. CO ₂ in 100 c.c. of water (mean).	Per cent. of saturation.				
Fontinalis	0·0080	4·91	8·1	22	0·0093	22.3.05
	0·0106	6·71	8·1	23	0·0133	17.3.05
	0·0124	7·85	5·7	23	0·0176	20.2.05
	0·0182	11·52	5·7	23	0·0237	15.2.05
	0·0236	14·93	5·7	23	0·0232	24.2.05
	0·0272	17·22	5·7	23	0·0218	21.2.05
	0·0326	20·63	5·7	23	0·0217	14.3.05
	0·0330	20·87	5·7	22	0·0230	20.3.05
	0·0338	21·39	5·7	23	0·0231	16.2.05
	0·0536	33·92	5·7	23	0·0223	14.3.05
Elodea	0·0028	1·58	8·1	19	0·0049	3.8.05
	0·0032	1·80	8·1	19	0·0083	4.8.05
	0·0054	3·05	5·7	19	0·0124	1.6.05
	0·0070	3·95	8·1	19	0·0205	1.6.05
	0·0078	4·40	5·7	19	0·0173	4.8.05
	0·0088	4·96	8·1	19	0·0276	1.6.05
	0·0090	5·08	5·7	19	0·0221	4.8.05
	0·0200	11·92	5·7	21	0·0226	21.8.08
	0·0202	12·04	5·7	21	0·0245	18.8.08
	0·0212	12·63	5·7	21	0·0252	20.8.08
	0·0279	16·11	5·7	20	0·0268	7.6.07
	0·0426	26·96	5·7	23	0·0216	26.5.05
	0·0490	37·50	5·7	30	0·0218	29.5.05
	0·0492	35·82	5·7	28	0·0249	30.5.05
Potamogeton ...	0·0080	4·63	5·7	20	0·0191	4.5.05
<i>Ceratophyllum</i> <i>demersum</i> ...	0·0014	0·77	8·0	19	0·0053	11.8.05
	0·0104	5·70	8·0	19	0·0321	5.8.05
	0·0120	6·58	8·8	19	0·0329	9.8.05

NOTES.—The values in Column 2 are the means between the amount of CO₂ in the water entering the chamber and the water leaving it. This is the most effective value of CO₂-content to take in relation to the assimilation. The numbers given in Column 3 are percentages calculated for the particular temperature of the individual experiment: they are therefore not strictly parallel with the absolute values in Column 2. (The data used were obtained from Landolt and Börnstein's Physico-Chemical Tables, p. 602, 100 c.c. of a saturated solution of CO₂ at 18° C. being taken to contain 0·1824 grm.) The light-intensities are calculated by the inverse squares of the distance of the front of the triple burner from the plant chamber, a distance of 13·0 cm. being taken arbitrarily as L.I. = 6 (*cf.* "Assim. and Resp., III," Sect. I (iii)).

In the weaker solutions of CO₂ the curve shows steadily increasing assimilation proportional to the increase of CO₂-supply. Here the light and temperature are in excess, but, at a certain point, sharply defined, increase of CO₂ is no longer followed by further increase of assimilation but the value of the assimilation remains at a fixed level of about 0·0227 (average of last seven values). This part of the curve is due undoubtedly to the limiting action of either the illumination or the temperature and presently experiments will be given which decide that it is due to the light.

Had a more intense light and higher temperature been fixed upon, then the ascending part of the curve would have been prolonged further and a fixed (but higher) level only attained with a greater concentration of CO_2 . With less light the limiting value would have been arrived at sooner.

The second noticeable feature of this curve is the long range over which

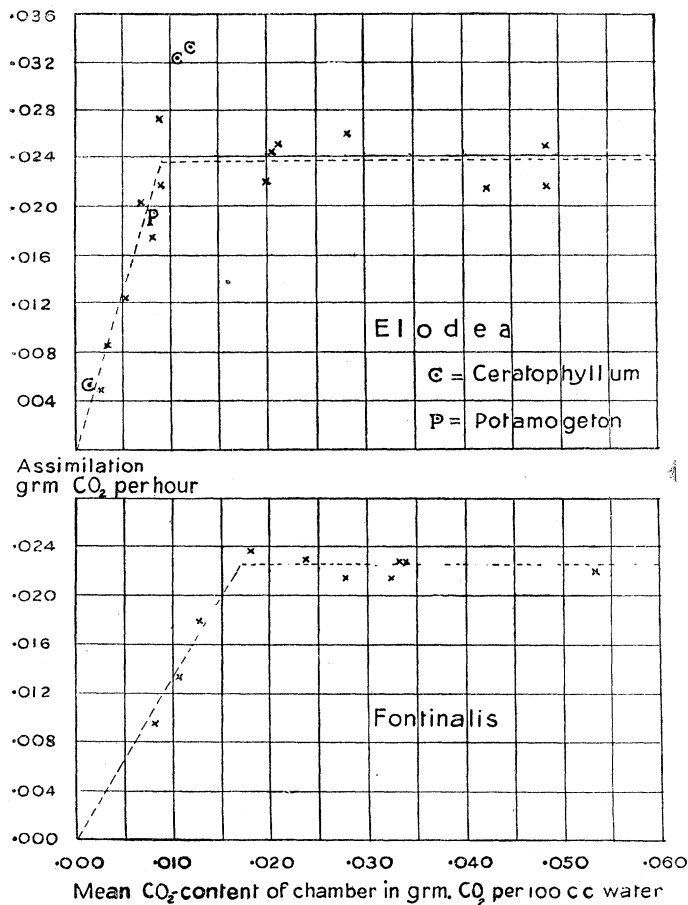


Fig. I

the constant limiting value is obtained. There is no sign of super-optimal depression of assimilation even with the strongest solution used.

In no previous experiments has the effect of such concentrated CO_2 been satisfactorily investigated and it affords strong support to our view that the whole conception of an optimum is out of place here, to find no signs of it even with an environment as rich in CO_2 as is an atmosphere containing 30 per cent. of this gas.

Of course with these aqueous solutions of CO_2 , diffusion of this gas from

the water in the chamber to the assimilating cells is much more sluggish than if the chamber were filled with a *gaseous* mixture containing 30 per cent. CO_2 , so that in the latter the chloroplasts would probably be bathed in stronger CO_2 than in the former medium. No doubt we should then find a narcotic effect of the CO_2 on the protoplasm, though an aqueous medium containing 30 vols. per cent. dissolved CO_2 does not produce this effect.

Of the effect of subjecting the plants to still stronger solutions of CO_2 we shall speak briefly presently.

In the middle of Table I are found the results of a long series of experiments with *Elodea* and these data are represented by small crosses in the upper part of fig. 1. With *Elodea* the same form of curve is revealed as with *Fontinalis*; the limiting value is here slightly higher, 0.0237 (average of last eight values),* and until it is reached increase of CO_2 causes a proportional increase of assimilation.

