

CARBON DIOXIDE ASSIMILATION AND LIMITING FACTORS

BY

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CHAPTER I.

INTRODUCTION.

The velocity, with which a green plant assimilates CO_2 , depends primarily on three environmental factors, to wit: the concentration of the CO_2 , the intensity of the light and the temperature.

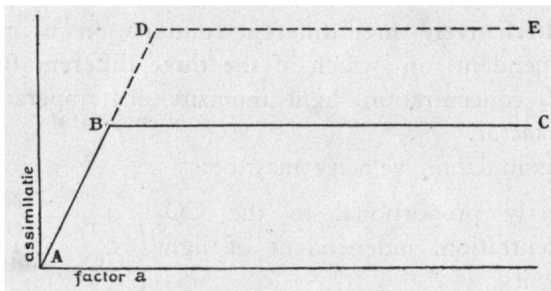


Fig. 1.

F. F. Blackman (1905) formulated this relationship 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”.

This formulation can be represented by the well known graph, to be found in all textbooks (fig. 1). I might call it *the ideal Blackman curve*. The ascending line AB repre-

sents the increasing assimilation velocity caused by the increase of factor a ; at B further increase is impossible, because a second factor b now becomes limiting. The curve from this point runs parallel to the abscissa (BC). If a larger amount of factor b had been present, the assimilation velocity would have increased up to D, before the factor b became the limiting one.

The point, at which there is a *transition* from the limitation by one factor to the limitation by another factor, I will call the *transition point*. It may be defined as *the point at which two factors exert an equal influence on the assimilation velocity*.

Concerning the validity of Blackman's formula opinions are still divergent. I do not want to discuss this question here, but for the present I will merely assume Blackman's formula to be a true description of the facts. The consequences to which this leads may now be pointed out.

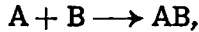
The first consequence is that the assimilation process shows alternatively three different combinations of properties, dependent on which of the three different factors: viz. CO_2 concentration, light intensity or temperature is limiting factor.

The assimilation velocity may be:

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

When temperature is the limiting factor, the so called *time factor* introduces a new complication. At higher temperatures the assimilation rate is no longer constant but decreases gradually. The higher the temperature the more rapid the decrease. This time factor, however, will not be considered here, since it does not occur at moderate temperatures.

Comparing the assimilation process with a simple chemical process *in vitro*, for instance the reaction:



we observe immediately an important difference. This process too is governed by three different factors, viz. the concentration of A, the concentration of B and the temperature. But these factors determine the reaction velocity not alternatively, but simultaneously.

The reaction velocity will be:

Directly proportional to the concentration of A,	}
Directly proportional to the concentration of B,	
exponentially increasing with the temperature ($Q_{10} = 2-3$).	

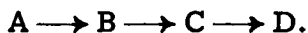
The reaction retains this combination of properties under any condition.

One may look in vain for a simple chemical process, which changes its properties in such a fundamental way, when altering the factors governing its velocity. So we can not hope for an explanation from this direction.

Things become different, if the assimilation is compared to a chain reaction, a process consisting of several subsequent reactions. Catenary processes are subject to the general rule, that the velocity with which the final product is formed is determined only by the rate of the *slowest* reaction of the chain.

For instance, it may be assumed that a substance A is

transformed into a substance B, this substance again into a substance C and this again into a substance D. Then in the catenary reaction



each subsequent reaction will have as starting material the final product of the foregoing reaction and can not proceed any faster than the rate at which this material is supplied. So a subsequent reaction never can have a greater velocity than the foregoing one.

But also a foregoing reaction can be hampered by the following reaction, as many reactions are retarded by the accumulation of their reaction products.

So it is quite comprehensible that in a reaction chain the slowest process determines the velocity of the whole. Physical processes, such as solution and diffusion, also follow this rule. Should it be possible to reduce the assimilation to a catenary process, this process would have to consist of at least three different reactions to which the three different combinations of properties previously enumerated would have to be applicable.

The three processes to be considered are:

1. a *diffusion process* by which the CO_2 travels from the external medium into the chloroplast;
2. a *photochemical process* by which the radiant energy is converted into chemical energy with the aid of the chlorophyll;
3. a *chemical process* in which light does not take part.

The first process might be the slowest if CO_2 were limiting, the second if this were the case with light and the third with temperature.

In accepting the above assumptions as a working hypothesis I will be obliged to show that the three combinations of properties, as found by Blackman, actually apply to these three processes. That this is possible may be argued as follows.

1. *The diffusion process.*

When a substance is traveling by means of diffusion over a certain path, the diffusion velocity is *ceteris paribus* directly proportional to the difference of concentrations of this substance at the beginning and the end of the path.

When the concentration at the end is 0, the velocity is therefore directly proportional to the concentration at the beginning. So when the partial pressure of CO_2 within the plastid is 0 (*i.e.* when all CO_2 is used immediately upon arrival there), the direct proportionality to the CO_2 concentration of the external medium is explained.

Light, of course, does not affect the velocity of a diffusion process. Temperature, on the contrary, has a decided influence on diffusion processes, much less, however, than on chemical processes. A $Q_{10} = 1.2$ to 1.3 is generally mentioned. This is therefore higher than a $Q_{10} = 1$, which should be expected according to Blackman's scheme. I will show in Chapter VI that in certain cases this apparent controversy is easily explained.

Besides I want to mention the difficulties of experiments with higher plants with CO_2 as the limiting factor, as the transverse section of the diffusion path is very inconstant because of the capricious behaviour of the stomata.

2. *The photochemical process.*

According to the laws of photochemistry the velocity of a photochemical reaction is directly proportional to the amount of radiant energy absorbed (Law of Grotthus—van 't Hoff, see Plotnikow 1920). Notwithstanding the fact that the distribution of light intensity in the leaf is very complicated, the absorption coefficient may be considered a constant (Ursprung, 1918 *b*). So in all parts of the leaf the light intensity will be directly proportional to the intensity of incident light. As long as the photochemical process is the "slowest" in all parts of the leaf, the total assimilation

velocity will vary in direct proportion to the intensity of the incident light.

According to Blackman's scheme the concentration of CO_2 does not affect the velocity of the photochemical reaction after a certain limit of CO_2 concentration has been passed. Granted, that CO_2 takes part in this reaction, the explanation of this fact is not quite self-evident. One is inclined to believe that on the spot, where the photochemical process takes place, the concentration of CO_2 has then reached its maximum and can not be increased any further by increasing the CO_2 concentration of the medium. So the assimilating agent, probably the chlorophyll, seems to have a high affinity for CO_2 , being able to completely saturate itself even at very low CO_2 concentrations.

Supposing, as Willstätter (1918) does, that the carbon dioxide combines with chlorophyll to form a dissociable compound:

carbon dioxide + chlorophyll \rightleftharpoons carbon dioxide-chlorophyll and that the photochemical reaction proceeds in this carbon dioxide-chlorophyll molecule, we may say that, at a certain light intensity, the reaction velocity will be determined by the amount of carbon dioxide-chlorophyll present.

When practically all chlorophyll is combined with carbon dioxide, it is obvious that the reaction velocity has reached its maximum at a given intensity of light. If there exists a very high affinity of chlorophyll towards carbon dioxide, this will occur at a very low carbon dioxide concentration. A further increase of this concentration will no longer result in an increase of the photochemical reaction velocity.

It is not surprising that temperature does not influence this process, as a large category of photochemical reactions show a Q_{10} of ± 1 (Plotnikow 1920, p. 62). So, if the photochemical phase of carbon dioxide assimilation belongs to this category, it is no exception to the rule.

3e. *The dark chemical process.*

In my assumption this process succeeds the photochemical one and uses up its reaction products. So the CO_2 probably does not take part in it; nor does light, as the process is a non-photochemical one.

Like every dark chemical reaction this process has a high Q_{10} . Indeed the majority of chemical reactions have according to Van 't Hoff's rule a Q_{10} varying between 2 and 3.

In what has been said above I have put forward a working hypothesis reducing the photosynthesis to a catenary process, based exclusively on Blackman's principle of limiting factors. A quantitative accordance with Blackman's formula was arrived at, except that a slight influence of temperature is to be expected when CO_2 is the limiting factor.

I do not mean to say that such a hypothesis has not already been put forward by others. On the contrary, all ideas given here have already been expressed by other authors.

Nevertheless, I wanted to give first of all this line of thought without citing further literature and to take Blackman's "limiting factors" as the only point of departure, first because it has served as a working hypothesis for my experiments, second to arrive at as sharply defined a circumscription of the problem as possible.

Now I may try, with the aid of the literature to arrive at an answer to the following questions:

1. Is my point of departure a reliable one?
 2. Are there other arguments to be found, confirming the conception that carbon dioxide assimilation is a catenary process?
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CHAPTER II.

DISCUSSION OF THE LITERATURE.

The Validity of Blackman's formula.

After the principle of limiting factors in carbon assimilation had been put forward by Blackman in 1905 and after it had been propagated by him and his co-workers¹⁾ it found a ready acceptance and gained a great influence in botany and elsewhere.

Its special aim was to contradict the conception, common in those days, that almost every physiological process could be represented by an optimum curve.

It is often called a theory but incorrectly so. Only Blackman's explanation of the temperature optimum by means of the combination of an ascending and a descending curve may be called a theory. For the rest, I only consider it as a circumlocution of the facts, which circumlocution may or may not be correct.

Is it correct? Several authors say it is not. A severe attack was made by Brown and Heise (1917 a and b, Brown 1918). They made the error of considering the assimilation process to be a simple photochemical process. They tried to prove the consequences, drawn from this conception, with the aid of data available in the literature. For instance, they say that at high light intensities the Q_{10} must be the same as at low intensities: "A photochemical reaction, with such intensities of light, would still be photochemical and show photochemical coefficients".

They suppose Blackman and his co-workers to have been guided in their experiments by a prejudicial idea. They insist that several factors must in reality have a simultaneous influence; also that the process may be represented by an optimum curve.

¹⁾ See: Blackman and Matthaei (1905), Matthaei (1904), Blackman and Smith (1911 a and b).

Smith (1919) in replying to these statements in Blackman's defense rightly remarks that the Brown-Heise conception of photosynthesis is far too simple. He alludes to the possibility, that photosynthesis consists of a chain of processes, in which dark, non-photochemical reactions also enter into play. He does not, however, go any further than alluding to this possibility.

In one respect, however, Smith has to agree with Brown and Heise, namely that at the transition point the assimilation curve has no sharp break, but is rounded. Near this point "the one (factor) not limiting may have some appreciable effect". Here he admits that Blackman's formula does not entirely fit the facts.

Boysen Jensen (1919) puts forward this same objection, based upon his own researches. For, with increasing light intensities, he finds a gradually bending assimilation curve. He is in favour of a modification of Blackman's principle in the same sense as in agricultural science Liebig's law of the minimum has been modified by Mitscherlich. Mitscherlich says namely that the crop production increases with an increase in one certain nutritive substance, but not directly proportionally with it; the amount of increase diminishes as the point, where the factor mentioned is no longer limiting, is approached.

Benecke (1921), drawing conclusions from his experiments on starch formation in *Elodea*, comes to the same result. He accepts the nomenclature of Mitscherlich: a factor may be in *absolute minimum* (= 0) or in *relative minimum* (= more limiting than any other factor). Never is only one factor limiting (except when it is in absolute minimum), always other factors exert a certain influence also.

Harder (1921) draws the same conclusions from his experiments on evolution of oxygen by assimilating water plants. His curves deviate even more from Blackman's scheme than do those of the above mentioned authors.

Lundegårdh also finds similar deviations from this scheme. He does not consider it, however, as a modification, but as an entirely new principle, called by him "Das Relativitätsgesetz der Assimilationsfactoren" (1924 a). In short it runs as follows: "Die relative Wirkung eines Factors nimmt mit seiner absoluten Intensität und zwar vielfach nach einer logarithmischen Kurve ab" (1927).

Moreover, in his later publications (1926, 1927) he records a very remarkable influence of temperature, differing so much from what is published by all other authors, that it seems worth while to discuss it briefly.

