Botany. — *Studies on Limiting Factors in Carbon Dioxide Assimilation.* By T. H. VAN DEN HONERT. (Communicated by Prof. F. A. F. C. WENT.)

(Communicated at the meeting of March 31, 1928).

The velocity, with which a green plant assimilates CO_2, depends upon three environmental factors, to wit : CO_2 concentration, light intensity and temperature. In a well-known article F. F. BLACKMAN (1905) has formulated this relation as follows :

"When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the "slowest" factor".

The graphical representation of this is the well-known curve : an ascending line, which represents the assimilation velocity as influenced by the increase of a factor *a*, which line suddenly and discontinuously becomes horizontal as soon as a velocity is reached, at which a second factor *b* becomes the limiting factor. The point at which this happens I will call the *transition point* (the point at which there is a transition of limitation by factor *a* to a limitation by factor *b*). We will therefore define the transition point as the point at which two factors exert the same influence on the velocity of the process.

Assuming BLACKMAN's formulation to be a true representation of the facts, we may arrive at three different combinations of properties characterising the assimilation process.

The velocity of the process may be :

1°. Directly proportional to CO_2 concentration, Independent of light intensity, ,, ,, temperature ($Q_{10} = 1$).	CO_2 "limiting".
2°. Directly proportional to light intensity, Independent of CO_2 concentration, ,, ,, temperature ($Q_{10} = 1$).	light intensity "limiting".
3°. Exponentially increasing with temperature ($Q_{10} = \pm 2$), Independent of CO_2 concentration, ,, ,, light intensity.	temperature "limiting".

Physical chemistry cannot account for these three combinations of properties as long as we consider the assimilation to be a *simple* process.

One may look in vain for a simple chemical reaction, which will change its characteristics in such a fundamental way by varying its dominating factors, such as temperature and concentration. The objections of BROWN

and HEISE (1917) against BLACKMAN's formulation are based upon similar considerations. The above mentioned objection disappears, when we consider the assimilation process as a catenary reaction, consisting of at least three distinct consecutive processes. For in a catenary process the velocity with which the final product is formed, is dominated by the *slowest* reaction. These reactions may posses entirely different individual properties, the velocity of the "total" process will have the properties of the reaction, which will be momentarily the slowest.

In this direction WILLSTÄTTER and STOLL (1918), BRIGGS (1920), THODAY (1922) and WARBURG (1920—1924) have been looking for the explanation of the phenomena.

BLACKMAN's formulation has not remained unattacked. Considerable discrepancies have been found by various workers ; the above mentioned discontinuous curve was found to be considerably rounded near the transition point. Various other formulations have been given to account for this experimental fact. (BOYSEN JENSSEN 1919, HARDER 1921, BENECKE 1921, LUNDEGÅRDH 1924.)

These workers used leaves or even entire plants in their experiments. The individual plastids of a single leaf, however, are in entirely different conditions. When the leaf is illuminated from above, the plastids near the upper surface will receive much more light than those near the lower surface.

Increase in light intensity will create a condition, at which the light is no longer a limiting factor for the plastids near the upper surface. Increasing the light intensity still further, the "transition point" will also be reached for the deeper layers, and finally for the lower surface of the leaf. We may therefore say that, even if BLACKMAN's formulation would hold for each individual plastid, one would still expect for the entire leaf a gradual transition from limitation by light to limitation by temperature or CO_2. The more the conditions of the plastids are different *inter se*, the more gradual this transition will be and the more the curve will be rounded.

Also the CO_2 concentration needs not to be the same for all plastids (SCHROEDER 1924). Moreover the capricious behaviour of the stomata materially hampers the experiments with CO_2 as limiting factor.

If we want to obtain a true picture, we will have to carry out our experiments in such a way, that *all plastids will be in the same condition as far as CO_2 and light are concerned*. We have recourse in the first place to unicellular organisms as WARBURG (1919) did, using in his experiments the unicellular green alga Chlorella.

Because of the very small dimensions of the cells, the CO_2 has only to diffuse over a very short distance to reach the plastid. In view of this fact, WARBURG is of the opinion, that the diffusion process will never hamper the velocity of the assimilation. We will discuss the validity of this assumption later in this paper.

WARBURG, in his experiments on the influence of light intensity, has taken the precaution to use very dilute cell suspensions. With this procedure

he approached the ideal condition of homogeneous environment for all cells.