With *Elodea* the assimilation values along the horizontal part of the curve are not so regular as with *Fontinalis*, but their agreement is perhaps as close as might be expected when it is noticed that the experiments range over three years and that identity for this value in successive experiments depends on repeating identically intense illumination of an identical area of leafy tissue.

The intensity of illumination varies with the square of the distance of the chamber from the light, and this had to be adjusted for each experiment but could not be measured directly, as the bath-window and cooling-screen intervened. An error of a few millimetres may have occurred and the light must have varied from changes in efficiency of the mantles. The uniformity of the area illuminated, 137 sq. cm., depended upon careful packing and distribution of the green shoots upon the silver grid; this was always covered as completely as possible, and the error from this cause is probably not great.

The difference of about 5 per cent. between the limiting values for the two plants has possibly no real significance at all and we may well hold that equal areas of different water-plants equally illuminated produce the same amount of assimilation, *provided* of course *that light is the limiting factor*. It may be recalled that this law was demonstrated in a previous paper† for a variety of types of land-plants and in that case also the agreement found experimentally was within 5 per cent.

* The sixth value, 0.0276, is excluded from this average because a more intense light, = 8.1, was then employed, which would give too high a value should light 5.7 be limiting.

† Blackman and Matthaei, "Assim. and Resp., IV.," 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 444.

Considering only the low concentrations of CO_2 , when CO_2 is certainly the limiting factor, there is, however, an interesting consistent difference between the assimilation-values for the two plants; one which holds for each concentration of CO_2 . *Fontinalis* assimilates just about half the amount achieved by *Elodea* for any given concentration of CO_2 , so it looks as if the obstacles to the diffusion of CO_2 up to the chloroplasts from outside were less in *Elodea* than in *Fontinalis*.

This difference seems to be a class distinction between the Bryophyte and the Phanerogam, for values obtained with plants of *Potamogeton* and *Ceratophyllum* are of the same order as those for *Elodea*, and quite different from those for *Fontinalis*. These experiments are given at the bottom of Table I, and the values for the real assimilation are incorporated in the top part of fig. 1. The four points marked P or C fall into perfect line with the crosses which represent the values for *Elodea*. The two highest values for *Ceratophyllum*, 0.0321 and 0.0329, are too high to be obtainable with light of 5.7 intensity, and it will be noted that the light was 8.0 and 8.8, due to its being placed closer to the plant. Thereupon the light ceases to be limiting, and the proportional relation to CO_2 -supply is continued further than with the weaker light of 5.7. *Ceratophyllum* in 0.0014 grm. CO_2 solution, however, must be limited by the CO_2 -supply, and so, although the light has the higher intensity, the assimilation value is down near that for *Elodea* in a similar solution.

The Bryophyte then is less efficient in utilising the CO_2 that surrounds it; possibly this may be the outcome of important structural differences, as submerged flowering plants have a well developed system of air-spaces containing an "internal atmosphere," while *Fontinalis* lacks this physiologico-anatomical characteristic.

This internal atmosphere certainly makes for greater efficiency in gaseous exchange generally, and the only doubt in accepting this theory of the cause of the difference between these plants arises from the fact that the leaves of *Fontinalis* are only one cell thick, and would therefore be expected to be highly efficient in assimilation.

A certain number of estimations of assimilation have been made with concentrated solutions of CO_2 , exceeding those dealt with in the present paper and approaching saturation. With these concentrations there may be vigorous giving off of gas-bubbles; but, on analysis, the gas turns out to be mostly CO_2 , and the corrected real assimilation is much depressed.

That, ultimately, depression of the assimilation sets in when the CO_2 is sufficiently increased is no evidence in favour of there being a *primary*

optimal amount of CO_2 for assimilation; rather it is a sign of a general narcotic effect of the strong CO_2 upon protoplasm, and has no specific relation to assimilation. It is quite a general phenomenon, and it has been shown by previous workers that many vital processes are depressed by an atmosphere containing 20 to 25 per cent. of CO_2 . Chapin* has given an account of previous work on CO_2 -narcosis, and has himself shown that the growth of shoots and roots of higher plants is thus affected. He finds that the growth of bean-roots is retarded by 5 per cent. CO_2 and inhibited by 25 per cent.; with the hypocotyl of *Sinapis*, 15 per cent. retards and 25 per cent. suspends growth.

We conclude then that in the curve expressing, in any given light, the relation of assimilation to the whole range of CO_2 -concentrations from zero to saturation, we may separate off the falling end-part of the curve as an effect of narcotic poisoning. This third and last phase thus contrasts with the first two phases, which are specific assimilation effects, the first rising in a straight line where the CO_2 is limiting and the assimilation proportional to it, and the second a horizontal line where the assimilation is limited by the light (or the temperature) and is independent of increase of the CO_2 -supply.

Section II.—SUB-NORMAL ASSIMILATION OF UNHEALTHY PLANTS.

We must now point out that the results just dealt with are not the whole body of experiments done, but are selected results, and this selection has to be justified.

Many observers have noted that water-plants are very sensitive to unfavourable conditions of environment, and we have found marked depression of vigour and assimilation-power brought about by keeping plants in vessels of water in the laboratory. Even plants freshly collected from a natural habitat do not always show the same uniform vigorous activity: these sickly plants give low assimilation values and have been excluded from our tables and figures, which contain all the high normal results obtained under each experimental condition.

We may now give some examples of this sub-normal assimilation. For instance, on July 29th, 1905, as a test experiment, pieces of *Elodea* were gathered of quite dull and unhealthy appearance, lacking the bright green colour of the normal plants. The assimilation of this material was measured in the highest intensity of light (8.8) and in water containing 0.0416 grm. CO_2 per cent. In these conditions normal material would have given a

* 'Flora,' 1902, *Ergänzungsband*, p. 348.

value of about 0.034 grm. CO_2 assimilated per hour (consult fig. 1), but this unhealthy plant did not exceed 0.008 grm.

Such unhealthy plants would naturally be avoided in collecting, but it is not only those whose appearance obviously indicates loss of vigour that give figures below the normal for assimilation. Plants which have been kept in the laboratory shade-greenhouse in tap-water for a few days always show a falling-off in assimilation, though their outward appearance remains unchanged. For example, on May 26, 1905, a quantity of *Elodea* was collected, some experimented upon the same day, and the rest kept in a large basin of tap-water in the shade-greenhouse until June 6. The value obtained on May 26 was 0.0216 grm. CO_2 per hour in 5.7 intensity of light and CO_2 -concentration of 0.0426 grm. per cent. (which figures in Table I as a normal value), while on June 6 under no less favourable conditions the value was only 0.0179, or about 17 per cent. less.