He finds namely, besides the above mentioned general shape of the assimilation curve, several definite optima and minima, which are superimposed upon it. Some of his co-workers obtain similar results using the same methods (Walther 1929, Stocker 1927). Should Lundegårdh's view be correct, the prevailing conceptions would have to be entirely altered. However, in Lundegårdh's methods there is much which may render the results doubtful.

The following objections may be raised.

1. All experiments lasted a very short time, often only a few minutes and were started immediately after the beginning of illumination. As a leaf is able to contain a considerable amount of CO_2 (Willstätter and Stoll 1918), first the CO_2 present in the leaf will be used before intake from the air occurs. No doubt the intake of CO_2 will not be constant in those first minutes.

2. At the higher CO_2 concentrations the assimilation was determined volumetrically by means of a gas analysis apparatus. In this apparatus, according to the description, no constant water vapour tension of the air sample has been procured, whereas in other such instruments (like those of Haldane and Krogh) great care is taken in this respect. When for instance a sample of air, saturated with water vapour is taken into the apparatus at 20°C . and

comes afterwards in contact with a 30 per cent. KOH solution, the water vapour tension may decrease from 17 mm. to 12 mm. mercury. This would mean a decrease in volume of ± 0.6 per cent. Nevertheless Lundegårdh claims that with this apparatus the mean error is only 0.003 per cent.

3. In some of the experiments (1924 b) the CO₂ containing air to be analysed was expelled from the assimilation vessel by means of *water*. In this water CO₂ may dissolve to a considerable extent.

Also to collect the air above glycerine (1927) would not appear to be a reliable method.

4. Each time a new leaf was used for the determinations. The assimilation was computed in terms of surface units. It would seem that individual differences between the leaves may not be excluded.

It seems to me that the above objections may give cause for some doubt regarding the fundamental value of the optima and minima, as found by Lundegårdh.

Warburg (1919), in his experiments with the unicellular alga *Chlorella* finds also an almost logarithmic curve for the assimilation as related to CO₂ concentration or light intensity.

Romell (1926) attacks on purely hypothetical considerations the principle of limiting factors. In the last Chapter I will return to this point.

Summarising, Blackman's rule seems to hold only approximately, but not perfectly. Near the transition point there seem to be great deviations causing a considerably rounding of the curve.

All authors except Warburg experimented with leaves or even with entire water plants. Now a leaf in itself is already a very complicated system. The individual plastids in it are submitted to very different conditions. When illuminating a leaf from above, the plastids at the upper surface receive many times the light received by those at

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

This may explain to a great extent the deviations from Blackman's scheme. The carbon dioxide supply also needs not to be the same for all chloroplasts, this being probably a second source of deviations. These two circumstances are pointed out clearly by Schroeder (1924).

Is carbon dioxide assimilation a catenary process?

This idea is not new, as already pointed out. Theories, like those of Willstätter and Stoll, Warburg and Briggs, are based on this hypothesis. There are a number of arguments which favour it. For the sake of clearness I will divide them into four groups.

1. Blackman's principle of limiting factors.
2. Phenomena of assimilatory inhibition and "Ermüdung".
3. Disproportionality of chlorophyll content and assimilation power.
4. Effect of chemical substances on assimilation.

ad. 1. In the introduction I made an effort to deduce in a logical manner from Blackman's formula the hypothesis

that assimilation is a catenary process. Arguments of the same sort are brought forward by Willstätter and Stoll and Briggs, who differentiate between a photochemical and a dark chemical process. A more detailed, though more hypothetical elaboration is made by Warburg. This author is also of the opinion that the assimilation velocity in the leaf is determined by a diffusion process, when the CO_2 is the limiting factor.

What I wrote on pag 150 about simple chemical reactions *in vitro* has been expressed almost in the same way by Thoday (1922). He says:

“Clearly the principle of limiting factors is not applicable to simple direct reactions of this type, between substances in solution. That it does hold for assimilation is in itself a demonstration of the complexity of the process. It is at least probable that to each of the external limiting factors corresponds a distinct stage of the process which is dependent upon it. The rate of the process as a whole is in reality determined by the rate of the slowest contributory stage, as pointed out by Briggs”.

Spoehr (1926) also remarks: “The principle of limiting factors has its analogon in the step-reactions of chemistry” (l.c. p. 97). Like me, he considers Blackman’s principle as a formulation: “This theory and its modifications, however, tell us little of the kinetics of the photosynthetic process; they are the first attempt at an accurate expression of the relation of this process to the various factors which influence it”. (l.c. p. 139).

Ad. 2. The best known instances of *assimilatory inhibition* and “*Ermüdung*” phenomena are the researches of Ewart and Pantanelli.

Ewart (1896, 1897, 1898 a and b) exposed green parts of plants to various deleterious agents, such as: high temperature (under humid and dry conditions), cold, dessic-

cation, partial asphyxiation, narcotics, acids, alkalies, accumulation of carbohydrates in the plant, strong plasmolytics, strong sunlight, long absence of carbon dioxide.

In nearly all these cases he found, by means of Engelmann's bacteria method a reversible checking of the assimilatory process. When the plants were returned to normal conditions, the power of assimilation gradually returned. Generally no change was observed in the green colour.

This phenomenon was called by Ewart: "assimilatory inhibition". He concluded that, though chlorophyll is indispensable to assimilation, "equally important factors of probably plasmatic origin also enter into play" (1896 pag. 457).

Pantanelli (1904), like Ewart working in Pfeffer's laboratory, studied the assimilation of water plants by means of the air bubble method. At high light intensities (1—64 times the intensity of direct sunlight) he observed a decrease in assimilatory power, which recovered to a considerable extent at lower light intensities. This phenomenon he called "Ermüdung". He considered it not to be explained by decomposition of the chlorophyll, as this substance will not show as rapid a recovery: "In der That sind die Ermüdungserscheinungen gewiss auf protoplasmatische Reactionen zurückzuführen" (l.c. pag. 188).

The experiments of Engelmann (1882—1884), Timiriaseff (1903), Ursprung (1917, 1918a) and others have demonstrated that only the light, which is absorbed by the chlorophyll, takes part in the assimilation process, that is to say, in the photochemical process, since there is a close relationship between absorption and assimilation at the different parts of the spectrum. So the chlorophyll may be considered to govern the photochemical reaction, to be the photochemical agent. When it is possible, according to Ewart and Pantanelli, to cause a decrease of the assimilation power without affecting the chlorophyll, this decrease

appears to concern another agent, governing a non-photo-chemical process.

Blackman's so-called *time factor* is obviously nothing more than a special case of inhibition: at high temperatures the velocity of the dark chemical process decreases. Blackman assumes it to be a decomposition of an enzyme by the high temperature. So this enzyme is to be considered as the dark chemical agent.

The phenomenon called "*Solarisation*" by Ursprung is perhaps the same thing. The starch production in leaves first increases with increasing light intensities, but is stopped partly or totally at high intensities, while the discharge of starch seems to go on. This phenomenon is also reversible. But, on the other hand, starch formation is not to be identified with the absorption of CO_2 or the evolution of O_2 . Moreover Schroeder and Horn (1922, Trude Horn 1923) suppose "*Solarisation*" to be an inversion of the starch caused by dessiccation.

Ad 3. Concerning the *ratio of the chlorophyll content and the assimilation power* Ewart (1896) already made some experiments. He stated that the green colour is first developed and only afterwards the assimilation power (Willstätter expresses some doubt as to the validity of his observations).

Miss Irving (1910) started work on this problem anew. According to her experiments when illuminating etiolated seedlings of barley and bean the green color is first developed. Only when the leaves are grass-green does the assimilation power begin to develop, which thereafter increases rapidly. She concludes: "It must be some other component part of the photosynthetic machinery, which controls the beginning of complete functional activity".

Willstätter, with his extraordinary knowledge about chlorophyll, was able to devise a colorimetric method to determine chlorophyll quantitatively.

Using this method he and Stoll (1918) investigated the connection between chlorophyll content and assimilation power in leaves of many different plants. The experimental conditions generally were such, that the temperature was the limiting factor, viz. 25° C., a very high light intensity (48000 Lux) and a great excess of CO₂ (± 5 per cent).

The amount of CO₂ in grams assimilated per hour, divided by the chlorophyll content in grams was called by them the *assimilation number* (Assimilationszahl). This number represents a measure of the ratio of the content of dark chemical agent to the content of photochemical agent (chlorophyll). For in their experiments the dark chemical process governed the assimilation rate.

Great differences in assimilation numbers were found by them. On the whole those numbers varied only from 5 to 8 in ordinary leaves, being somewhat higher in leaves with a strongly developed assimilation power (for *Helianthus* 14.0-16.7). But yellow varieties, poor in chlorophyll, exhibited very much higher assimilation numbers (50—120) up to 20 times higher than the green forms of the same species.

This led them to conclude that there must exist a chemical agent, not identical with chlorophyll, but something apart. They called it "enzymic factor".

The theory of assimilation, put forward by them, is briefly as follows:

The carbonic acid, not as CO₂ but in the form of H₂CO₃ enters into combination with the Mg atom of the chlorophyll molecule. In fact, Willstätter and Stoll were able to demonstrate the combination of carbonic acid with chlorophyll in a colloidal chlorophyll solution.

Now the photochemical process consists of the absorption of radiant energy by the carbonic acid-chlorophyll compound, whereby it changes into a form with higher energy content, viz. formaldehyde peroxide.

The chemical process is the splitting off of oxygen by a catalase-like enzyme, causing the formation of formaldehyde. As they found always an assimilation quotient 1, they drew the conclusion that the reduction-level of formaldehyde is reached immediately and that no intermediant reaction product is accumulated.

The formaldehyde formed is subsequently split off from the chlorophyll and is polymerised to sugars.

After this little digression on Willstätter's theory, a few words are to be said about the assimilation numbers which he finds in etiolated leaves.

As contrasted with Miss Irving's results Willstätter finds at the first appearance of the chlorophyll very high assimilation numbers. This would prove the "enzymic factor" to be already present before the appearance of the chlorophyll, while Miss Irving finds exactly the reverse. This contradiction is explained afterwards in a satisfactory way by Briggs (1920). He used Blackman's palladium black method, by which it is possible to keep a constant chlorophyll content during the experiments. Het stated that in seedlings of *Phaseolus vulgaris* there exists a factor controlling the assimilation rate, this factor *not* being chlorophyll. It appears the 8th day after germination, independent of the chlorophyll content and reaches its maximum within a few days. It was called by Briggs the *internal factor*.

If the seedlings are etiolated during more than 8 days, the "internal factor" is already abundant and high assimilation numbers are found. If they are etiolated during a shorter period only, the "internal factor" is not developed as yet, resulting in an absence of assimilation power.

Willstätter seems to have etiolated his plants longer than Miss Irving did. This explains the contradiction.

Briggs also considers the assimilation to be a chain of consecutive processes. His former opinion that the "internal factor" must belong to a photochemical stage, being some-

thing quite other than Willstätter's enzymic factor, is afterwards retracted by him (1923 a). He now also believes the "internal factor" to belong to a dark chemical stage,

It appeared to him that two types may be distinguished among seedlings (1923 b). In the first type the assimilation power is present as soon as the chlorophyll appears (*Helianthus* type); in the second type this is not the case (*Phaseolus* type).

Ad 4. The greater part of the researches concerning *the influence of chemical substances on assimilation* need not be mentioned here. A summary may be found in Stiles monograph (1925, p. 119).

In this connection, however, Warburg's publications are of great interest (1919, 1920). Warburg made his experiments with suspensions of the unicellular green alga *Chlorella*. One of the advantages of this material is its small dimensions, resulting in an easy penetration of CO_2 and other substances into the chloroplast.

Warburg, too, considers the assimilation to be a catenary process; in his opinion it is possible to study the photochemical and dark chemical stage separately, by causing light or temperature respectively to be the limiting factor.

He made experiments on the influence of different chemical compounds, viz. urethanes and hydrocyanic acid, on the velocity of the two above named processes.

Urethanes are surface-active substances, having the property of decreasing the surface tension of water when dissolved in it. This surface action is accompanied by an accumulation of the substance in the surface boundary, which may displace more or less the normal constituents of this surface.