In my opinion this ideal has not been reached entirely by him. Probably a part of the cells is shaded by the rest.

A second objection to WARBURG's on the whole brilliant method is his use of buffer mixtures in order to vary the CO_2 concentration. The cells have to be exposed to various acidities, which introduces a new complication.

In this paper a method is described in which these two objections are avoided.

The problem may be put as follows :

1°. Which is the relation in the individual plastid between assimilation velocity on one hand, CO_2 concentration, light intensity and temperature on the other hand ?

2°. May the assimilation be reduced to a catenary process ?

This is a preliminary account.

Shortly a more extensive account will appear (1928).

Methods.

a. The object.

As experimental object I selected a filamentous alga, belonging to the genus Hormidium, isolated from material growing on a garden wall. The alga is easily cultivated in flasks on purely inorganic media. In this case the bacterial development is minimal. The alga has the property to form shiny pellicles, one cell thick, floating on the liquid media. This property has been made use of in the following way.

The algal material, which has to serve for an experiment, is oculated upon the surface of a liquid medium, which is put into a receptacle. This receptacle has a bottom, formed by a glass plate. The walls are formed by an oblong ring of ebonite, a rubber ring serving as a water-tight seal between bottom and wall.

Before starting the experiment, the liquid is siphoned off for the largest part from below the pellicle of algae, which pellicle remains with very little liquid on the glass plate. This glass plate will serve, after removal of the ebonite ring, as the bottom of the assimilation vessel (fig. 1, 15).

b. Apparatus.

The technical requirements of the method are the following. In the assimilation vessel we must be able to vary or to keep constant CO_2 concentration, as well as light intensity and temperature individually and arbitrarily. The CO_2 concentration in the assimilation vessel has to be determined to .001 % accurate. This concentration has to be the same in all parts of the vessel. Production and consumption of CO_2 (respiration and assimilation) must be determined accurately.

The CO_2 determinations were carried out with a slightly modified KROGH's apparatus (1920), the modification mainly consisting in the size

of the gas sample which was 10 cc instead of 50 cc, which sample could be analysed with an accuracy of 1 cmm or .001 %. Only CO_2 determinations were carried out, the oxygen was not considered.

The experimental arrangement is half-schematically represented in fig. 1, the explanation of which is as follows.