Again, *Elodea* gathered on June 8 which gave full active assimilation when measured on the same day gave on June 14 in 5.7 light and CO_2 strong enough not to be limiting (0.0318 grm. per cent.) only an assimilation value of 0.0167 grm. CO_2 per hour when at least 0.0240 would be normal. This falling off of nearly 30 per cent. was unaccompanied by any microscopic sign of change, and was brought on by five days' stay in a basin in the laboratory and 24 hours in the dark in the actual assimilation-chamber* before the assimilation value was measured.

It is reasonable to conclude that among a large number of random gatherings of healthy-looking *Elodea* from natural habitats some will be depressed below their normal vigour, and therefore it seems more instructive to eliminate from the final comparison of assimilation values, those which fall considerably below the general level under similar conditions.

Section III.—THE INTERACTION OF LIMITING FACTORS.

When the two series of experiments described in the first section were carried out in 1905 we were not certain whether it was the light or the temperature employed that limited the assimilation of both *Elodea* and *Fontinalis* to approximately the same value in all the higher concentrations of CO_2 . The temperatures lay between 19° and 23° C., except in two cases, and the light intensity was usually 5.7. Inspection of the table would incline us to the view that it was the light that was limiting because, in the two cases where temperatures of 28° and 30° C. were employed with *Elodea*, no

* This long enclosure in the chamber may have been specially deleterious; it was never repeated.

higher assimilation was noted than with much lower temperature at the same light intensity, 5·7.

There were also other small indications in the same direction.

When this work was taken up again in 1907 and 1908 definite experiments were made to determine what limit was actually set to assimilation by the light and the temperature employed respectively in the long series of earlier experiments. Five such experiments, all made with *Elodea*, may now be described briefly, and these will serve to exemplify the general principle that is employed for experimentally determining which of the possible factors is actually limiting the function in any specific case. This principle may be formulated as follows:—*When several factors are possibly controlling a function, a small increase or decrease of the factor that is limiting, and of that factor only, will bring about an alteration of the magnitude of the functional activity.*

Each of our experiments accordingly will involve the change of some one factor in the middle of the experiment to test whether the assimilation responds to this change or not.

Experiment A: CO₂-Supply the Limiting Factor.—In this case the light intensity was 8 and the temperature 19° C. throughout the experiment. The

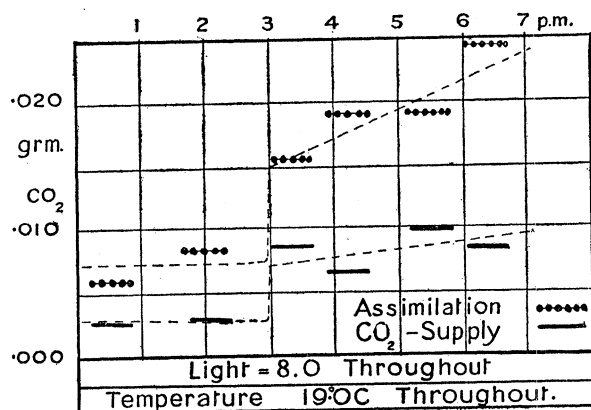


Fig. 2 Exp. A.

CO₂-supply was at first 0·0032 gm. CO₂ per 100 c.c. solution, and at 3 P.M. it was suddenly increased. The diagram in fig. 2 shows the course of the assimilation. As in all experiments in this section the abscissæ are time and the ordinates grammes CO₂; two separate curves are given, one for the assimilation (in grammes CO₂ assimilated per hour), and the other for the CO₂-supply in grammes CO₂ dissolved per 100 c.c. of the solution.*

* It is, perhaps, slightly unfortunate that the weight of CO₂ assimilated is greater than the number given for the CO₂-supply. This, of course, is only a question of units, as the plant has in each hour *three* hundred cubic centimetres of the solution to draw from.

The mean of the two assimilation estimations, each representing 40 minutes, taken before 3 o'clock is 0.0073 gm. CO_2 per hour. With so low a CO_2 -supply we expect this factor to be the limiting one, and at 3 P.M. the supply bottle of CO_2 -solution was opened and filled up with a stronger solution. Owing to excessive generation of CO_2 in the tower, and perhaps also to imperfect mixing in the supply bottle, the final strength of the new affluent was only slowly attained, continuing to increase until 7 P.M. The behaviour of the assimilation after the change shows clearly that, in this particular combination of factors, the CO_2 -supply actually was limiting, for the assimilation increases suddenly at 3 P.M., and goes on rising continuously till 7 P.M., about in proportion to the altering the CO_2 -supply.

Experiment B: CO_2 -supply the Limiting Factor.—In this case the temperature was 19°C . throughout, and until 5 P.M. the light was 6. At 5 P.M. the light was increased to 8.3. It was intended to maintain the CO_2 -supply at 0.0090 gm. per 100 c.c. throughout, but, as the diagram of fig. 3 shows, its concentration declined in the last half of the experiment.

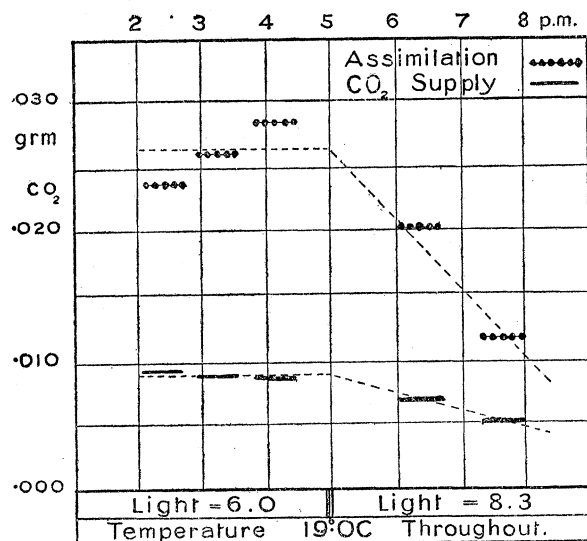


Fig.3 Exp. B.

The assimilation values for the first half average 0.0265, which might possibly be limited by either the light or the CO_2 (see fig. 1). On increasing the light it becomes clear that the latter is true, because not only is there no increase of assimilation, but there is a decrease due to the fall that has set in with regard to the CO_2 -concentration.