Now many biochemical reactions, if not all, are heterogeneous reactions; the substances are reacting in a surface. A surface-active substance is able to displace them by block-

ing the surface, as it were, causing a decrease of the reaction velocity. So urethanes are to be considered as a "test" for surface reactions.

Warburg remarks that hydrocyanic acid has a great affinity for heavy metals. A reaction, which is catalytically accelerated by a heavy metal, will be checked by hydrocyanic acid, since the catalyser is blocked by it. Therefore hydrocyanic acid is considered by Warburg to be a test for heavy metal catalysis.

Warburg found that the assimilation velocity is materially decreased by urethanes in very low concentrations, both when light or when carbon dioxide are limiting factors. In the series of homologues of the urethanes he found an increased effect with increasing molecular weight, which is coincident with increasing surface-activity.

Hydrocyanic acid, on the contrary, has an effect only when temperature is limiting, not when light is limiting.

The different behaviour in this respect is a new indication of the existence of two different processes, controlling the assimilation velocity in these two cases.

Before 1924 Warburg's idea concerning the state of affairs was somewhat as follows:

1. By the intake of light energy the chlorophyll is changed into a substance with a higher energy content; it becomes the "photochemical primary product." This is the real photochemical process.

2. By a dark chemical process the CO_2 is changed into a substance which is able to take from the "photochemical primary product" the chemical energy contained in it. So the CO_2 has become an "acceptor" of this energy. The reaction by which this "acceptor" is formed he calls *Blackman reaction*. This reaction determines the assimilation rate when temperature is the limiting factor. It takes place in a surface boundary, is catalysed by a heavy metal (probably

iron) and therefore is checked by urethanes as well by hydrocyanic acid.

3. The acceptor enters into a reaction with the "photochemical primary product" and undergoes a reduction. This reduction of the acceptor determines the assimilation velocity when light is the limiting factor. It takes place in a surface boundary and is checked by urethanes but not by hydrocyanic acid. When treated with surface-acting substances the cells absorb the light energy in a normal way; however, a smaller part of it is transferred to the acceptor, as this substance is partly displaced from the surface.

For the arguments I refer to Warburg's original publications (1919, 1920, 1921). I mention this theory in connection with Willstätter's, only to call attention to the fact that we have here two well-considered theories, based on physiological facts, which differ in the following respect:

In Willstätter's theory the carbon dioxide undergoes *first* a photochemical and *afterwards* a dark chemical reaction. In Warburg's theory it is just the reverse; the carbon dioxide itself does not take part in the photochemical reaction, until it has previously been changed by the dark chemical process.

In 1924 Warburg gave up his acceptor theory without a struggle and accepted Willstätter's theory as being the most probable one. In his experiments with *Chlorella* it appeared to him that the influence of hydrocyanic acid and urethanes on the "Blackman reaction" is almost the same as on the decomposition of H_2O_2 (oxidase- or catalase-activity) by the same organism (Warburg and Uyesugi 1924).

Also the dependence upon temperature was the same for both reactions (Yabusoe 1924). Warburg drew the conclusion that both reactions are caused by the same agent. So the "Blackman reaction" should be an oxidase-reaction, which wholly conforms with Willstätter's theory.

Although this conception seems to be probable, it seems to me that it is by no means established that carbon dioxide

takes part in the photochemical reaction. The proof is still to be furnished.

The above discussion of the literature is not at all complete. In the last years two excellent surveys, covering the whole field of photosynthesis, have been published by Stiles (1925) and by Spoehr (1926), to which I might refer. An account of the literature, much more complete than I intend to give here, may be found in these two books.

I mentioned only as much as was necessary to confirm or criticise my initial conception. We may conclude that, without doubt, there exists besides the photochemical reaction a dark chemical reaction. The names: *temperature factor* (Blackman), *enzymic factor* (Willstätter), *Blackman reaction* (Warburg) and *internal factor* (Briggs) are all concerned with this reaction or the agent, to whose activity it is to be ascribed. The names *inhibition* (Ewart), "*Ermüdung*" (Pantaneli), *time factor* (Blackman) and perhaps *solarisation* (Ursprung) refer to a decrease in activity of this process.

The great independence of this reaction in regard to the photochemical one makes the supposition of a dark chemical agent in addition to the photochemical agent — the chlorophyll — to be a very probable hypothesis. It is likely to be of an enzymic nature as temperatures above 30° C. affect it very much (Blackman's time factor and theory of the temperature optimum, 1905).

I am not convinced that this process, about which we are arguing is in itself a simple reaction. The considerable inconstancy of the temperature coefficient, which decreases with increasing temperature, as Warburg stated for the "Blackman reaction" may indicate a complex nature of this process. The data necessary, however, to a further analysis are lacking at present. So in my opinion it is permissible to consider all the above enumerated phenomena as referring to one and the same dark chemical process.

Of course the true assimilation process is preceded by a transport of CO_2 from the external medium into the chloroplast. It is generally accepted that a partial closure of the stomata may decrease the assimilation velocity. Therefore in certain circumstances the transport of CO_2 may determine the assimilation rate. This justifies the hypothesis that at low concentrations of CO_2 a diffusion process is the "slowest" process of the chain reaction.

In the first part of this Chapter I tried to explain that the scheme of limiting factors did not hold in every respect for leaves, but that it might possibly hold for the single chloroplast.

Warburg made the only experiments on this subject. As said before, he used the unicellular alga *Chlorella*, the cells of which are, as it were, to be considered as a single chloroplast with a cell wall. As the CO_2 has to be transported over a very minute distance only, this process would in no case hamper the assimilation velocity, according to the author's opinion. So the total process would be simplified here by one degree as the diffusion process would be eliminated as a possible "slowest" reaction. Warburg believed that the pressure of CO_2 inside and outside the cell might be considered to be equal and, by varying it, its influence on the real assimilation process inside the cell might be studied.

The variations in carbon dioxide concentration he obtained by suspending his algae in buffersolutions of carbonate and bicarbonate. By altering the ratio of these two salts he was able to vary the carbon dioxide concentration within very wide limits. The assimilation he determined by a manometer device, making use of the different solubilities of O_2 and CO_2 in these solutions.

Warburg's method is very ingenious and refined. It seems to me, however, that it is not altogether without objections. In the first place, the ideal condition of a uniform environment for all cells is not quite reached by him in

the case of light intensity. He took the precaution, it is true, to use very dilute cell suspensions which absorbed only 10 to 20 per cent of the incident light, in cases when the light was the limiting factor. If the maximum difference of light intensity amounted to only 10 to 20 per cent for all cells, this would be no serious objection. But it is highly probable that a relatively small percentage of the cells is materially shaded by other cells and that these may obtain only 50 % or even less of the required light intensity. In this case the error would become much greater, even though the total absorption of the light be only 10 to 20 per cent.

For, if the greater part of the cells have reached the "transition point" at a certain light intensity, twice the amount of light or even more will be required for the shaded cells to reach the same point.

A second objection to the method of Warburg, which is on the whole brilliant, is the use of buffer mixtures to vary the CO_2 concentration. The cells have to be exposed to various acidities, which may not be entirely harmless and which introduces a new complication.

A method has been elaborated by me, in which these two objections are avoided. By means of this method it has been my purpose to find an answer to the following two questions:

1. What is the relation in the individual plastid between assimilation velocity on the one hand and CO_2 concentration, light intensity and temperature on the other hand?
2. What is the nature of the individual reactions of the catenary process, which determine the assimilation velocity?

CHAPTER III.

MATERIAL USED IN THE EXPERIMENTS.

A suitable object was found among the unicellular terrestrial algae, which are to be seen everywhere on trees and walls.

Cultures of this material were started in the laboratory and kept on various nutrient media, with the purpose of finding a species which could be cultivated in a single cell layer.

I did not consider it very probable that I would be able to keep cultures entirely free of bacteria during the experiments. So I looked for an organism, which would propagate in purely inorganic media, to secure a minimum development of bacteria. An article of E. G. Pringsheim (1926) was my guide in this work.

Various solid substrata were used: agar, silica-gel and gypsum.

The water applied in making the nutrient media was always distilled in glass apparatus without metallic parts, to avoid traces of copper and other heavy metals, which are highly toxic to algae (oligodynamic action). The use of Jena glass apparatus and receptacles hardly appeared necessary.

The agar was washed before use to eliminate diffusible organic substances. It was put for two days in running tap water and afterwards allowed to stand one day in distilled water.

A medium was made, having the following composition, as recommended by Beyerinck (1898):

Agar.....	2	per cent.
NH ₄ NO ₃	0.05	" "
KH ₂ PO ₄	0.02	" "
MgSO ₄	0.02	" "
CaCl ₂	0.01	" "
FeSO ₄	0.0001	" "

It was sterilised and put into Petri dishes. Silica-gel plates were made according to Pringsheim: water-glass is diluted to a specific gravity of 1.08 by means of an areometer. Each time 10 cc of this fluid is put into a glass beaker containing 7 cc of 1 N hydrochloric acid and quickly mixed

with it. Then it is immediately poured into Petri dishes where it gelatinizes within a few minutes. (Pringsheim takes a slightly different ratio, viz. equal volumina of water-glass spec. gr. 1.08 and hydrochloric acid spec. gr. 1.1 diluted to 1/10, giving a normality of ± 0.55).

These plates are put in running tap water for one day to wash out the remaining hydrochloric acid and are subsequently covered with a nutrient solution. The nutrient salts are allowed to penetrate into the silica-gel for some hours, after which time the solution is decanted.

It appeared to me that in the long run, the silica-gel was more suitable to my purpose than agar substrata. For, if the cultures are not pure, there is always some development of fungi and bacteria in the agar plates, in spite of the fact that the agar is thoroughly washed. On silica-gel substrata, however, hardly any development of fungi or bacteria was observed.

For gypsum substrata small flat gypsum discs are cast, put into Petri dishes and surrounded with 10 cc of a nutrient solution.

From several walls and trees a small quantity of the green substance was scratched off and shaken with water in a test-tube. This suspension was diluted several times and finally poured out over the nutrient substratum. After a moment the water was carefully poured off.

After a few weeks a great number of little green spots appeared in the culture dishes, being colonies of various algae belonging to the genera *Protococcus*, *Chlorella*, *Hormidium*, *Stichococcus* and others. With a pointed knife such a colony may be lifted from the substratum, shaken with distilled water to form a suspension and poured over a fresh substratum. In such a way it was not difficult to obtain cultures containing only a single species.

In the beginning it was my idea that certain *Protococcaeae* should be suitable to my purpose. But by chance I

detected a much better object. After some five weeks I observed some green wisps on the nutrient solution, surrounding one of the gypsum discs in the Petri dishes which increased, came together and so in the end covered the fluid entirely with a thin green layer.

It proved to be a filamentous alga, *Hormidium* spec.¹⁾ It has the property of developing on the surface of a liquid medium, forming shiny pellicles. The filaments are doubled back upon themselves when growing, forming finally a continuous pellicle, which is kept almost entirely floating upon the surface by capillary action. The pellicle is composed of only a single cell layer. In transmitted light it has a very peculiar appearance; at different places the bundles of filaments are oriented in different directions, causing alternating light and dark spots, due to diffraction of light.

Afterwards I found that this property of *Hormidium* was already known many years ago, described by Chodat in 1913 and even used by Pascher (1914) in his *Flora* as a species character. Because of this property *Hormidium* was an ideal object for my investigations. By means of silica plates cultures, containing *Hormidium* only, were obtained in the manner described above, it was then cultivated further on liquid cultura media.

Since Willstätter (1918, note pag. 168) and Benecke (1921) had mentioned a specific retarding influence of ammonium salts on assimilation, I preferred to use nutrient solutions without ammonium. Therefore I altered Pringsheim's (1921) solution and after a number of preliminary experiments I found one on which the algae developed very well.