The algae are placed in the assimilation vessel 15. Through this vessel

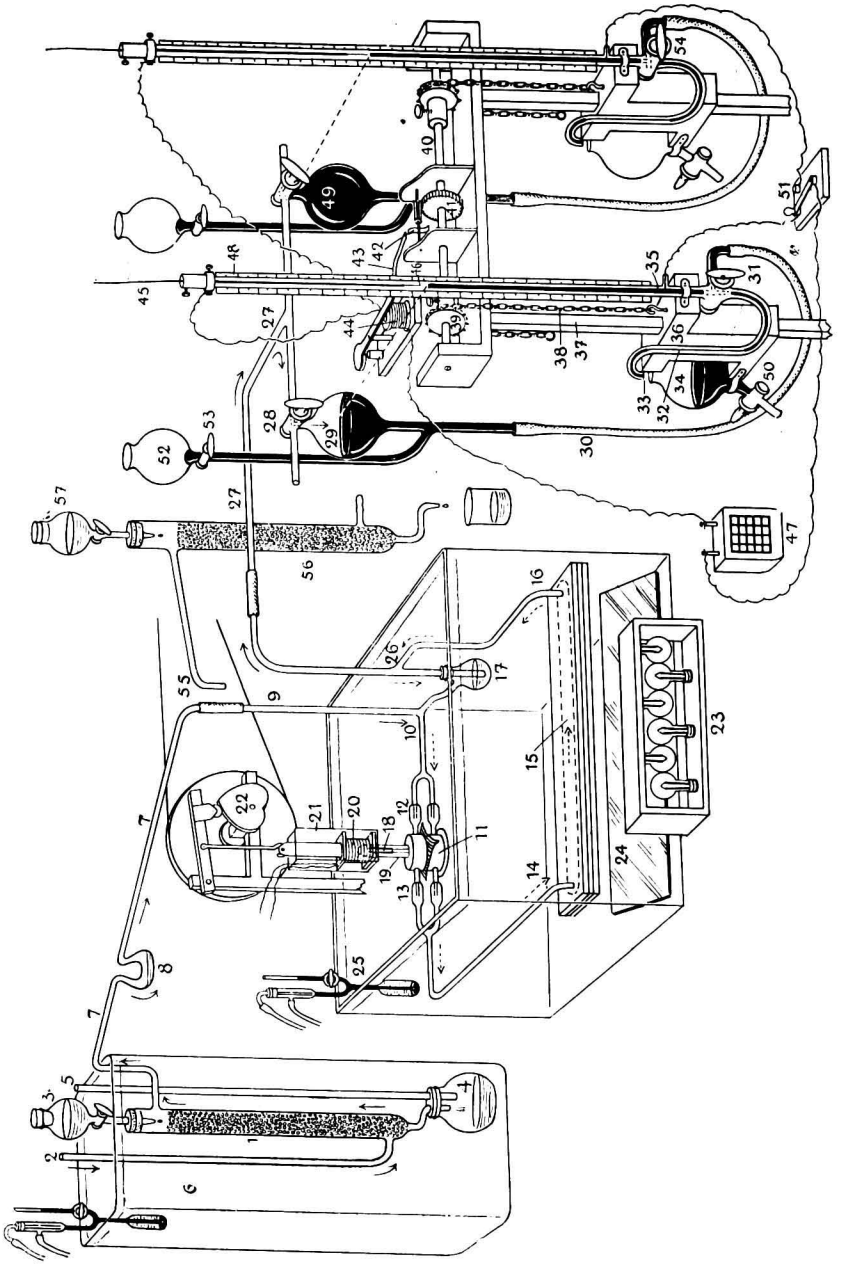

Fig. 1.

an air current passes. This air has passed through the CO_2 generator 1, in which it has been supplied with a constant, known amount of CO_2.

The air which leaves the assimilation vessel is collected above mercury in the aspirator 29. This mercury is running out of the aspirator with constant velocity. Air samples are taken from this aspirator and analysed.

If the above mentioned air current should enter the assimilation vessel at 14 and should leave it at 16, a considerable gradient in CO_2 concentration from 14 to 16 should exist; the concentration would not be the same in different parts of the vessel. For this reason circulation has been applied, the air being thoroughly mixed by means of a circulation current of much greater velocity than the supply current. By this means a minimal gradient of CO_2 concentration is obtained in the assimilation vessel.

The supply current as well as the discharge current are indicated in fig. 1 by drawn arrows, the circulation current is indicated by dotted arrows.

As it proved impossible to install the circulation apparatus inside of the assimilation vessel, it has been mounted outside the vessel as a small suction-pressure pump 11, which immediately drags along in a swift current all CO_2 containing air, which enters the system at 10.

After having entered, the CO_2 is partly used by the algae in 15, the remainder is carried off at 26. The air, which leaves the system at 26 has the same CO_2 concentration as the air in the assimilation vessel; the assimilation in cmm CO_2 per hour is computed from the difference in concentration of entering and discharged air.

The *generator* consists of a long glass tube 1, filled with small glass beads. An aqueous solution of Na_2CO_3 and $NaHCO_3$ (in definite proportion, together .5 grammol p. L.) drips from vessel 3 into the generator. This solution is discharged into vessel 4, which may be emptied from time to time by siphoning through tube 5. This complex is placed into thermostate 6, in which the water is kept very accurately at 30° C. by means of a microburner and a toluene regulator.

The air enters the generator by tube 2 and passes unhampered through the openings between the beads, by which the solution drips down, (principle of countercurrent). The air is in equilibrium with the solution when it leaves the generator.

For air, in equilibrium with such a solution of Na_2CO_3 and $NaHCO_3$, has at a certain temperature, a definite CO_2 concentration. For several mixtures of different proportion of both salts, this CO_2 concentration has been determined at 30° C. by a number of gasanalyses.

Na_2CO_3	grammol p.L.	0.325	0.259	0.182	0.126
$NaHCO_3$	,, p.L.	0.175	0.241	0.318	0.374
	CO_2 vol %	0.090	0.204	0.497	1.006

In the entrance tube 7 a small reservoir 8 has been blown, which contains a few drops of acidulated 25 % NaCl solution, in order to lower the

vapour tension of the air to prevent the formation of condensation water at other places of the system.

The *circulationpump* 11 consists of two small cylinders, between which a rubber membrane is clamped. At both sides of each cylinder there are small valves 12 and 13, which only admit the air in the direction right to left. The centre of the membrane is connected to a rod, which supports at its upper end a small piece of iron 18, enclosed within a small glass tube 19, which is sealed at the top.