Experiment C: Light the Limiting Factor.—An experiment was carried out at a temperature of 29°C . and a CO_2 -supply of 0.0490 gm. per 100 c.c.,

both of these factors being very high. In the first half the light intensity was only 4.2, being increased to 5.7 at 6 P.M. (fig. 4). The assimilation from 4 to 6 P.M. averaged only 0.0183, much lower than either CO₂-supply or temperature would account for, and so it was expected that the light was limiting. This was proved in the second half of the experiment, because on increasing the light the assimilation rose, giving an average of 0.0220 between

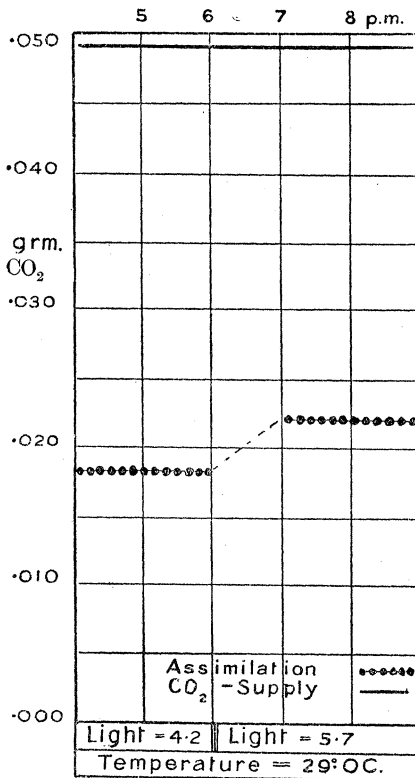


Fig. 4 Exp. C.

7 and 9 P.M., which Table I shows us is a possible limiting value for the light of 5.7.

We have attempted to prove directly that the average value of 0.0237 in the series on Table I is limited by light by starting an experiment in some of the conditions there shown and subsequently *increasing* the light still further, in the expectation of getting still greater assimilation.

There is, however, a difficulty about doing this, because rates of assimilation much higher than 0.0240 are not very long maintained before a decline sets in. This is a general phenomenon in assimilation which has

elsewhere* been attributed to the "time factor," which term we may continue to use as a non-committal name for the collective action of certain internal depressant factors still obscure.

An increase of illumination in the middle of an experiment superposed on a natural decline of assimilation might partly or wholly neutralise this decline and yet not lead to an actually increased assimilation. We have, indeed, shown that the progressive decline in time is less marked when the light intensity is increased than when it is constant, but the experiments are not significant enough to be given in detail here.

Experiment D: Temperature the Limiting Factor.—The light was of 5·7 intensity throughout and the CO₂-supply 0·0230 to 0·0215. The experiment started at a temperature of 7° C., which was maintained by the careful addition of ice to the bath at short intervals; at 4.12 P.M. the bath was quickly warmed up and kept at about 21° C. from 5 to 7 P.M. (see curve of temperature of chamber in fig. 5). At the low temperature the assimilation

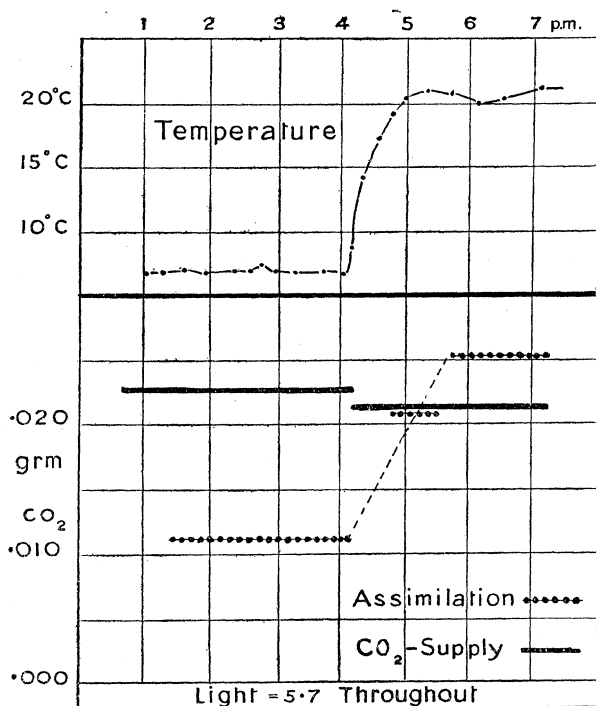


Fig. 5 Exp. D.

remained at 0·0115 hour after hour, not half the value that this combination of light and CO₂ permitted in 1905. It is clear then that this value marks

* "Optima and Limiting Factors," p. 282.

the limit set by the temperature of $7^{\circ}\text{C}.$, and on raising the temperature the assimilation rose to 0.0252; the conditions of 1905 being thus reproduced, the assimilation rose to a value which falls into line with those of that year in Table I.

Experiment E: Temperature the Limiting Factor.—A second experiment similar to D was then carried out in order to get the limiting value for some other temperature. In this case the light was 5.7 and the CO_2 -supply 0.0212, and at first the chamber was kept at a temperature of about $13^{\circ}\text{C}.$ Fig. 6 shows that the assimilation kept at 0.0177 for three hours under these

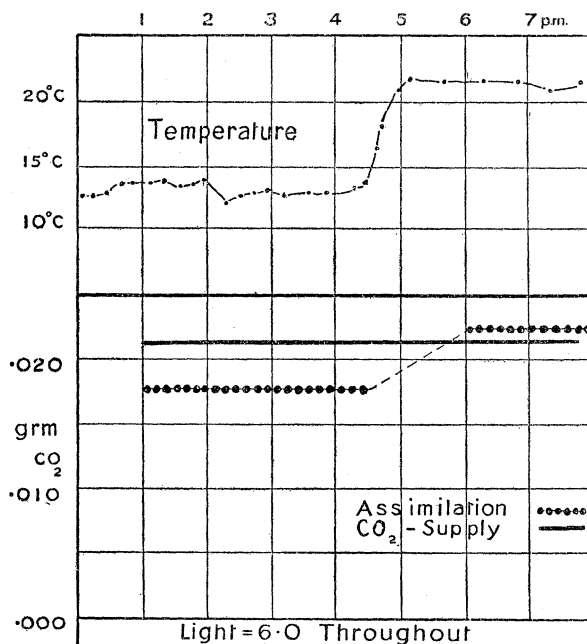


Fig. 6 Exp. E.

conditions. At 4.30 p.m. the temperature was raised to $21^{\circ}\text{C}.$, and then between 6 and 8 p.m. the assimilation increased to 0.0226, a low, but possible, value for the light as limiting factor, as Table I shows.

The bearing of these experiments upon the problem of the nature of the limiting factor in the two long series in 1905 is clear.

By Experiment D we have established 0.0115 as the "specific temperature maximum" for the temperature of $7^{\circ}\text{C}.$, and by E the value of 0.0177 for the temperature of $13^{\circ}\text{C}.$ Assuming that assimilation in water-plants increases logarithmically with temperature, which can hardly be doubted on the evidence available for land-plants, we can from these two data calculate

the coefficient of increase for 10° C. The coefficient arrived at is $K_{10} = 2.05$.*

We are now in a position to calculate the *primary* curve of assimilation and temperature in Elodea (see fig. 7, middle curve), and this will hold *actually* for medium temperatures. From inspection of this we see that the lowest temperature used in 1905, namely, 19° C., should allow an assimilation of at least 0.0267. The average limiting value obtained was, however, less, being 0.0237, so that though this is not much below the temperature limit it is just enough so for us to consider it certainly proved that the light of 5.7 intensity and not the temperature (19° to 23° C.) was the limiting factor in the 1905 series of experiments.