A number of single salt solutions were made and kept

¹⁾ Mr. J. Heimans, Amsterdam, was kind enough to determine the alga for me. Probably it is a form of *Hormococcus flaccidus* (Kütz) Chodat = *Ulothrix flaccidus* Kütz = *Hormidium flaccidum* Kl. = *Stichococcus flaccidus* (Näg.) Gay.

in store, viz. KNO_3 5 per cent, CaSO_4 saturated solution = 0,2 per cent, MgSO_4 1 per cent, KH_2PO_4 , per cent and FeSO_4 0,1 per cent. This last solution had to be prepared fresh before use, since the ferrous salt is rapidly oxidized, forming a precipitate of insoluble ferric salt.

The above solutions were added to about 400 cc distilled water in the following quantities and succession (the last column giving the final composition of the solution):

FeSO_4	0.1 per cent	5 cc. . .	0.001 per cent
MgSO_4	1 " "	5 cc. . .	0.01 " "
CaSO_4	satur. sol.	10 cc. . .	0.004 " "
KNO_3	5 per cent	10 cc. . .	0.1 " "
KH_2PO_4	2 " "	5 cc. . .	0.02 " "
distilled water up to 500 cc.			

I believe to have noticed that the order of the addition of the salts is not without influence on the nutritive value of the solution.

The solution was not sterilised. The algae were cultivated in flasks of 100 cc. capacity, containing \pm 15 cc. of the solution. The flasks were stoppered with cotton wool.

Transfers were made by means of a nickel chrome needle, the eye of which had a diameter of from 5 tot 10 mm. This eye, when put in the fluid below the algae and lifted, bears a film of the culture solution which is covered on either side with a tiny layer of algae. When it is brought into contact with the surface of the solution in a new culture flask, the algae immediately spread over its surface and may multiply again here.

I put the culture flasks into a glass basin, covered by a glass plate. The basin was kept before a window. Direct sunlight was screened off by means of transparent paper. In the beginning I considered it necessary to introduce some gaseous carbon dioxide into the glass basin every day. After some time the carbon dioxide of the air in the

room appeared to be sufficient to supply the algae by diffusion through the cotton wool stoppers.

After two or three weeks the total surface of the fluid is covered with the algae. Thereafter growth does not proceed any further. Apparently the nutrient solution is exhausted or decomposition products of the metabolism are checking further development.

In a culture, which is in bad conditions for this reason,

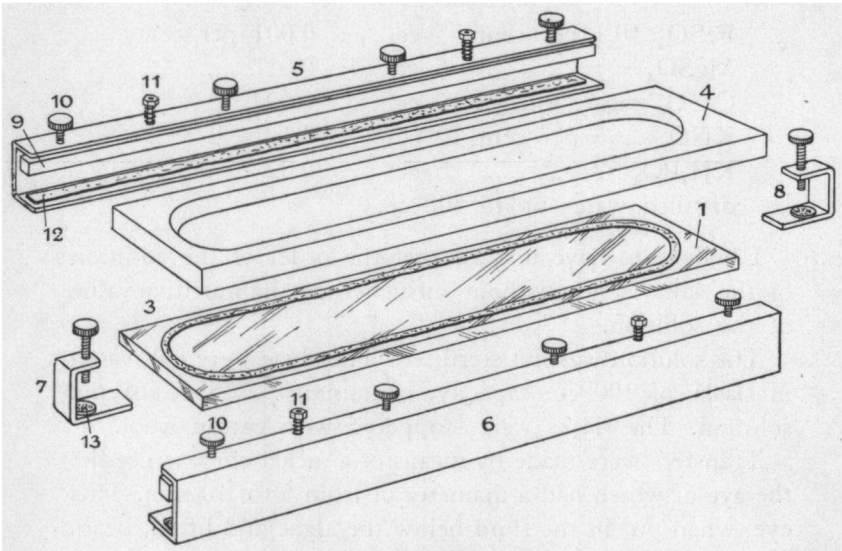


Fig. 2.

or because of high temperature or excessive illumination, the filaments begin to sink. After some time almost the entire quantity of algae is suspended in the fluid like a flaky green precipitate.

Algae to be used in experiments were transferred two or three days previously on the surface of a culture solution, contained in a special receptacle (see fig. 2).

This receptacle is 24 cm. long and 4 cm. broad. It consists of a glass plate 1, used as a bottom and an ebonite ring 4,

which serves as a wall. A rubber gasket 3, cut from the inner tube of a bicycle tire, is clasped between them by means of the clasps 5, 6, 7 and 8, thus serving as a water tight seal between bottom and wall. The clasps 5 and 6 consist of an U-shaped, white lacquered piece of copper; a copper rod 9, plated with nickel is connected to it in a movable manner. Two small bars 11 are fastened to this rod, keeping it suspended by means of lithe spiral springs. By turning the screws 10 the rod may be pressed down and the ebonite ring and the glass plate clamped together. Rubber strips 12 and cork disks 13 protect the glass from the metal; otherwise it is soon broken.

This receptacle is put in a horizontal position and filled half full with a nutrient solution. A suitable quantity of algae is transferred into it.

When an experiment is to be made, the solution is siphoned off from beneath the algae by means of a small siphon. This is done also when the solution is to be renewed. It has to be done very carefully, for the layer of algae remains in its place only as long as the opening of the little siphon is below the surface of the solution. But when the receptacle is nearly empty, air bubbles are siphoned off also. At this moment the algae on the surface are drawn towards the siphon and run off with the fluid, causing a considerable loss of material. This can be prevented by means of a small loop of a paraffined thread, which is placed floating upon the surface of the solution around the opening of the siphon. In this way the surface film around the siphon is separated from that of the remainder of the vessel and the latter no longer reacts when air bubbles are sucked through.

It is also possible to push the algae towards the middle of the receptacle with two paraffined pieces of paper, before siphoning off the solution. In the greater part of the experiments this procedure was used.

When the fluid is sucked off, the algae remain lying on a

thin film of solution on the surface of the plate 1. This plate is the one which is to serve as the bottom of the assimilation vessel (see next chapter).

CHAPTER IV.

APPARATUS.

The methods have been briefly described elsewhere (1928). A more detailed account will be given here. The technical requirements are the following.

In the assimilation vessel we must be able to vary or to keep constant the CO_2 concentration as well as the light intensity and temperature individually and at will. The CO_2 concentration has to be the same throughout the assimilation vessel and has to be determined with an accuracy of 0.001 per cent.

When considering that in the atmospheric air, the normal medium of the algae, the CO_2 concentration amounts to only about 0.03 per cent, it is clear that such a degree of accuracy is necessary.

Intake and evolution of CO_2 (assimilation and respiration) have to be determined accurately.

The CO_2 concentration was determined by gas analysis. The frequently used apparatus of Haldane is accurate only till 0.01 per cent. Krogh (1920), however, has constructed an apparatus, with which it is possible to analyse the CO_2 and O_2 content of an air sample of 50 cc. with an accuracy of 0.001 volume per cent.

My gas analysis apparatus was copied from Krogh's apparatus with slight modifications, consisting mainly in the size of the air sample, which was 10 cc. instead of 50 cc..

A brief description may follow here. For a detailed discussion of the construction I refer to Krogh's article.

The Gas Analysis Apparatus.

The principle is quite simple. By lowering the mercury within a calibrated burette 1 (fig. 3) a sample of air is taken up and its volume is measured.

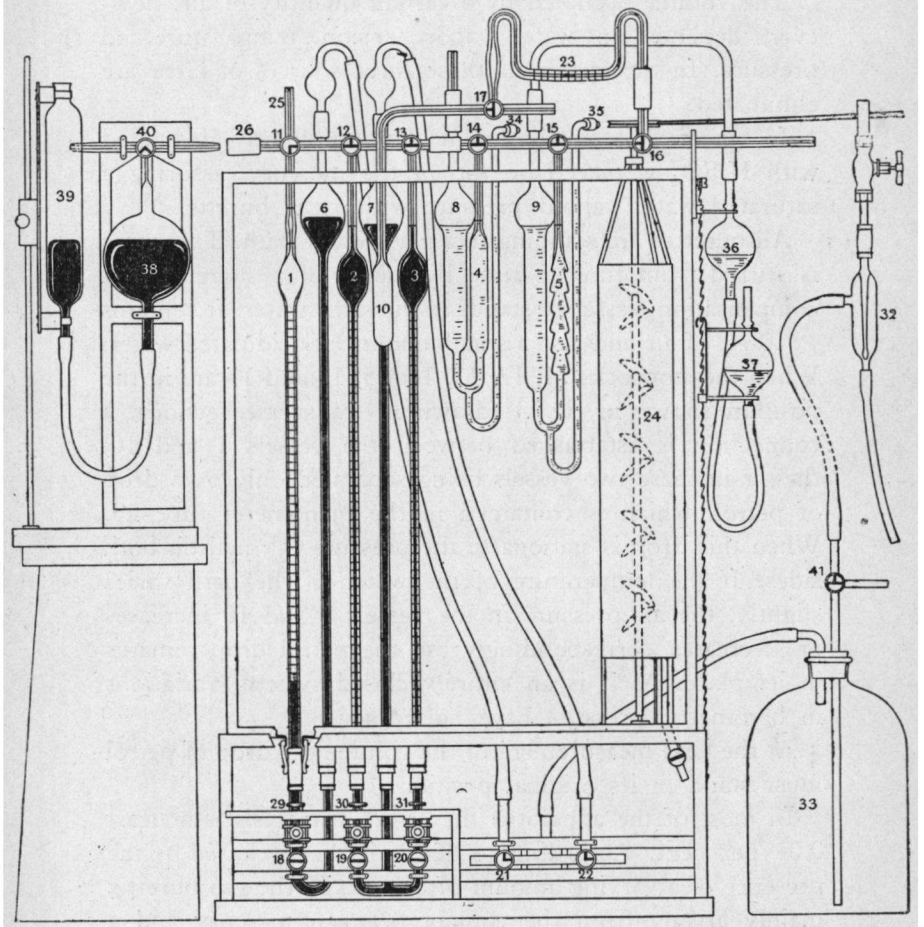


Fig. 3.

Then it is brought into contact with a 10 per cent solution of KOH in pipette 4 where the CO_2 is absorbed, after which

the volume is measured again. The decrease in volume corresponds to the CO_2 content of the air sample.

In the same manner the O_2 is determined. It is absorbed in pipette 5 by a solution of pyrogallic acid in caustic potash.

The volume occupied by a certain quantity of air, however, depends on water vapor tension, temperature and pressure. In the apparatus these three sources of error are eliminated.

In burette 1, a small amount of water slightly acidulated with H_2SO_4 is placed on top of the mercury, assuring a saturated water vapour pressure within the burette.

All burettes are standing in a glass water bath. The water is stirred from time to time by means of a stirrer 24. A compensation vessel 10 stands in the same water. It contains ± 10 cc. of air and also a small amount of acidulated water. When the stopcocks 11, 12, 13, 14, 15, 16 and 17 are in the position shown in fig. 3 (drawn in transverse section), a connection is established between the vessels 1 and 10, the air in these two vessels being separated only by a drop of petrol, which is contained in the manometer tube 23. When this drop is stationary, the pressure is equal on both sides. If the temperature of the water in the bath varies slightly, the air pressure in the vessels 1 and 10 increases or decreases correspondingly; so the petrol drop remains in its place. As it is an entirely closed system, variations in barometric pressure have no influence.

At the next measurement of the volume the drop of petrol must stand in its original position. ~

In most of the apparatus the error of analysis is at least 0.01 per cent. According to Krogh this is caused by the presence of a varying amount of detritus in the gas burette, mainly arising from the contact between mercury and a moving rubber tube, and also by the inconstancy of the volume of water, contained in the gas burette. For at each analysis a small part of this water is distilled over into the

absorption fluids, which have a lower water vapour tension than pure water.

The following two improvements have been applied by *Krogh*.

The mercury is not moved up and down by means of a leveling bulb with a connecting flexible rubber tube, but by variations of air pressure. By means of the water vacuum pump 32 and the vacuum flask 33 the air pressure above the mercury in 6 is lowered through stop-cock 21. As at its lower end vessel 6 is connected with burette 1, the mercury is sucked out of the burette. By turning stopcock 21 the air in 6 may be connected with the atmospheric air and the mercury may be run back into burette 1. At the required moment the mercury flow may be stopped by closing tap 18.