The rotation of the cordate-shaped disc 22 causes the electric spool 20 to move upwards and downwards around tube 19. This movement induces a similar movement in the small piece of iron, which, transmitted to the membrane, causes the pump to function. The electric spool is protected from the water in the thermostate by the cover 21, which is shown transparent in the figure.

The electromagnetic device was necessitated by the great permeability of rubber for CO_2. A rubber membrane in one-side contact with atmospheric air or with water allows a considerable diffusion of CO_2, which would cause a loss of this gas by leakage.

In the pump, described above, the power transmission takes place through glass, which is permeable to magnetism but not permeable to CO_2. For the same reason the rubber connections of the glass tubes throughout the system were made such as to secure contact between the glass tubes.

The assimilation chamber 15 consists of two glass plates (bottom and lid), between which is pressed a rubber ring $\pm$ .8 mm. thick. The height of the assimilationchamber is therefore .8 mm.

The rubber ring was cut from the inner tube of a bicycle tire. The thin layer of algae rests on the bottom of the chamber.

The illumination (23) takes place by means of 1—6 lamps of 50 candlepower (Philips ½ Watt 6—8 volt automobile lights) which burn on a storage battery.

The voltage may be regulated by means of a variable resistance. In this way the light intensity is kept constant to about 1 %. For higher intensities I used a Philips projection lamp ($\pm$ 1650 candlepower) the intensity of which is obviously more variable, with the fluctuations in the city-current.

The light is projected perpendicularly from below upon the algae by means of a mirror; other light is screened off by means of black paper.

The assimilation chamber and the circulation apparatus are mounted together on a frame made of cupper, well plated with nickel. This frame can be put in the thermostate 25, in which the temperature can be changed arbitrarily by adding warm or cold water. Within 15 minutes the temperature may be made constant up to about .1° C. by means of a microburner and adjustable toluene regulator.

To the circulating apparatus belongs a small gas washing bottle 17, containing a small amount of distilled water. By observing the air bubbling

through it, the rate of the circulating current may be controlled. In this way the water vapour tension of the air is kept saturated and the dessiccation of the algae is prevented.

The air leaves the circulation apparatus at 26 and enters the aspirator through the tube 27.

The aspirator regulates the rate of the supply and discharge current by means of mercury, which is running out of the glassvessel 29. Through the entire system of tubes the air in this vessel is at 2 in free communication with the atmospheric air. The rate at which the air enters the vessel 29 is the same as the rate at which it is sucked into the generator at 2, enters the circulation apparatus at 10 and leaves it at 26.

The mercury has to be run out at a constant and adjustable velocity. This is attained in the following manner. The mercury flows successivily through the rubber tube 30, the three-way tap 31 and the capillary glass tube with a very narrow part 32, where the current meets a strong resistance. Finally it drops at 33 in the vessel 34, in which it is collected.

The rapidity of this mercury current is determined : 1° by the height of the mercury collumn above the opening 33, 2° by the constant resistance in 32.

When the mercury level in 29 is going down the rapidity of the current will diminish, unless the opening 33 is going down at the same rate. This is attained by the following automatical device. At the three-way tap 31 a capillary tube 35 is attached, forming communicating vessels with 29. So in 29 and 35 the mercury meniscus is at the same level.

The capillary tube 35, the tap 31, the resistance tube 32 and the receptacle 34 are mounted together on a log of wood 36, which can be moved along the bar 37 and is suspended by a chain 38. This chain is hanging on a cogwheel, that can be fixed with a screw on the spindle 40. By turning this spindle, the wing 42 is brought in a swift turning movement by means of a number of cogwheels 41 (only two of them are given in the figure). This wing (and the spindle with it) is stopped, when the lever 43 is pulled down. This occurs when a faint electric current passes the electromagnet 44.

In the capillary tube 35 a piece of iron-wire 45, provided with a tiny platinum needle 46, is put from above. If this needle comes into contact with the mercury, a current from the accumulator 47 passes the electromagnet 44, causing the wing 42 and the spindle 40 to be fixed. If subsequently the mercury level in 29 (and therefore also in 35) is going down, the contact with the platinum needle is broken, the wing is released and the log 36 begins to move slowly downward, causing a swift movement of the wing. When the platinum needle reaches the mercury again, the movement is stopped at once.