The Inter-relation of these Limiting Factors.—We will conclude this section by showing how the knowledge of the interaction of limiting factors on assimilation here set out will give us the power of predicting the value of the assimilation that will take place in any selected combination of the three external factors.

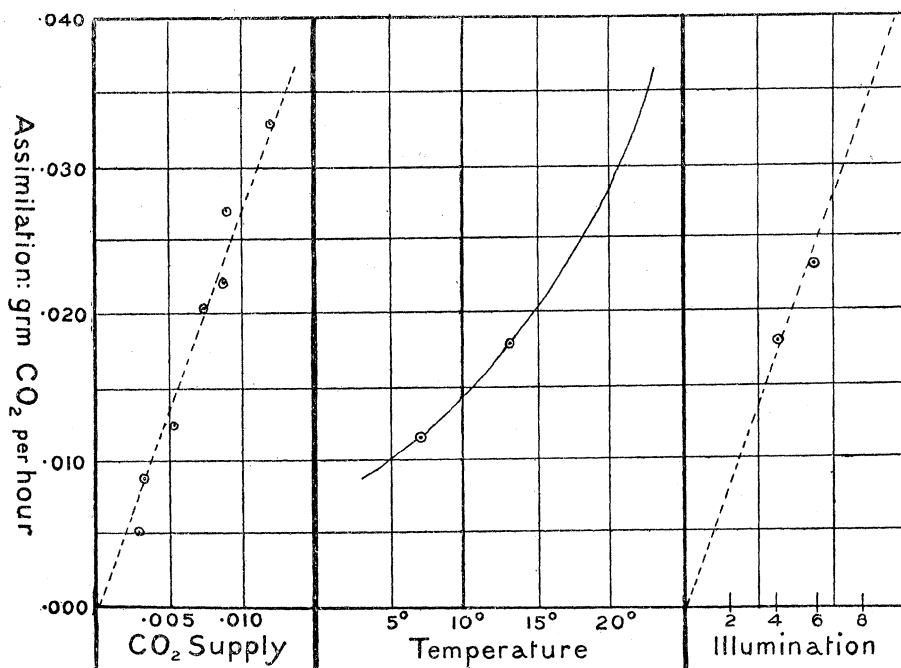


FIG. 7.—Schema of inter-relation of factors and assimilation in Elodea.

Fig. 7 shows for Elodea the relation between different magnitudes of

* This is nearly identical with the coefficient $K_{10} = 2.1$ arrived at for Cherry Laurel, "Optima and Limiting Factors," p. 283.

assimilation and each of the three factors, CO₂-supply, temperature, and illumination separately.

The two latter curves are based on the data set out in this section, the particular points established being marked with circles; the curve for CO₂-supply is taken from fig. 1. We can by inspection tell exactly what minimum of CO₂, temperature, and light will be required for any one of the values of assimilation on the ordinates by following the horizontal line right across the diagram and noting where it intersects the three curves respectively.

Reciprocally, given any hypothetical combination of different magnitudes of the three factors, we have only to note in the diagram the values of assimilation corresponding to each of them, and we can foretell that the value of assimilation actually attained in that combination will be *the lowest of the three values*.

Section IV.—CRITICAL ANALYSIS OF THE RESULTS OF PREVIOUS WORKERS.

Instead of prefacing the present paper by the usual summary of previous work, we have thought fit to expound our own point of view first, and we now proceed to some critical account of the work of other investigators. This order is adopted because we differ from them in the interpretation to be put upon the facts of assimilation rather than in the nature of the facts themselves. We may confine ourselves to the works of Reinke,* 1883; Treboux,† 1903; and Pantanelli,‡ 1903.

Reinke counted the bubbles given off by *Elodea* in spring water with a little added CO₂, when it was exposed to a series of lights of different intensity§ in rapid succession. After the plant had been about one minute in a given illumination the number of bubbles liberated in two successive quarter minutes was counted, and then the intensity of the light was altered and a new reading started. Increasing the light from 1/24 to 1/2 sunlight

* J. Reinke, "Untersuchungen ü. d. Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen," 'Bot. Zeit.,' 1883, p. 697.

† O. Treboux, "Einige stoffliche Einflüsse auf die Kohlensäure-Assimilation bei submersen Pflanzen," 'Flora,' 1903, p. 53.

‡ E. Pantanelli, "Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen," 'Jahrb. f. wiss. Bot.,' 1903, vol. 39.

§ Reinke placed his plant, within a dark room, in the path of a beam of sunlight concentrated by a large lens of long focus. By adjusting the distance of the plant beyond the focus of the lens he obtained positions corresponding to a series of definite illuminations, *i.e.*, 1/36, 1/25, 1/16, 1/9, 1/4, 1/1, 4/1, 9/1, 16/1, 25/1, 36/1, 49/1, and 64/1, these being fractions of the direct sunlight falling at the moment on the lens from a heliostat. Pantanelli used also the same method of obtaining definite intensities of light.

caused generally a proportional increase in the rate of bubbling, but further increase of light, even up to 64/1 sunlight, gave no further increase of bubbling, but a maintenance of the same uniform rate as at 1/2 sunlight.

To us this is a perfect example of the action of CO₂-supply as a limiting factor; the temperatures were between 20° C. and 28° C., and therefore too high to be limiting with this small (though indeed unknown) CO₂-supply.

Reinke naturally, at that time, regarded the long level part of the curve as being in direct relation to the intensity of light and as being a *prolonged optimal effect*.

It should be mentioned here that Pfeffer, in 1897,* suggested that possibly the photo synthetic work of the Elodea in Reinke's experiments was limited by the inadequate CO₂-supply, and that if adequate CO₂ were supplied, an optimal relation between light and assimilation might become evident. This first suggestion of a specific factor limiting the activity of assimilation was not developed further.

Treboux, in Pfeffer's laboratory, worked chiefly at the effect of drugs, acids, and salts upon the bubbling of Elodea, using the light of one or two Auer burners at a temperature of about 16° C. in a dark room; he has, however, in addition a section upon the influence of the quantity of dissolved CO₂. His procedure was to change, every fifteen minutes or so, the water in which the Elodea was bubbling for a water containing twice the previous quantity of CO₂. All the CO₂-concentrations were low, beginning at 0.1 vol. CO₂ per cent., and doubling up to 12.8 vols. CO₂ per cent. His series of bubble-countings show that at first the rate doubled with the doubled CO₂-supply, but that abruptly the increase ceased, and further concentrations of CO₂ gave continually only the same value.