In such a way the mercury may easily be moved up and down and it is not in contact with moving rubber tubes.

The second, still more important improvement is the application of three different gas burettes. The first (1) serves to move the air to and from the absorption pipettes and to saturate it with water vapour. The second (2) serves to measure the volume before and after the absorption of CO_2 ; the third (3) to measure the volume after the O_2 has been absorbed.

The burettes 2 and 3 also contain a small quantity of slightly acidulated water (± 2 cmm), just sufficient to secure a saturated water vapour tension of the air sample and so small that the water is to be seen as a small line along the mercury meniscus as it stands in the bulb.

When the mercury is lowered the water remains entirely in the bulb, adhering to its wall. If the quantity of water is too large some of it goes down into the narrow, calibrated lower end and hinders accurate reading of the position of the mercury meniscus.

The capacities of burettes 1 and 2 are upwards of 10 cc.

The capacity of the narrow lower part of burette 2 is only 0.4 cc or 4 per cent. of the total capacity. This part has been calibrated into units of 1 cmm. It is possible by estimation to measure the volume to within 0.1 cmm.

When the CO_2 of the air sample has been absorbed, it is again possible to determine its volume in burette 2, since the CO_2 content of the air sample seldom amounts to more than 1 per cent. When O_2 has been absorbed however the volume is decreased by ± 21 per cent. and can no longer be determined in burette 2. This is therefore done in burette 3, which has a capacity of about 8 cc.

In my apparatus the lower part of this burette is calibrated in parts of 5 cmm. making it possible to read the volume to within 0.5 cmm. or 0.005 per cent.

The upward and downward movement of the mercury in 2 and 3 is accomplished in the manner already mentioned by means of vessel 7 and stopcock 22.

This is sufficient to obtain an approximate adjustment of the mercury meniscus. For accurate adjustment the following device is used.

Above the stopcocks 18, 19 and 20 side tubes have been attached. These are provided with short pieces of vacuum rubber tubes, closed by glass stoppers.

By tightening or loosening the screw cocks 29, 30 or 31 the mercury content of the burettes may be altered and a very accurate adjustment may be attained. These screw cocks are fastened on a little frame in order to keep the rubber tubes in a fixed position and to assure a steady position of the meniscus in the burette.

The 10 per cent. KOH solution in the absorption pipette 4 and the communicating vessel 8 has been previously saturated by shaking with atmospheric air at room temperature.

The alkaline pyrogallic acid solution in pipette 5 and vessel 9 is, following Haldane's procedure, a solution of 10 gm pure pyrogallic acid in 100 cc. saturated KOH solution

(sp. gr. 1.55). I used for this purpose „Pyral” of Hauff and Co. The solution must have been saturated with nitrogen (air free of CO_2 and O_2). To accomplish this it is vigorously shaken with air in a small bottle for about twenty minutes.

The CO_2 and O_2 are readily absorbed; by slightly opening the stopper of the bottle at intervals the nitrogen is kept at atmospheric pressure. Care has to be taken not to warm the bottle with the hand while shaking.

The absorption pipettes may be emptied, cleaned and refilled through the tubes 34 and 35. The upper ends of these tubes are bent backward and closed with a piece of rubber tube and glass stopper. In this manner the water in the bath never comes in contact with the alkaline absorption fluids.

Vessel 8 communicates with the outer air through a capillary tube, allowing only a very slight diffusion of atmospheric carbon dioxide towards the KOH solution, which may be kept a long time.

The absorption fluid in vessel 9 is separated from the outer air by a KOH solution in the communicating vessels 36 and 37, as may be seen in the figure.

The Construction of the Apparatus.

The gas analysis apparatus and the other instruments were made by Mr. P. A. de Bouter, mechanic of the Botanical Laboratory in Utrecht. Many technical difficulties were solved in consultation with him. I wish here to express my thanks for his valuable assistance.

For constructing the gas burettes three glass tubes, each having a nearly uniform bore throughout its length, were selected from among a large number.

They were provided with the bulbs 1, 2 and 3 and their capacity was determined by filling with mercury, which was afterwards weighed. By narrowing or widening the glass bulbs over a flame the desired capacity was finally attained.

Subsequently the burettes had to be calibrated. In this purpose a glass stopcock, the discharge opening of which had been drawn out to a fine point, was fused to the lower end of each burette. The burette was filled with purified mercury; a quantity of it, contained between two little lines above and below the bulb, was run off and its volume was determined by means of weighing.

The little marks were applied with a glass hair and a mixture of black lacquer and turpentine, dried to a sticky consistency.

During the testing procedure the mercury must be kept at a constant, known temperature. For this purpose a glass cylinder, filled with water, was applied around the bulb, and the stopcock at the lower end was protected by cotton wool from the warmth of the hand.

The capacity of the lower part was determined in the same way and the space between two lines provided with graduations.

Burette 1 was divided from 6.4 to 10.3 cc. into graduations of 10 cmm, burette 2 from 9.57 to 10.03 into graduations of 1 cmm. and burette 3 from 7.0 tot 8.3 ccm. into graduations of 5 cmm.

The accuracy of the calibration was controlled in the manner already described. It is unnecessary to surround the slender tubes with a water bath. A reading of the room temperature is sufficient. To adjust the slight errors found in the calibration a correction table was made for each burette.

The entire gas analysis apparatus mainly consists of three units. The first unit is composed of the burettes 1, 2 and 3 with the stopcock 11, 12 and 13 and is fastened to the edge of the bath by means of a copper frame. The burettes pass through holes in the bottom of the bath. A water-tight connection is made by means of soft rubber tubes, exerting no pressure on the glass tubes, as shown for burette 1 in fig. 3.

The second unit is formed by the absorption pipettes 4 and 5 with the vessels 8 and 9 and the stopcocks 14, 15 and 16. It is fastened to a separate frame and can be removed separately.

The third unit is formed by the compensating vessel 10, the stopcock 17 and the manometer tube 23.

These three pieces are connected together with vacuum tubes in such a way as that the glass tubes are in end to end contact with each other.


It may be useful to subsequent experimentors to mention some errors in construction, which I made in the beginning.

In the first place there appeared to be very slight leakages in the stopcocks. The greater part of the stopcocks on the market are not adapted to this purpose. By carefully grinding with emory powder this defect could be partly removed; stopcocks 16 and 17 were replaced by others, especially ordered for this purpose. Since stopcock 11 closed well, leakage to or from the outer air was excluded. As it was always possible in presence of considerable pressure differences to separate the absorption pipettes by two stopcocks from the vessel in which the high or low pressure was applied, the undesirable sucking up of the absorption fluids into the stopcocks could be avoided.

A second fault was the relatively large air volume contained in the capillary tubes outside the water, in comparison to the volume of the burettes (± 14 per cent.). This caused errors due to changes in room temperature. Therefore the volume of these tubes was made equal on both sides of the manometer tube by enlarging tube 17. After this correction the room temperature had only a very slight influence on the position of the petrol drop. Furthermore the tubes were wrapped for the most part in cotton-wool, as a protection against the warmth of the hands, when the stopcocks had to be handled.

A third error was made in the calibration of the tubes.

I overlooked the fact that the calibration must correspond to the real volume of the burette, to which is added the inner volume of stopcock 11.

For, when an air sample is to be taken in, the stopcock 11 is put into position  and some of the air to be analysed is driven through 26, 11 and 25, to displace the air present in it with the air to be analysed. When 11 is put back into its original position, its content of air for analysis is already brought into the apparatus, before any air has been taken up by lowering the mercury.

To eliminate this little error, a small enlargement was made above the O-line in burette 1, and above it a second line, the volume between the lines being equal to the inner volume of stopcock 11. Before each analysis the meniscus of the water above the mercury was adjusted at the lower line; at each following volume measurement (in burette 2 or 3) the level was adjusted at the upper line.

The air samples were brought to the analysis apparatus in a gas container 38, the mercury in which may be moved up or down by means of a leveling vessel 39.

In nearly all my experiments I confined myself to CO₂ determinations; generally the O₂ was not considered.

Since I did not know in advance, however, whether CO₂ determinations would be sufficient, I preferred to be able to make O₂ determinations also.

After the precautions and corrections mentioned, CO₂ determinations accurate to ± 0.001 per cent. were finally attained (see the duplicate determinations in the tables, which seldom deviate more than 0.002 per cent.).

The reading of the mercury level in the burettes was made with the aid of a magnifying-glass.

Parallax errors were eliminated in the following manner. A white piece of paper provided with a slit was attached to the front of the magnifying glass. When looking through it at the mercury in the burette, keeping the slit in a hori-

zontal position, the image of the slit is seen as a dark spot on the mercury column. When a reading is taken this spot has to be just below the mercury meniscus. Following this procedure the eye of the observer is always in the same position with reference to the meniscus.

Procedure in making a CO₂ determination.

We assume the apparatus to contain only CO₂-free air, all menisci to be at their O-position (in burette 1 at the lower line) and the stopcocks to be in the position as indicated in fig. 3. The position of the petrol drop is determined and stopcocks 16 and 17 are put into position λ . Stopcock 11 is now put into position $_ |$; the gas reservoir is connected to tube 26 (contact between the glass) and the niveau vessel is lifted a few cm. upward to cause a somewhat higher pressure within the gas reservoir. Stopcock 40 is then opened for several short moments to wash out the space between 40 and 11, now stopcock 11 is put into position $_ |$, the vacuum pump 32 is started, stopcock 41 is put into position $_ |$ and 21 into position $_ |$. Stopcock 40 of the gas reservoir is again opened. By opening stopcock 18 an air sample of ± 10 cc. is sucked in, and 18 is closed again. The mercury in the gas reservoir and the niveau vessel is put exactly at the same level by moving vessel 39 upward or downward. Stopcock 40 is closed and 11 is turned to position $_ |$. Now stopcock 12 is turned to position $_ |$, 14 to position \sphericalangle , 21 and 22 to position $_ |$. Stopcocks 18 and 19 are cautiously opened and the air sample is transferred from burette 1 to burette 2. The water meniscus in 1 is adjusted at the upper line.

The rough adjustment of the air pressure in 2 is done by means of the KOH-meniscus in pipette 4; to accomplish this stopcock 12 is put into position $_ |$ and 14 into position $_ |$. Usually the KOH-meniscus then moves on and is brought back to its former position by adjusting screwcock

30. Stopcock 14 is put back into position \perp and 16 and 17 are cautiously opened alternately. Screwcock 30 is turned until no displacement of the petrol drop is observed; then 16 and 17 are put both into position \dashv and by turning 30 the petrol drop is returned exactly to its former position.

In the mean time stirrer 24 is moved by an electric motor, to secure an even temperature throughout the water.

The menisci in 1 and 4 are examined and the position of the mercury in 2 is read off. Usually after 2 or 3 minutes the volume of the air sample has become constant.

Stopcocks 16 and 17 are put into position \sphericalangle , 14 into position \dashv , 12 into position \perp and 22 into position \perp . Stopcock 19 is now opened and the air sample is driven from burette 2 into pipette 4, after which stopcock 12 is put into position \perp . By moving the mercury in burette 1 up and down about ten times, the space between the stopcocks is washed out and every trace of CO_2 present is absorbed. When determining very high CO_2 concentrations the space between stopcock 12 and the meniscus in burette 2 is washed out also, but in general this is superfluous. The air sample is now brought back into burette 1 and then again in burette 2, its pressure is adjusted in the manner already described and its volume is measured. The decrease of volume corresponds to the CO_2 content. When necessary corrections are applied to compensate for the calibration errors in the burette and the CO_2 content is calculated in terms of volume per cent.

Through stopcock 16 the sample is now driven out of the apparatus and the meniscus in burette 1 is returned to the lower line, after which procedure the apparatus is ready for a new analysis.

If the CO_2 content as well as the O_2 content has to be determined, this is done in an analogous manner, the only difference being that the apparatus must contain nothing but nitrogen before the analysis is started. The washing out of

the space between the taps takes a longer time when the O_2 is absorbed; I moved the mercury up and down in burette 1 from 30 tot 40 times. Krogh, in his own apparatus, does it 6 times only, probably because the dead space in his instrument is comparatively much smaller.