In this way the mercury level is kept at a constant height above the opening 33. This height is determined by the position of the platinum needle in the capillary tube 35, which can be read off on a scale 48. To

each position corresponds a certain height of the mercury column and therefore a certain rate of the current; for a number of positions throughout the scale the corresponding rate of the current has been tested empirically. So the rapidity of the mercury current can be regulated at will from 0 to 160 cc an hour, accurate to ½ % at the mean velocities.

At the beginning of an experiment the CO_2 content of the air, entering the aspirator 29, has not yet become constant. Not before a volume of air, four times as great as the content of the circulation system (this is about 17 cc), has been sucked through, an equilibrium has been established between supply, consumption and discharge of CO_2. The CO_2 content of the discharged air thus being constant, this air may now be used for analysis.

Therefore the current of air is conducted to a second aspirator 49, provided with the same arrangement as the former one and adjusted at the same velocity of mercury current, after some manipulations the air current may be diverted from 29 to 49 in a single moment, without interruption, by opening tap 54 and closing tap 31 at the same time.

The air in the aspirator 29 now may be discharged. Therefore the mercury is run out of tap 50 and poured into the receptacle 52. Subsequently tap 28 is opened to the left and by opening tap 53 the mercury runs back into aspirator 29, driving out the air.

When determining the respiration, I mostly used air containing no CO_2. For that purpose tube 9 is connected with tube 55 of generator 56; through this generator a 2 % KOH solution drips from vessel 57 along a number of small glass beads. From the air, passing between the beads in opposite direction, the CO_2 is absorbed.

The experiments.

By assimilation is meant the CO_2 consumption under illumination to which is added the CO_2 production in the dark.

In order to obtain comparable values under different conditions, the values obtained in the experiments have to be reduced to a certain unity of living material. Usually this is 1 gram of dry weight.

In my experiments, however, the quantities used were extremely small (1—3 m.gr.) and could not be weighed with sufficient accuracy. So I have chosen another unity, to wit the quantity which assimilates 100 cc CO_2 an hour at certain, well defined conditions. These conditions are : excess of CO_2, illumination by 6 lamps (light intensity 6.18) and temperature 20° C. At those conditions the temperature is limiting factor. By preliminary experiments it was shown that the CO_2 concentration was in excess (that means without influence on the velocity of assimilation) when more than .040 vol. % CO_2 was present.

The unity of material in question contains upwards of 40 m. of cell threads and is weighing about 1 m.gr. when dried at the air.

During the experiments, which often took a long time, the living material increased by growth. Small corrections were to be applied for it.

The influence of the light intensity. The intensity of each separate lamp was tested by using the algae themselves as a photometer. At the low intensity of one lamp the light is limiting factor and the assimilation is directly proportional to the light intensity. The intensity of one of the lamps was arbitrarily taken as unity and the intensities of the other lamps expressed in terms of this unity.

Fig. 2 is a graph representing the influence of the light intensity on the assimilation velocity at 20° C. and the CO_2 concentration in excess. The

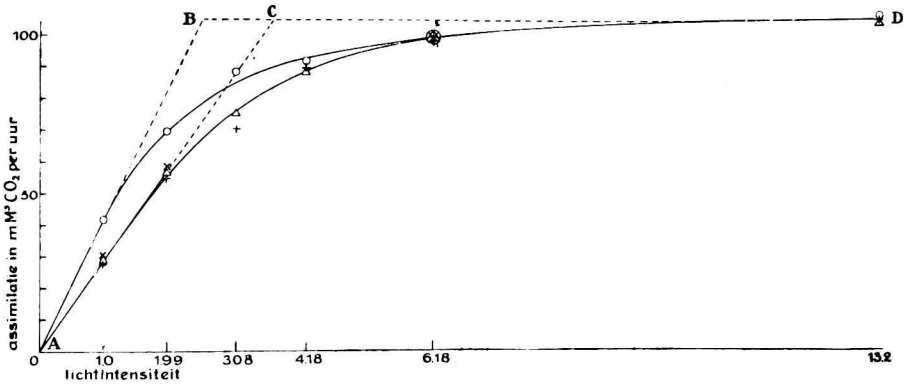

Fig. 2. The relation between the light intensity and the assimilation rate (cmm per hour).

marks +, △ and × are representing three experiments with algae grown upon a fresh culture medium. The marks O indicate an experiment with algae upon an old, exhausted medium. As the results are different in those two cases, the algae are to be cultivated in constant conditions in order to obtain comparable results. Therefore I took care to use only material on fresh culture media.