On carrying out a similar experiment with a doubled light throughout (Experiment XVIII), the progressive doubling of assimilation was continued one stage longer, and then a maximal value was reached—at about 3 vols. CO₂ per cent.—and maintained unaltered.

Treboux concludes (1) that assimilation is proportional to CO₂-supply, and (2) that with weak lights the CO₂-optimum shifts proportionally with the light-intensity. Treboux's observations are to us also a clear case of the CO₂-supply at first being a limiting factor to the assimilation, and then the feeble light in its turn acting as one. These results are just the converse of Reinke's, and both are according to expectation.

The work of Pantanelli, also finished in Pfeffer's laboratory, must be considered in more detail. It is the latest and fullest work which deals with the effect of the factors of light-intensity and CO₂-supply upon assimilation

* 'Physiology,' vol. 1, sect. 59.

in water-plants. Pantanelli's ultimate object was the investigation of the effects of intense light upon chloroplasts and their power of assimilation. He is interested to see whether the decline of assimilation in "ultra-optimal" light is correlated with bleaching of chlorophyll, aggregation of plastids, and inhibition of protoplasmic circulation; and he rightly attaches great importance to the functional activity of the colourless protoplasm of the plastid. Too readily, however, he shelves complex phenomena as due to "irritability," and interprets his effects generally in terms of fatigue of the plastid by the strong stimuli of intense light. He would define the optimum as the maximal intensity of function which can be carried on for an appreciable time without fatigue.

We propose to show how, in our opinion, the data which Pantanelli presents in illustration of his principles can be more clearly and satisfactorily interpreted from the point of view of interaction of limiting factors. Let us take first the curve which he puts forward for the relation of the rate of bubbling to different intensities of light. On Plate 4, *Elodea*, *a*, Pantanelli gives a graphic diagram which represents the bubbling in a series of lights ranging from 1/36 sunlight to 64/1 sunlight, being the mean of all the experiments carried out in less than 15 vols. CO₂ per 100 c.c. water.

The enclosed part of the diagram in our fig. 8 represents Pantanelli's curve in slightly modified form (*cf.* p. 408); the abscissæ are intensities of light, and the ordinates the times in seconds for the liberation of 10 bubbles.

Pantanelli's interpretation of his results being based upon the conception of optima, he naturally finds it difficult to apply such interpretation precisely over a wide range of conditions. He has no thought of abandoning this point of view as untenable, and he is therefore driven to hold that the optimum of one factor may be shifted considerably by alterations in the magnitude of the other factors, so that the position of the CO₂-optimum becomes purely relative to the magnitude of the light-intensity, and so on.* This can only be regarded as a transitional point of view and cannot be even a temporary halting place.

The lights he investigated ranged from 1/36 sunlight to 64/1 sunlight (see footnote, p. 408) and the CO₂-supply from 1 to 50 vols. per 100 c.c. of water. As regards the factor of temperature we have unfortunately inadequate information, because Pantanelli's research was carried out before

* He says: "The light optimum for *Elodea*, etc., is about 1/4 sunlight in spring water, and shifts towards stronger light with increase of the CO₂-content, and towards weaker light with decrease of CO₂"—p. 224. "With 1/4 light CO₂-optimum is about 10 vols. CO₂ per cent.; with 1/1 light, 15 vols. CO₂ per cent.; and with 4/1 light, about 20 vols. CO₂ per cent."—p. 195.

Miss Matthaei* had caused the great significance of this factor to be generally recognised. Pantanelli is satisfied with stating that his experiments were all done between 22° C. and 30° C., within which range temperature was then believed to be without any determining effect on assimilation. In the course of each single experiment, however, the temperature of the water is stated to have varied very little.

In Pantanelli's experiments the small sprig of *Elodea*, 1 cm. long, was contained in a large cubical glass vessel of CO₂-laden water, and this was moved along through a series of increasing or decreasing light intensities, staying exactly 10 minutes in each position.

The first point for comment is that the rate of bubbling did not remain constant minute after minute in each position, but nearly always fell off steadily all the time. This effect is slight in weak lights but strongly marked in intense lights.

This falling off in time may be partly due to external factors† and partly to internal causes which may be aggregated as the "time factor."

An effective cause of decline is to be found in the protoplasmic disturbance produced by the intense radiation of lights 16 to 64 times the intensity of direct sunlight. The excess light absorbed by the chlorophyll will cause considerable local heating of the plastids, which will depress their activity generally, as may also its direct photo chemical action. In accordance with this view it is only with these higher intensities of light that the falling off is so marked. Further, the effect is cumulative in Pantanelli's procedure because *Elodea* is not exposed to light 64/1 without having first stayed ten minutes in each of the lights 9/1, 16/1, 25/1, 36/1, and 49/1. Thus the assimilation value for each intensity is depressed by the sum of the injurious effects of all the previous members of the series, and cannot be brought forward as the value of assimilation really corresponding to that intensity of light.‡

With such a series of changing values, there arises at once the question as to which value is to be taken as best representing numerically the assimilation proper to that set of conditions. Pantanelli arbitrarily assumes that

* Matthaei, "Assim. and Resp., III," 'Phil. Trans.,' 1904, B.

† One may wonder whether the stagnation of the water and slow diffusion of CO₂ had anything to do with these falling values. Prolonged experiments in one vessel of water certainly need artificial stirring. In Pantanelli's apparatus the water could be made to flow through the vessel, but it is not clear that it was his practice to have it so. F. Darwin and D. M. Pertz have shown how striking is the effect of stirring ("On the Effect of Water-currents on the Assimilation of Aquatic Plants," 'Camb. Phil. Soc. Proc.,' 1895, vol. 9, p. 76).

‡ No attempt is made to get information on this matter by determining, for comparison, the rate of bubbling when *Elodea* is exposed straight away to 64/1 without passing slowly up the whole series of lights.

ten minutes is the proper time to allow for the chloroplast to readjust itself to a new set of conditions. For this there does not seem to be any real experimental justification in his work, and in his experiment on p. 182, where the plant continued 15 minutes in each light, the values continue to fall steadily for the whole 15 minutes.

To us it seems that the better value to take would be the earliest value that is not subject to any special suspicion. The initial values recorded in each light-intensity are in all cases also the highest values, when the series of lights is an ascending one, and it is the initial values of bubbling that we have taken in the presentation of Pantanelli's curve given in the inset diagram in our fig. 8.

The first part of this curve is represented by a double track, because the experiments always began with the *Elodea* in light 1/1, and then proceeded by ten-minute stages down to light 1/36, after which the vessel, with its sprig of plant, is returned by the same stages to 1/1, and so on to 64/1. In further dealing with this curve, we propose to simplify this by taking the mean of the up and down series from 1/1 to 1/36 and back (see note to fig. 8). In such weak lights there is no injurious effect, and the plant returns practically to the same value for 1/1 as it started with.