Several analyses of atmospheric air (taken on the roof of the laboratory) are given here.

TABLE 1.

	CO_2	O_2	N_2
	0.028	20.951	79.021
	0.030	20.934	79.036
	0.030	20.926	79.064
	0.033	20.885	79.082
	0.027	20.921	79.051
Mean	0.030	20.923	79.051

Krogh, when analysing air samples of a Copenhagen street, found a CO_2 content varying from ± 0.030 per cent. to 0.039 per cent. and a N_2 content, varying from ± 79.040 to 79.055 per cent. Though my O_2 determinations were not as accurate as I desired, the above analyses show a good agreement with Krogh's determinations, indicating that my apparatus was properly calibrated.

When the burettes are to be cleaned, the mercury is lowered to just below the above mentioned side tubes. The pieces of rubber tubes are removed and with another rubber tube the side tubes are connected to a rectangularly bent glass tube, through which a solution of potassium bichromate in dilute sulfuric acid is run into the burettes. Then the rubber tube is closed by a screw cock. After remaining for one day in the burette, the potassium bichromate solution is run off and the burette is carefully washed with distilled water. This water must run off cleanly from the wall of the burette

and may not adhere to it in the form of drops, which is the case when the burette has not been thoroughly cleaned. Finally the burette is dried by a current of air, filtered through cotton wool.

In order to place the acidulated water on top of the mercury in the burettes, the stopcock at the upper side is removed and the mercury is raised to the upper end of the tube. With a very small pipette, the point of which is bent at a right angle, the acquired amount of water is put on top of the mercury, which is then lowered slowly by means of the screw cock.

To lubricate the stopcocks I used a mixture consisting of 1 part unvulcanised rubber, dissolved in 5 parts of vaseline at a temperature of 100° C.

The experimental Equipment.

The experimental equipment is shown in semi-diagrammatic drawing (fig. 4) and will be described here. It had to answer the requirements already mentioned in the beginning of this chapter.

The algae are placed in the assimilation vessel 15. Through this vessel an air current passes, which has previously been drawn through a CO₂ generator 1, in which it has been supplied with a constant, known amount of CO₂. The air leaving the assimilation vessel is collected above mercury in the aspirator 29. This mercury is discharged from the aspirator at a 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 CO₂ concentration from 14 to 16 would 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.

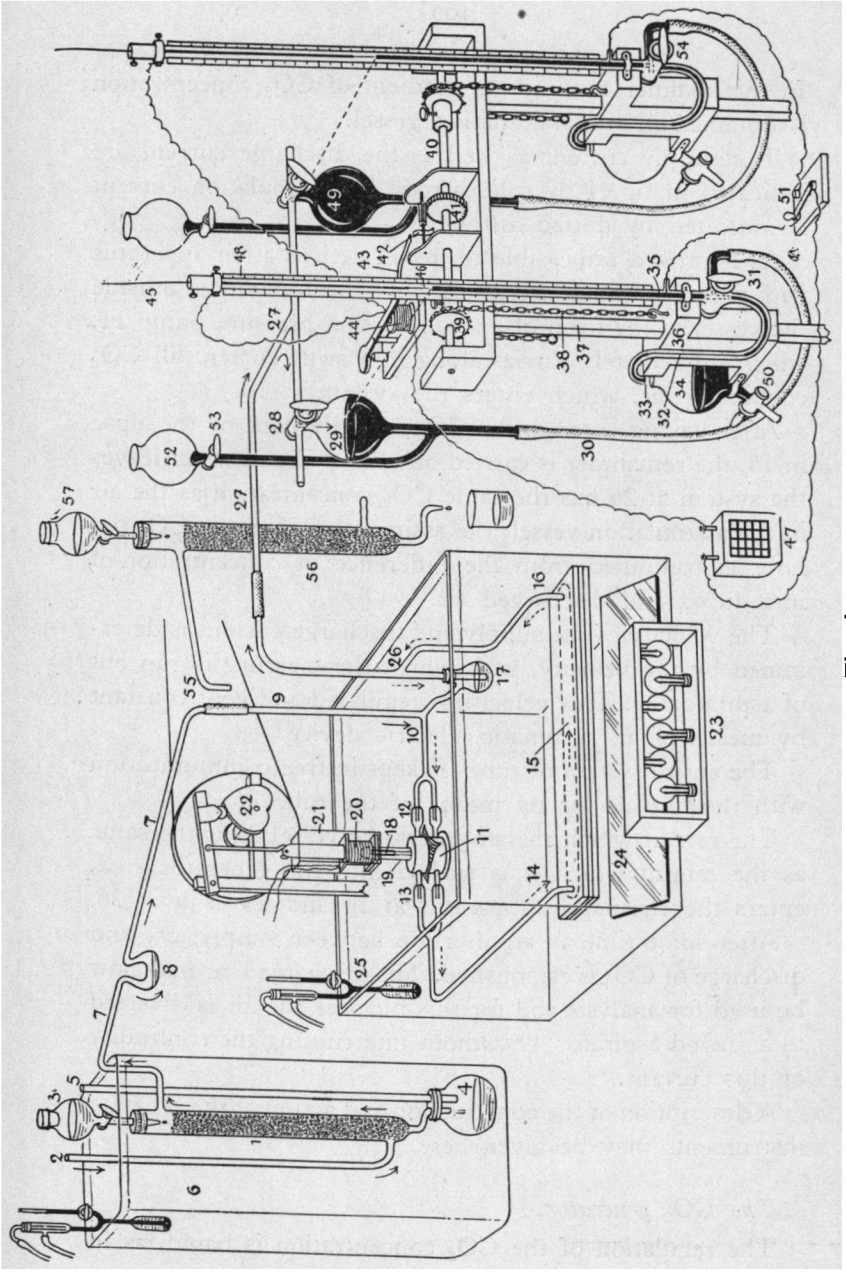


Fig. 4.

In this manner a minimum gradient of CO_2 concentration is obtained in the assimilation vessel.

The supply current as well as the discharge current are indicated in fig. 4 by solid arrows, the circulation current is indicated by dotted arrows.

As it proved impossible to install a circulation apparatus within the assimilation vessel, it has been mounted outside the vessel in the form of a small suction-pressure pump 11, which immediately carries 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 *mm.* CO_2 per hour is computed from the difference in concentration of introduced and discharged air.

The velocity of the supply and discharge current is determined by the velocity, with which the mercury is run out of aspirator 29. This velocity is regulated and kept constant by means of an automatic, electric device.

The entire system of tubes is kept in free communication with the outside air by means of the tube 2.

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.

After some time an equilibrium between supply, use and discharge of CO_2 is established. The discharged air may now be used for analysis and for this purpose the air is diverted to a second aspirator 49 without interrupting the continuity of this current.

A description of the construction and action of the separate instruments may be given here.

The CO_2 generator.

The regulation of the CO_2 concentration is based, as in

Warburg's method, on the use of buffer mixtures of carbonate and bicarbonate.

The generator is represented in fig. 5. It consists of a long glass tube 1, which is 40 cm long and $1\frac{1}{2}$ cm in diameter. It is filled with glass beads of 2 mm. diameter. An aqueous solution of Na_2CO_3 and NaHCO_3 in definite proportion, together amounting to 0.5 grammol p. L., drips from vessel 3 into the generator. Vessel 3 is closed by a cork, but not air-tight.

The solution is discharged into vessel 4, which may be emptied from time to time by siphoning through tube 5. This complex is placed into a water bath (fig. 4, 6) in which the water is kept very accurately at 30°C by means of a microburner and a toluene regulator (variations of temperature $\pm 0.02^\circ\text{C}$.).

The dotted line 7 in fig. 5 indicates the level of the water in the water bath.

The air enters the generator by tube 2 and passes unhampered through the openings between the beads, through which the solution drips down (principle of countercurrent). The air is in equilibrium with the solution when it leaves the generator. Air, in equilibrium with such a buffer mixture of Na_2CO_3 and NaHCO_3 , has at a certain temperature a definite CO_2 concentration, which becomes higher as the concentration of the NaHCO_3 is increased and that of the Na_2CO_3 decreased.

Tube 6 is lined with paraffine to prevent the ascending of the solution within by capillary action, as it would otherwise rise beyond the reach of the water bath.

It appeared to be impossible to regulate the rate of dripping by means of a stopcock. Therefore, after adding the buffer mixture in 3, a little cotton wool stopper is applied at 8 with a pair of forceps and pressed tightly into the tube, in such a way that one drop falls every 4 or 5 seconds. This may be controlled at 9. In the S-shaped tube 10 it is warmed

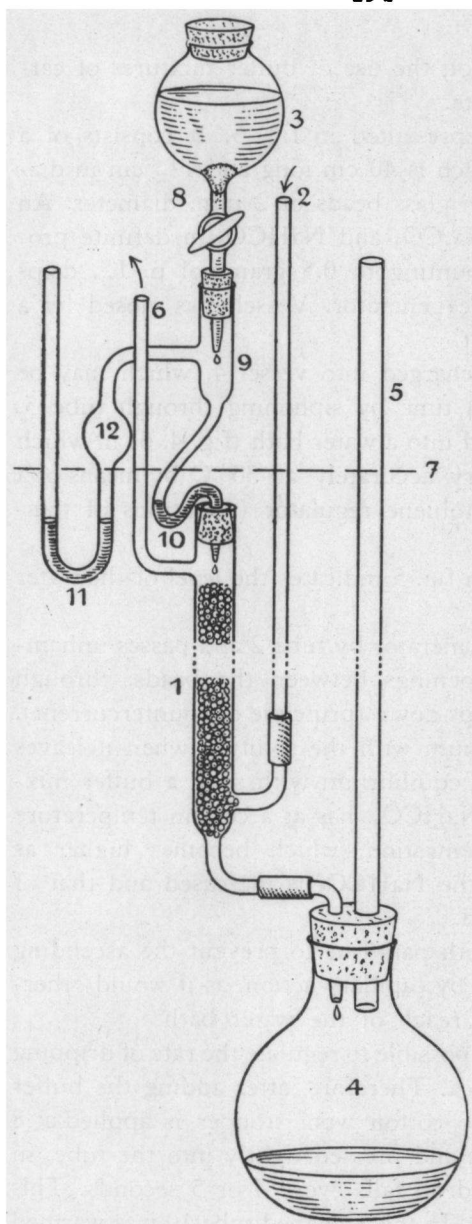


Fig. 5.

to the desired, constant temperature, before entering tube 1.

Several times it happened that I omitted to siphon off in time the fluid in vessel 4. Then instead of air the buffer solution is sucked up through the generator into the circulation system and even into the aspirator. Cleaning the apparatus after such an accident involved a great deal of work. To avoid such mishaps, a safety tube 11 was applied, containing a small amount of water. As soon as buffer solution is sucked up from vessel 4, the air pressure in 1 is decreased, causing the outer air to bubble through tube 11 into the enlargement 12.

To tube 6 a tube is connected, which conducts the air mixture towards the circulation system. This conducting tube (fig. 4, 7) is provided with

an enlargement 8, which contains a few drops of acidulated 25 % NaCl solution in order to lower the water vapour tension of the air. In this way the formation of condensation water at other points in the system was prevented.

When determining the respiration I used for the most part CO₂ free air. For that purpose tube 9 (fig. 4) was connected with tube 55 of generator 56. Through this generator a 2 per cent. KOH solution drips slowly down from vessel 57 through a column of small glass beads.

The air, passing between the beads in opposite direction is freed of CO₂.

The circulation system.

By this term I mean the assimilation vessel, together with the air circulation apparatus, as represented separately in fig. 6. It is mounted on a copper frame, carefully plated with nickel and may be lifted as a unit out of the waterbath (fig. 4, 25).

The assimilation vessel (see fig. 6 and 7) consists of two glass plates 1 and 2 (bottom and cover). As mentioned in Chapter III the glass plate 1 has served as a bottom to the receptacle, in which the algae are cultivated. These algae now rest on a thin film of solution on this plate.