As fig. 2 shows, the maximal assimilation velocity at 20° C. is nearly reached, when illuminating with 6 lamps (light intensity 6.18), for doubling this intensity results in an increase of 5 % only.

The figure also shows that in normal cases the assimilation velocity is directly proportional to the light intensity up to 1.99 (for up to this intensity the curve is a straight line). Therefore at this intensity the light is still limiting factor.

The influence of the temperature. At a high intensity of light (6.18) and excess of CO_2 a Q_{10} of 1.87 was found between 12° and 20° C.

At a low intensity of light (1.0) and the same temperature interval a $Q_{10} = 0.90$ was found, not much differing from unity.

The influence of the CO_2 concentration. As has been pointed out, all values are reduced to a quantity of algae, which assimilates 100 cmm CO_2

an hour at a light intensity of 6.18 and a temperature of 20° C. As one may see at fig. 2 the same quantity assimilates 57 cmm CO_2 an hour at a light intensity of 1.99. The experiments about the influence of temperature showed, that at 12° C. and a light intensity of 6.18 this same quantity of algae assimilates 61 cmm CO_2 an hour, of course with CO_2 in excess.

These facts were used to calculate the experiments about the influence of the concentration of CO_2 (see fig. 3).

These experiments were started with an excess of CO_2, there-upon the CO_2 concentration was decreased till it became limiting factor, to establish its influence on the assimilation rate. This was done under three different conditions, viz.

1°. at 20° C., light intensity 6.18 (fig. 3, O)
2°. ,, 12° C., ,, 6.18 (fig. 3, +)
3°. ,, 20° C., ,, 1.99 (fig. 3, △)

In this way the influence of light and temperature was established in that part of the curve, where the CO_2 concentration is limiting factor. As may be seen in fig. 3, this part is a straight line nearly to the transition

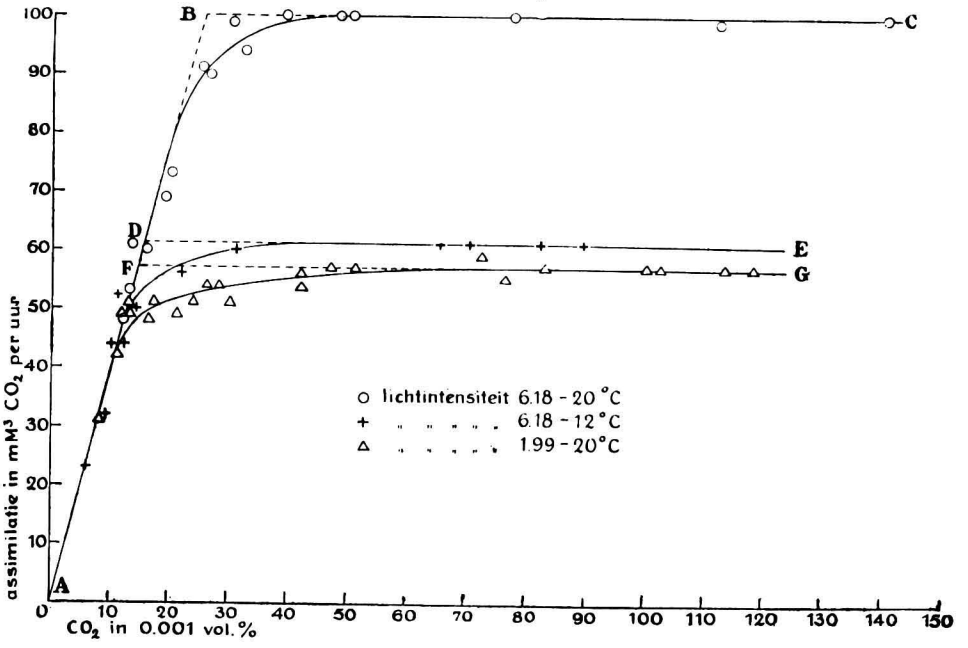

Fig. 3. The relation between the carbon dioxide concentration (expressed in units of .001 volume per cent of the air) and the assimilation rate (ccm per hour) at different conditions of temperature and light intensity.

point. In this part temperature and light intensity are obviously without any influence on the assimilation velocity (as far as the accuracy of the experiments allows to conclude); for here (on the line AF) the curves

for high and low light intensity as well as for high and low temperature coincide altogether.

Discussion of the Results.