For lights 1/1, 4/1, and 9/1 the same initial value (within ± 2 per cent.) is obtained in all three cases, afterwards the initial values begin to get lower and lower, not due to a primary optimal point having been passed, but to cumulative depression from prolonged exposure to intense radiation.

If this is a sound point of view, one would expect that the high initial values would be kept up further along the series if the plant stayed a less time at each stage. Pantanelli's own results show that this is so, for on p. 181 there is a small diagram of a set of experiments in which the plant stayed only three minutes in each position. Here the initial values up to 36/1 are practically the same as at 1/1, and those at 49/1 and 64/1 are only a little less.

Even the curve of initial values, as modified in the inset to fig. 8, does not give the right aspect of the relation investigated, and this is due to the distorted scale of abscissæ and ordinates adopted.

It will be noted that from 4/1 to 64/1 the abscissæ are proportional to the light-intensity, but for intensities below 1/1 down to 1/36 a logarithmic scale is adopted, which would put the zero of light at an infinite distance. Further distortion is produced by the fact that the ordinates do not represent *intensity of assimilation* (i.e. rate of bubbling), but the time taken by 10 bubbles to form, which is the inverse of the

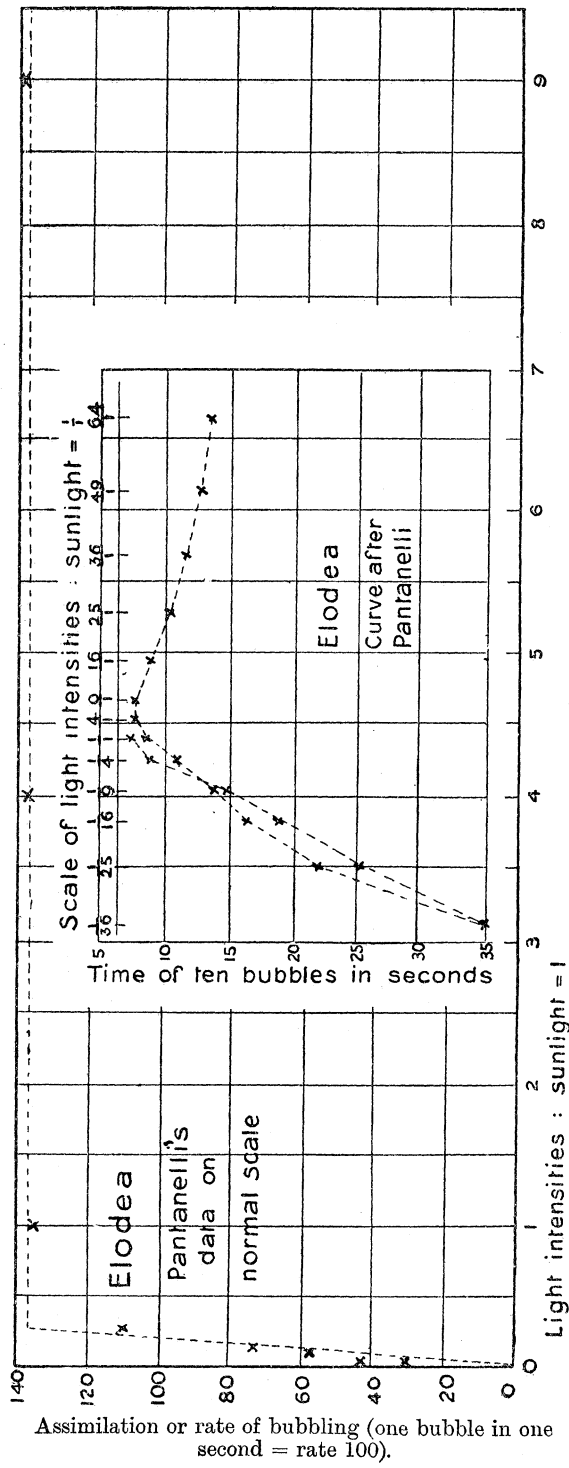


Fig. 8

The enclosed diagram gives, on a much reduced scale, Pantanelli's curve (Plate IV, Elodea, a), simplified by taking the initial value only for each intensity and omitting the subsequent ones. His exact data are appended to show the derivation of our outer curve from them.

Light Intensities.		1/36	1/25	1/16	1/9	1/4	1/1	4/1	9/1	16/1	25/1	36/1	49/1	64/1
Time of ten bubbles in secs., Initial Values. (Pantanelli.)		19.2	15	12.3	9.6	7.7	7.3	7.2	8.2	10	11	12	13	13
Rate of bubbling: Inverses of above: 10 bubbles in 10" = Rate 100. (B. and S.)		29	42	58	73	111	135	137	139	122	100	91	83	77

rate, and here again the zero of bubbling would be at an infinite distance. Such methods of presenting facts are obscurantist rather than graphic, and we have been compelled to construct the larger outer curve of fig. 8 to give the corrected relation of the initial assimilation-values in the different intensities of light.

Inspection of this curve at once shows that we are dealing with a limiting factor relation such as we have found in our own work. From intensity $1/36$ to $1/4$ the assimilation increases in direct proportion with the increase of light and then a limit is reached. Possibly the limit is set by the CO_2 -supply, which did not exceed 15 vols. CO_2 per 100 c.c. of water (0.03 grm. CO_2 per cent.). On the other hand, it may have been due to the temperature, but we cannot go into this matter as there is no clue to the temperature at which this particular batch of experiments was carried out.

In spite of this uncertainty, it is impossible not to conclude from the form of the curve that a limiting factor is at work. Consequently, no higher assimilation is ever reached, though the light is increased twenty-seven fold, up to $9/1$.

Pantanelli's curve of the relation of bubbling-rate to light-intensity is therefore only really a curve of this nature as regards its first ascending part— $1/36$ to $1/3$ light. The next part, $1/3$ to $9/1$, has nothing to do with light, but represents the assimilation corresponding to the limited CO_2 -supply or temperature. Finally, we regard the third falling part (lights $16/1$ to $64/1$, shown only in the inset diagram to fig. 8) as not a specific assimilation effect at all, but as exhibiting the cumulative effect of depression of protoplasmic activity.

As a corollary to his investigation of the light-optimum, Pantanelli also made experiments on assimilation, with constant illumination and varying CO_2 -supply, to obtain information upon the location of the CO_2 -optimum.

This, he says, he found much less easy to investigate, as simple bubble-counting is not satisfactory in strong CO_2 -solutions. He therefore only gives details of three single serial experiments in which, starting with a constant light-position, say $1/4$ sunlight, the bubbling rate of a shoot of *Elodea* was observed in a succession of cylinders of water with different amounts of dissolved CO_2 , the plant staying 10 minutes in each medium.