The same rubber ring which was used as a water tight connection (fig. 2, 3) now serves as the side wall of the assimilation vessel. This ring is \pm 5 mm. broad and 8 mm. thick; so the height of the assimilation vessel is about 8 mm. The ring is thinly coated with vaseline and clasped between bottom and cover by means of two clasps 5 and 6, made of copper well plated with nickel. In this way an air-tight connection is obtained and an assimilation vessel is formed, which is 24 cm. long, 4 cm. broad and 0.8 mm. high.

At both end of the cover holes are bored to receive the ends of supply and discharge tubes. The manner in which

an air-tight connection is established with these tubes may be seen in fig. 7. In this figure the different parts are indicated with the same numbers as in the figures 2 and 6.

The supply tube 14 is surrounded by a cone-shaped rubber stopper 15, which is pressed tightly against both the

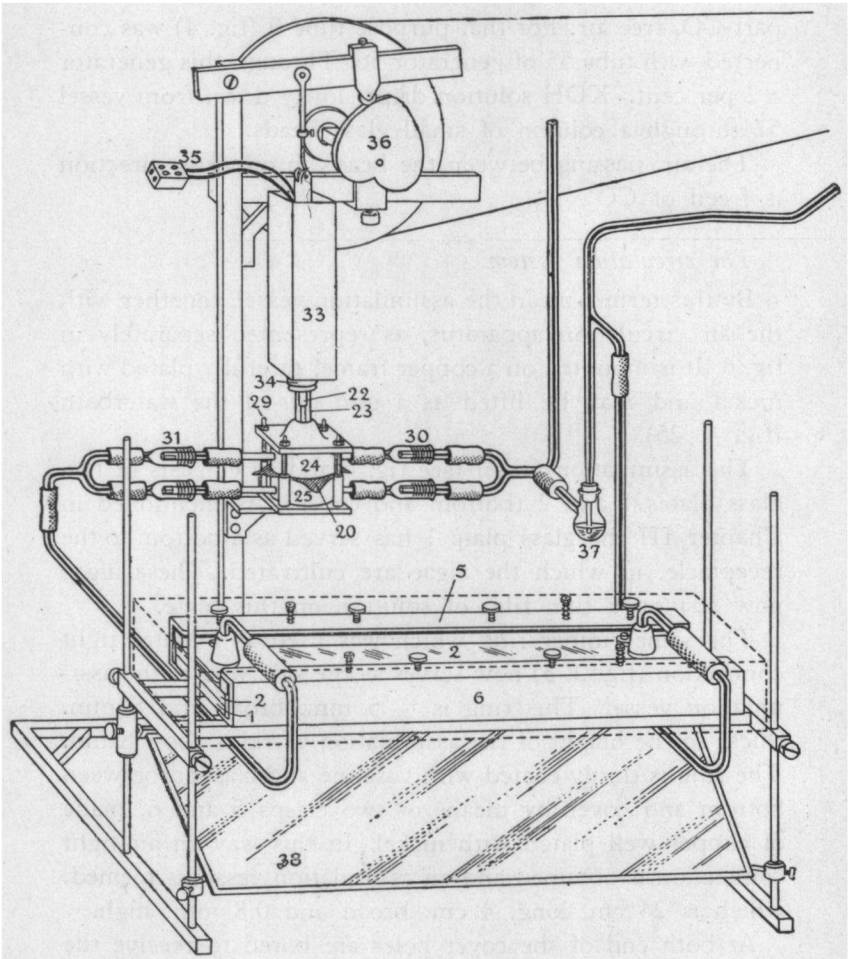


Fig. 6.

cover and the tube by a fitting of nickeled copper 16, which has the form of a hollow cone and is screwed to the cover by means of the machine screws 17. The discharge tube is fitted in the same manner.

The rubber ring 3 is running just outside of the opening of tube 14, but inside of the screws 17.

The assimilation vessel may be made dark within a light-tight copper box, well plated with nickel (drawn with dotted lines in fig. 6). The box as a whole is formed by two parts. The first part consists of a front-wall 18 (fig. 7) and two side-walls and is fastened to the frame. The two side-walls

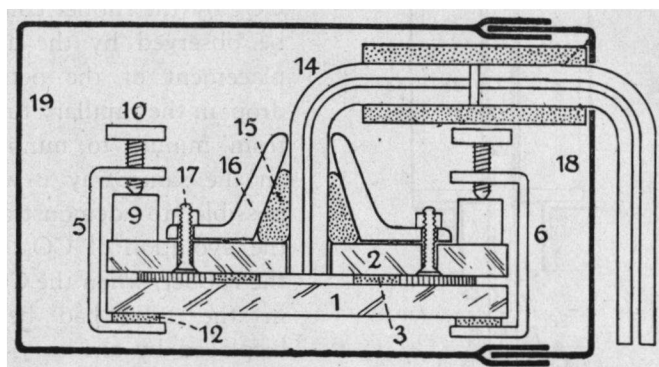


Fig. 7.

are provided with projecting parts to support the assimilation vessel. This is made in such a way that the second part 19, which constitutes the upper, lower and back side of the box, may be easily slid over the assimilation vessel.

The *circulation-pump* is represented in fig. 6 and in cross section in fig. 8. Originally I devised for this purpose a little apparatus consisting of two rubber membranes which were alternately moved up and down like pistons. But it appeared to me, that a rubber membrane in unilateral contact with the atmospheric air or with water allows a considerable diffusion of CO_2 , which may cause a loss of this gas by

leakage. This is correlated with the fact that CO_2 is "soluble" in rubber.

In order to form an idea about this "solubility" I made the following experiment. A piece of rubber was put into a glass tube which was sealed at one side and filled with gaseous CO_2 . The open end was connected to a capillary tube which contained a small drop of petrol and the glass tube was put into a water bath at 20°C .

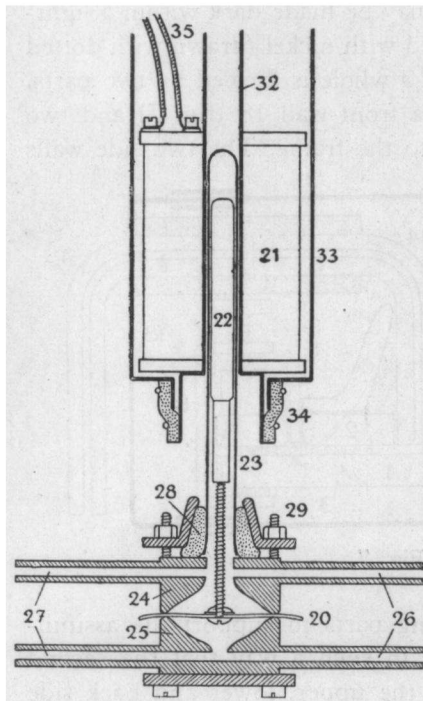


Fig. 8.

The open end was connected to a capillary tube which contained a small drop of petrol and the glass tube was put into a water bath at 20°C . The rate of absorption of CO_2 by the rubber could be observed by the displacement of the petrol drop in the capillary tube from minute to minute. In the same way it was possible to demonstrate the evolution of CO_2 by the rubber, when the CO_2 in the tube had been displaced by air.

A comparative experiment was made with a sheet of rubber, cut from a bicycle inner tube and a thick piece of vacuum tubing, in order to decide whether the CO_2 dissolves

only in the outermost layer or penetrates into the rubber. The results are given in table 2. In this table it may be seen that the rate of absorption decreases quickly in the case of the thin rubber; whereas in the case of the thick rubber it decreases slowly. Apparently the piece of bicycle tire, which is only 1 mm. thick, is saturated throughout in a short

time. In the case of the thick rubber tube this requires a much longer time. It is evident that the CO_2 penetrates with a considerable speed into the rubber.

Besides it may be noticed that after an hour the piece of bicycle tire had taken up an amount of CO_2 , equal to $\frac{3}{4}$ of its own volume.

So rubber is not an ideal material with which to make CO_2 -tight connections. A better material, however, is difficult to find. Coating with vaseline has very little effect. Several other substances, such as celloidine and even solid paraffine, appeared to absorb CO_2 to a considerable extent.

In the case of my circulation pump this difficulty was solved by keeping the membrane, which acts as a piston, separated from the water of the water bath (see fig. 8). The power transmission, required to raise and lower membrane 20, is accomplished by means of an electric helix 21. This helix is moved up and down and carries with it a piece of soft iron 22, contained in glass tube 23 which is sealed at its upper end. This piece of iron is connected by a little rod to the centre of the membrane.

This membrane 20 is clamped between two small cylinders 24 and 25, each provided by an inlet and outlet tube 26 and 27, which bear the check valves 30 and 31.

The air-tight connection between the glass tube 23 and the upper cylinder 24 is again accomplished by the use of a cone-shaped fitting like the one previously described. It is tightly pressed down by turning the nuts 29, by which means also the rubber membrane is clamped between the two cylinders.

The membrane must not be stretched too much. The piece of soft iron must be freely movable within its glass cover, so that it rattles when tapped.

The electric helix is protected from the water in the bath by a cover, consisting of two parts 32 and 33, connected together by a rubber tube 34. By means of the wires 35 and

a lamp-resistance unit, the helix is connected to the city-current.

With the aid of an electric motor, which also moves the stirrers of both water baths, the cordate-shaped disc 36 (fig. 6) is rotated and the pump driven. The pump makes ± 88 strokes per minute and creates a circulation current of 5 L. per hour.

The *check valves* must have a very small resistance. The manner in which this is accomplished is illustrated in fig. 9. The end of a glass capillary tube 1 is ground in such a way, that the central opening 2 projects to a slight extent. The end is then held in the flame for some moments to glaze its

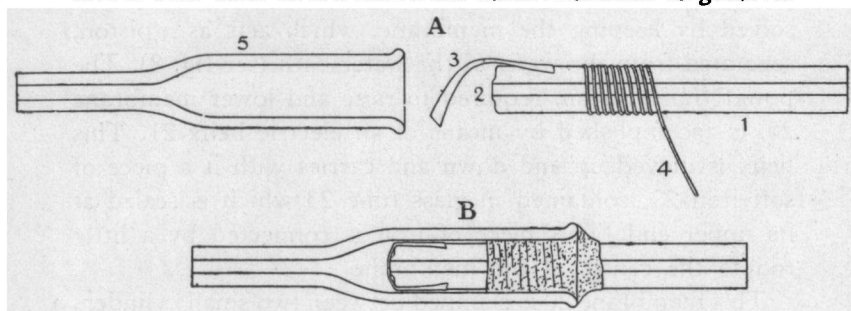


Fig. 9.

surface. Subsequently a slit of extremely thin rubber 3 is fastened over the opening by means of a mixture of melted wax and resin at both ends of the slit, so as to barely close the opening. A pressure difference of a few cm. water in one direction must be sufficient to drive air through it. The same pressure in the other direction must cause the opening 2 to be entirely closed.

The capillary tube 1 is wrapped with a piece of thread 4 so that it fits snugly in tube 5.

With the melted wax and resin mixture, which is poured into the thread packing, the two tubes are fastened to each other as may be seen at B.

A small *gas washing bottle* 37 (fig. 6) containing a small quantity of distilled water, is included in the circulation system. The rate of circulation may be observed from the rate at which the air bubbles through it. It assures a saturated water vapour pressure of the circulating air and prevents the algae from being desiccated.

The different parts of the circulation system are connected to each other by pieces of vacuum tubes in such a way as to secure a contact between the glass, because of the above mentioned high permeability of rubber to CO_2 . The rubber gasket, forming the side walls of the assimilation vessel, was the principal source of danger of CO_2 leakage. This leakage, however, appeared to be very slight and could be taken in consideration by the application of small corrections.

The water bath (fig. 4, 25), in which the circulation system can be placed, consists of a basin with walls of plate-glass and is provided with a stirrer.

The basin is filled with tap water, which must be renewed frequently as bacterial development soon makes it turbid. Even distilled water did not remain clear for a very long time.

The temperature of this water bath could be altered arbitrarily by adding warm or cold water and could be made constant to less than 0.1°C . within 15 minutes, by means of a microburner and an adjustable toluene regulator. For the regulation of temperatures at or below the room temperature, the water bath was provided with a lead tube, bent several times. Through this tube cold tap water was circulated when necessary.