Examining the results in connection to what was said in the first pages, it is clear that BLACKMAN's formulation gives a rather good description of the assimilatory behaviour of the single cell. In the figures 2 and 3 the "ideal BLACKMAN curves" are indicated by dotted lines; the greatest deviation shows fig 2, where it amounts 25 to 30 % (points *B* and *C*). I might ascribe it to the fact that, even in a single chloroplast, a considerable amount of light is absorbed. So the intensity of light is not the same at the different parts of the chloroplast. At decreasing intensity the light will therefore not become limiting at the same time in the different parts of the plastid. The same objection, which is valuable for leaves to such a great extent, seems not to be quite eliminated here.

This curve, however, agrees considerably better with BLACKMAN's scheme than the curve obtained by WARBURG (1919) with Chlorella. So I might conclude that in WARBURG's experiments a part of the algae is shadowed by the others.

The curves in fig. 3 show a nearly ideal BLACKMAN scheme. The deviations in the transition points *B, D* and *F* are upwards of 10 %. Probably those deviations are partly due to the different carbon dioxide supply in the different parts of the chloroplasts, causing an earlier CO_2 limitation in one part than in the other.

How are the above mentioned facts to be explained? Will it be possible to reduce the assimilation to a catenary process?

The answer upon these questions can be in the affirmative, when the assimilation is assumed to consist of: 1^0. a diffusion process, by which the CO_2 is transferred from the external medium into the chloroplast, 2^0. a photochemical process, 3^0. a dark chemical process.

Each of these consecutive processes may determine separately the rate of the assimilation, when 1^0. the concentration of CO_2, 2^0. the intensity of the light, 3^0. the temperature are limiting factor.

When this holds good, the three processes must possess the three combinations of properties, mentioned at the beginning of the article.

The velocity of a diffusion process is *ceteris paribus* directly proportional to the difference of the concentrations at the beginning and the end of the path along which diffusion is proceeding. When the CO_2 concentration is 0 at the end — inside the chloroplast — the diffusion velocity will be directly proportional to the concentration at the beginning of the path — outside the cell. Such a linear dependency shows the line *A F B D* in fig. 3.

Of course the light does not affect this process. However one should expect an influence of the temperature, as a Q_{10} of 1.2 to 1.3 is generally mentioned for diffusion processes.

On second thought this consideration is not correct. We may consider the cellwall and the protoplasm, through which the CO_2 is diffusing, as a water-like medium. Before entering the cell, the CO_2 has to dissolve into the outmost layer of the cellwall.

It is highly probable that in this outmost layer the CO_2 concentration is in equilibrium with that of the adjacent air. The solubility of CO_2 in water, however, decreases with increasing temperature. When this change of solubility is taken into account, from the apparent $Q_{10} = 1$ as found in my experiments a real $Q_{10} = 1.3$ can be deduced.

Still another argument may be mentioned. It was possible to measure approximately the total length of the cell threads used in some experiments, and to calculate the total surface available to the penetration of the CO_2. This surface was about 69 cm for the said unity of cell material. Since the velocity of the process and the difference of concentration are given, one may calculate, by means of the diffusion constant of CO_2 in water, the thickness of a water layer offering the same resistance to diffusion as the cells do.

This appears to be 8 μ. Now the cells have a thickness of about 8 μ and the chloroplasts are 4 μ thick at some places. Since it is highly probable that in the cell wall and the protoplasm the CO_2 has a smaller diffusion constant than in water, the accordance may be called satisfactory.

I do not agree with the opinion of WARBURG, who believes that in Chlorella the resistance to diffusion of CO_2 may be neglected, the more so as in WARBURG's experiments the CO_2 becomes limiting at about the same concentration as in mine.

As was argued herebefore, the diffusion velocity is directly proportional to the CO_2 concentration outside the cell, as long as the concentration inside the cell remains 0. Conversely when accepting that we have to do with a diffusion process, we can say that the CO_2 concentration inside of the cell remains 0, as long as there is a rectilinear dependence and that this concentration increases above 0, as soon as the rectilinear dependence ceases. Now fig. 3 shows that the deviation from the straight line *A B* begins only when the maximal assimilation velocity is nearly reached.

At a CO_2 pressure inside the cell, corresponding to .001 % CO_2 in the air, the assimilation is already functioning at nearly full speed. One is forced to accept that on the spot, where the reaction, subsequent to the diffusion proceeds, nearly a maximal amount of CO_2 is already available.