Pantanelli gives (p. 194) the initial and the final timings for the liberation of 10 bubbles, during the 10 minutes stay in each concentration of CO_2 , for the most satisfactory of his experiments, but there is no curve. We have constructed one (fig. 9) from his data, selecting, in each strength

of CO_2 , the more rapid of his two rates, as sometimes the final rate is recorded as quicker than the initial.*

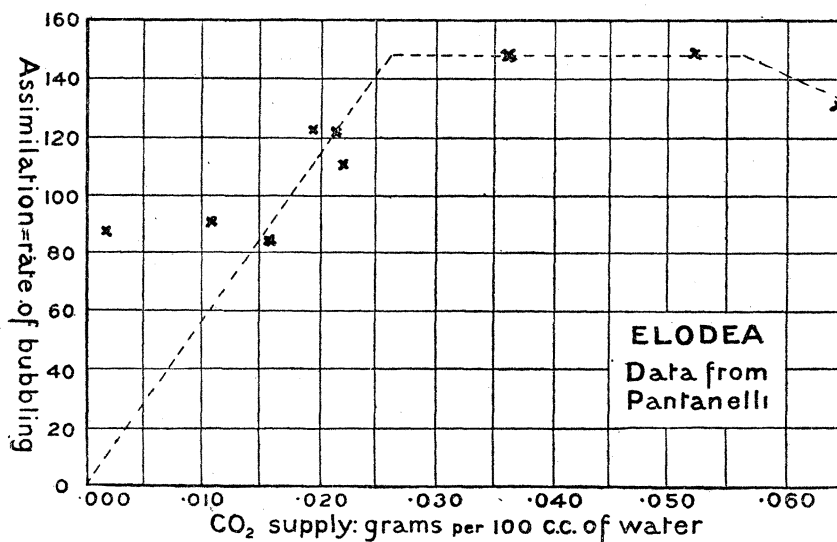


FIG. 9.

The data are not very regular, but it cannot be doubted that the diagram is of the same nature as our own fig. 1. Up to about 0.020 gm. CO_2 per 100 c.c. the assimilation increases proportionally with the CO_2 -supply; then a limit is set by the $1/4$ light-intensity, and the assimilation remains constant till about 0.06 gm. CO_2 . After this the narcotic effect of strong CO_2 seems to begin, as bubbling in 0.065 gm. CO_2 falls distinctly lower (this is just outside the range of our experiments in Section I). Further than this, as Pantanelli points out, the investigation cannot be pushed by bubble-counting alone as the physical liberation of CO_2 from the strong solution is so great.

In this series, with intensity of light = $1/4$, Pantanelli places the optimum at 0.02 gm. CO_2 (10 vols. CO_2 per cent.)†; in a similar series, with $1/1$ light, at 0.03 gm. CO_2 , and in the third series, with $4/1$ light, at 0.04 gm. CO_2 .

We trust that our detailed consideration of Pantanelli's work justifies itself

* The bubble rate in " CO_2 -free" water (= 0.002 gm. CO_2 per 100 c.c.) is impossibly high, but it is entered in the diagram; the rate in the strongest CO_2 (No. X) is obviously physical and erratic, and is omitted from the diagram.

† On p. 193 Pantanelli has an interesting footnote in which he treats with incredulity Treboux's location of the CO_2 -optimum in his particular experiments at so low a value as about 3 vols. CO_2 per cent. This is obviously explained by the fact that Treboux used such a weak light (see p. 404), that the plant was inevitably prevented from utilising much CO_2 .

by proving that there have been no facts brought in support of the conception of optima in connection with assimilation which are not more harmoniously interpreted on the theory of the interaction of limiting factors.

Section V.—CONCLUSIONS.

1. The experiments on assimilation in water-plants dealt with in this paper are carried out by a new method which takes account of the alteration of the gases in solution as well as of the gases liberated as bubbles.

2. The aim of this study is to demonstrate the *nature* of the relation between assimilation and the chief environmental factors; (1) CO₂-supply, (2) light-intensity, and (3) temperature. The relation is such that *the magnitude of this function in every combination of these factors is determined by one or other of them acting as a limiting factor.*

3. The identification of the particular limiting factor in any definite case is carried out by applying experimentally the following general principle. *When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the magnitude of the function.*

4. Two long series of experiments with *Elodea* and with *Fontinalis* have been carried out in which the light and temperature remained constant while the CO₂-supply varied through a wide range (from 0.0025 to 0.0540 grm. CO₂ per 100 c.c. of water).

In both series the same phenomena is observed; at first the assimilation increases steadily in proportion to the increase of the CO₂-supply and then abruptly this increase comes to a stop, at a value of about 0.023 grm. CO₂ assimilated per hour. This is shown to be the limit set by the particular intensity of light adopted and, without more light, no further increase of CO₂ will bring the assimilation above this level.

5. The form of curve obtained is a typical "compound limiting factor curve"; there is no sign of an optimum or of ultra-optimal depression of assimilation even when water one-third saturated with CO₂ is employed.

The first phase only of this curve exhibits the significant *primary relation* between CO₂-supply and assimilation, and it is clear that here the magnitude of assimilation varies in direct proportion to the CO₂-supply.

6. Incidentally it comes out that the Bryophyte, *Fontinalis*, is consistently less efficient in the intake of CO₂ than the submerged Phanerogam, *Elodea*. This is possibly correlated with the absence of any "internal atmosphere" in the former.

7. From the data obtained in this research there is constructed a diagram,

fig. 7, by which it is possible to foretell what value of assimilation in *Elodea* will be attained in any combination of medium magnitudes of the three factors of the environment. In this diagram, against the different values of assimilation as ordinates, are ranged three separate curves showing the degrees of CO₂-supply, temperature and illumination, which are respectively essential for the attainment of each value of assimilation. For any hypothetical combination of the factors, it follows, by the principle of limiting factors, that if the three functional values corresponding potentially to these be ascertained from the diagram, then the actual magnitude of assimilation attained with that combination of factors will always be *the smallest of the three potential values*.

8. The experiments in this paper deal with such moderate intensities of assimilation as may be fairly well maintained for several successive hours. With more intense assimilation the values soon fall off by the action of internal factors grouped at present as the *time-factor*. Experiments in which this additional factor has to be reckoned with will be considered in a later paper.

9. Section IV contains a critical account of the work of previous investigators who interpreted their results on the assumption that there was a *primary optimum* in the relation between assimilation and each external factor. The substantial work of Pantanelli (1903) led him to the conclusion that the position of the optimum for any one factor shifts with the magnitude of the other concurrent factors. This can only be regarded as a transitional point of view and from this we have advanced to the conclusion that *the whole conception of optima in this connection is inapplicable* and breaks down completely on careful analysis. It is shown in detail that all the experiments of previous workers are more harmoniously interpreted from the point of view of interacting limiting factors than by the conception of optima.