Obviously the assimilatory agent (either chlorophyll or an enzyme) has an enormous affinity to CO_2, being able to saturate itself almost quantitatively with CO_2, even at a pressure of .001 % or $\frac{1}{100000}$ atm.

The rate of the photochemical process, governing the assimilation velocity when the light is limiting factor, is directly proportional to the light intensity, according to the laws of photochemistry. With many photo-

chemical processes in vitro it has in common a $Q_{19} = 1$, as pointed out by several authors. The independence of the concentration of CO_2 needs no further explanation, after what has been said above about the assimilatory agent.

About the dark chemical reaction of the catenary process much has been written. Little is known, however, about its nature. The expressions "Blackman reaction" (WARBURG) and "enzymic factor" (WILLSTÄTTER and STOLL) refer to this reaction. It is a non-photochemical process, so the light does not affect it. It has a high, "chemical" temperature coefficient. As for the CO_2, this probably does not take part in it, but a reaction product of the photochemical process. Consequently it is independent of the CO_2 concentration.

As a summary we may say, that it is possible to study three parts of the assimilatory mechanism separately, without interfering with the organisation of the cell, only by altering the environmental factors.

Finally I determined the so-called *assimilation number* of Hormidium. WILLSTÄTTER and STOLL defined it as the amount of grams CO_2, assimilated per hour, divided by the amount of grams chlorophyll.

I determined the chlorophyll content of my algae spectrophotometrically, according to a method of WEIGERT (1916) by means of a "Color Analyser" of KEUFFEL & ESSER Co. This instrument was kindly given at my disposal by Prof. Dr. N. SCHOORL. As an average of five determinations I obtained an assimilation number of 6.75 at 20° C. and a lightintensity of 6.18.

The above-said unity of cell-material contained on an average .0271 m.g. of chlorophyll.

LITERATURE.

1. W. BENECKE: Beiträge zum Problem der Kohlensäureassimilation. Zeitschr. f. Bot. **13**, p. 417. 1921.

2. F. F. BLACKMAN: Optima and Limiting Factors. Annals of Botany **19**, p. 281. 1905.

3. P. BOYSEN JENSEN: Studies on the Production of Matter in Light- and Shadow-Plants. Botanisk Tidsskr. **36**, p. 219. 1919.

4. G. E. BRIGGS: The Development of Photosynthetic Activity during Germination. Proc. Roy. Soc. London **91**, B, p. 249. 1920.

5. W. H. BROWN and G. W. HEISE: The application of Photochemical Temperature Coefficients to the Velocity of Carbon Dioxide Assimilation. Philippine Journ. of Sc. C. Bot. **12**, p. 1. 1917.

6. R. HARDER: Kritische Versuche zu BLACKMAN's Theorie der begrenzenden Factoren bei der Kohlensäureassimilation. Jahrb. f. wiss. Bot. **60**, p. 531. 1921.

7. T. H. VAN DEN HONERT: Koolzuurassimilatie en Beperkende Factoren. Doctor's Thesis, Utrecht, 1928.

8. AUG. KROGH: A Gasanalysis Apparatus accurate to 0.001 % etc. Bioch. Journ. **14**. 1920.

9. H. LUNDEGÅRDH: Der Kreislauf der Kohlensäure in der Natur. Jena, 1924.

10. H. SCHROEDER: Die Kohlendioxydversorgung der Chloroplasten. Flora **117**, N.F. 17 p. 270. 1924.

11. D. THODAY: Carbon Assimilation, South African Journ. of Sc. **19**, p. 52. 1922.

12. O. WARBURG: Ueber die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen I. Bioch. Zeitschr. **100**, p. 230. 1919.

13. O. WARBURG: Ueber die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen II. Bioch. Zeitschr. **103**, p. 188. 1920.

14. O. WARBURG: Theorie der Kohlensäureassimilation. Naturwissenschaften **9**, p. 354. 1921.

15. O. WARBURG und T. UYESUGI: Ueber die BLACKMANsche Reaction. Bioch. Zeitschr. **146** p. 486. 1924.

16. F. WEIGERT: Ueber Absorptionsspectren und über eine einfache Methode zu ihrer quantitativen Bestimmung. Ber. d. d. Chem. Ges. **49**, I, p. 1497. 1916.

17. R. WILLSTÄTTER und A. STOLL: Untersuchungen über die Assimilation der Kohlensäure. Berlin, 1918.

Utrecht. March 1928. *Botanical Laboratory.*