The illumination.

The illumination takes place by means of from one to six 50 candle-power lamps (Philips $\frac{1}{2}$ Watt 6—8 Volts automobile-lights), which are connected with 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 per cent. For higher light intensities I used a Philips projection lamp (± 1650 candlepower), the intensity of which is obviously much more variable as it was connected with the fluctuating city-current.

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

I preferred this manner of illumination to illumination from both sides. The great difference in diffraction index of the air and the cylindrical filaments of the algae would probably have given rise to a lens-action, causing greatly different light intensities in the different parts of the cell.

The aspirator.

The aspirator regulates the rate of the air current by means of mercury, which is run out of a vessel at a constant, adjustable rate.

The manner, in which this was arrived at, is schematically represented in fig. 4. Out of the vessel 29 the mercury flows through a rubber tube 30, a three-way tap 31 and subsequently through a capillary glass tube with a constriction 32.

In this part the mercury current meets a strong resistance. Finally the mercury drips at 33 into the vessel 34 in which it is collected.

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

When the mercury level in 29 goes down, the rapidity of the current will diminish, unless the opening 33 descends at the same rate. This is attained by the following automatic device.

At the three-way tap 31 a capillary tube 35 is attached, which forms communicating vessels with 29. Therefore the mercury menisci in 29 and 35 are at the same level. Capillary tube 35, tap 31, resistance tube 32 and receptacle

34 are mounted together on a block of wood 36, which is movable along a bar 37. This block is attached to a chain 38 suspended over a cogwheel 39, which cogwheel may be fixed with a screw to the shaft 40. By turning this shaft, the propeller 42 is given a swift rotating movement by means of a number of cogwheels 41 (only two of them are shown in the figure). This propeller, and also the shaft 40 connected with it, are stopped when the lever 43 is pulled down. This occurs, when a weak electric current passes through the electromagnet 44. In this manner shaft 40, cogwheel 39 and block 36 are also stopped.

In the capillary tube 35 an iron wire 45 provided with a tiny platinum needle 46 is inserted from above. If this needle comes into contact with the mercury, a current from the storage battery 47 passes through the electromagnet 44, causing the propeller 42 and the shaft 40 to stop. If subsequently the mercury level in 29 (and therefore also in 35) goes down, the contact with the platinum needle is broken, the propeller is released and the block 36 begins to move slowly downward, causing a swift rotation of the propeller. As soon as the platinum needle again reaches the mercury meniscus, 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 tube 35, which position may be read off on a scale 48. Each position corresponds to a definite height of the mercury column and thus to a definite rate of flow. For a number of positions throughout the scale the corresponding rate of mercury flow has previously been tested. So the velocity of the current can be regulated at will from 0 to 160 cc. per hour with an accuracy for the mean velocities of 0.5 per cent.

At the beginning of an experiment the CO_2 content of the air which enters the aspirator 29 has not yet become constant. Only after a certain volume of air has been passed

through the circulation system is an equilibrium attained between supply, consumption and discharge of CO_2 and a constant CO_2 content of the discharged air attained. This air may now be analysed and is for this purpose conducted to a second aspirator 49, provided with the same arrangement as the former one and adjusted at the same velocity of mercury flow. To this end the following preparations are made. The cogwheel 39 is fastened to the frame with a screw (not shown in the figure), so that it can no longer rotate and is released from the shaft 40. The corresponding cogwheel on the right side is now fixed to the shaft. The stopcock above 49 is opened towards tube 27 and the switch 51 is moved to the right.

By opening stopcock 54 and closing stopcock 31 simultaneously the air current may now be diverted from 29 to 49 without its flow being interrupted.

The air in vessel 29 may now be discharged. To accomplish this the mercury, collected in the receptacle 34, is drawn from tap 50 and poured into the vessel 52, subsequently stopcock 28 is turned to the left and by opening stopcock 53 the mercury runs back into aspirator 29 and displaces the air.

Since the right part of tube 27 has not yet been washed out with the air under investigation, it is desirable to divert the air current back again to 29 before collecting the air sample, which has to be analysed.

The collected air is transported to the gas reservoir 38 (fig. 3) by means of a short rubber tube.

Care is again taken to wash out the intervening space. For the sake of simplicity several details have been omitted in fig. 4. For instance tube 27 is not fused together with the vessels 29 and 49, but is attached to it with rubber tubes in such a way, that the glass tubes are in end to end contact with each other. The tube 30 in reality consists of four glass tubes with connections of thick rubber tubing.

A continuous rubber tube, even if it is a vacuum tube, has not a sufficiently constant volume, if it is subjected to different pressures of mercury.

At the lower end of the capillary tube 35 a construction has been made, so that the mercury can flow into or out of this tube only very slowly. This prevents undesirable fluctuations of the mercury meniscus in this tube at every shock or vibration.

The mercury current resistance tubes 32 are connected also to the stopcocks 31 and 54 by rubber tubes. They may be detached for cleaning. Originally these resistance tubes were made by fusing in a flame a small part of a capillary tube until only an extremely small bore remained. After several trials the desired resistance was attained. In such an abrupt constriction, however, a slight film of extraneous material will soon be adhering, even if the mercury has been carefully purified. With a magnifying glass tiny air bubbles then may be observed in this region, which may cause a considerable decrease in velocity of the flow of the mercury.

Therefore these resistance tubes were replaced by others, a 15 cm. portion of which is formed by a tube of ± 0.3 mm. bore. It is preferable to avoid curves in this narrow part.

These tubes need not be cleaned very often. The cleaning is done by sucking through the tube a solution of potassium bichromate in dilute sulfuric acid, afterwards washing carefully with distilled water and drying by means of a current of air. No trace of fluid may remain in the tube.

The resistance of the tube was tested at different heights of the mercury column by measuring in a burette the quantity of mercury, flowing during a given period of time. For each resistance tube a curve was constructed, representing these data graphically. If the velocity of the current is taken as the abscissa and the height of the mercury column as the ordinate, the curve runs somewhat convex to the abscissa.

A slight accelerating influence of increasing temperature

was found (± 0.08 per cent per 1° C.). If this influence is taken into consideration, an accuracy of 0.1 per cent may be attained. I have contented myself, however, with an accuracy of 0.5 per cent for the mean velocities (± 100 cc. per hour). The velocity may be checked during the experiments by counting the number of mercury drops per minute.

The aspirator always must be exactly level.

The experimental equipment, described in this chapter, answers the requirements mentioned at the beginning of the chapter. In the assimilation vessel the supply of CO_2 may be varied first by altering the composition of the buffer solution in the generator, or second by altering the velocity of the air current by means of the aspirator. The difference in CO_2 content of introduced and discharged air may be considerable, so it is possible to determine the CO_2 consumption accurately within several per cent. Nevertheless the gradient of CO_2 concentration within the assimilation vessel is at a minimum because of the swift circulation current, this concentration being almost equal to the concentration of the discharged air to be analysed.

The acidity of the solution, on the surface of which the algae are lying, is altered only to a very slight extent by the different CO_2 concentrations.

By the presence of KH_2PO_4 the reaction of this solution is already slightly acid, even in the absence of CO_2 . On addition of a very small quantity of the weak carbonic acid the hydrogen ion concentration will be augmented but very slightly.

In order to vary the temperature in the assimilation vessel the bath, in which it is placed, is brought to a higher or lower temperature, in the manner already described.

Variations in light intensity are accomplished by using a greater or smaller number of lamps or by means of the powerful projection lamp. The manner in which the diffe-

rent light intensities are calculated is described in the next chapter.

CHAPTER V.

PRELIMINARY EXPERIMENTS, SOURCES OF ERROR, CALCULATIONS AND CORRECTIONS.

The buffer solutions.

In 4 L. of tap water a solution is made of NaHCO_3 and Na_2CO_3 in different proportions, in total amounting always to 0.5 grammol p. L.

Special precautions as to the purity of the salts are hardly necessary in this case, since the carbon dioxide value of each new solution is determined by taking the average of at least five duplicate determinations.

These solutions are kept in 4 L. bottles, stoppered with parafined corks and provided with a siphon. Although the CO_2 concentration of the air, in equilibrium with these solutions at 30°C ., was always determined experimentally, I nevertheless approximated these in advance by calculation using the two dissociation constants of the carbonic acid (k_1 and k_2) and the absorption coefficient of CO_2 in water (α).

From the equations

$$\frac{[\text{H}^+] \cdot [\text{HCO}_3^-]}{[\text{CO}_2]} = k_1 \quad \text{and} \quad \frac{[\text{H}^+] \cdot [\text{CO}_3^{--}]}{[\text{HCO}_3^-]} = k_2,$$

in which the expressions within the brackets indicate the number of grammolecules p. L., the value for the CO_2 concentration in the aqueous solution may be determined:

$$\text{CO}_2 = \frac{k_2}{k_1} \cdot \frac{[\text{HCO}_3^-]^2}{[\text{CO}_3^{--}]}$$

and in the air with which it is in equilibrium:

$$\text{CO}_2 = \frac{1}{\alpha} \cdot \frac{k_2}{k_1} \cdot \frac{[\text{HCO}_3^-]^2}{[\text{CO}_3^{--}]}$$

If one assumes, according to Kolthoff (1923), that $k_1 = 3 \times 10^{-7}$ and $k_2 = 6 \times 10^{-11}$ (for 30° C. probably too low), and $\alpha = 0.66$ and if one further assumes the concentrations of HCO_3^- and CO_3^{--} to be the same as those of NaHCO_3 and Na_2CO_3 , one may approximate the CO_2 concentration in the air in grammolecules p. L., which value may be converted into volume-units.

These calculations were next checked experimentally by gasanalysis for solutions with different proportions of the two salts (total concentration 0.5 grammol p. L.). For this purpose the CO_2 generator was connected directly to the aspirator, the intervening space washed out with a part of the air, which was to be investigated and a sample of air subsequently collected was analysed.

The speed of the air current used in the experiments was about 100 cc. per hour and the buffer solution was introduced at a rate of about one drop per 5 seconds. The CO_2 concentration of the air, which was passed through, proved to be independent of the above velocities.

In table 3 are assembled the CO_2 analyses of four such solutions. In this table, as in all of the others, the CO_2 concentrations are given in units of 0.001 volume per cent.

One sees in this table that a very constant CO_2 content of the air may be attained. Furthermore the duplicate determinations indicate the accuracy of the CO_2 analyses.

The solutions may be kept for months without change in their CO_2 values.

The CO_2 concentration in the assimilation vessel.

When the circulation system contains CO_2 free air and one begins to introduce air, containing a definite amount of CO_2 , the CO_2 concentration increases at first rapidly and then more slowly, finally approaching the asymptote, which has the CO_2 value of the incoming air.

It is possible to determine, both by calculation and by

experiment, how much air must be passed through the circulation system, before an approximately constant CO_2 concentration is attained and the deviations become so small, that they may be neglected.

Calculation.

The entire circulation system may be represented diagrammatically by the vessel A (fig. 10). The CO_2 containing air enters at B and is immediately mixed with the air in the system. It is assumed that the mixing takes place instantaneously. The mixed air leaves the system at C. In the beginning the CO_2 concentration of the vessel is 0. We may now let:

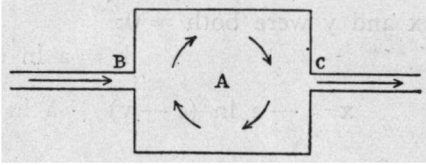


Fig. 10.

x = volume of the air passed through the system,

c = CO_2 concentration of the introduced air,

a = volume of the system,

y = CO_2 concentration within the system.

The CO_2 concentration y is *increased* by the introduction of CO_2 at B and at the same time *decreased* by the withdrawal of CO_2 at C.

If dx air is introduced, it will contain cdx CO_2 , which is distributed throughout the volume A . The increase in the CO_2 concentration is therefore

$$dy = \frac{c}{a} dx.$$

The discharged air has a concentration y ; ydx CO_2 is withdrawn. The resulting decrease in the concentration is therefore

$$dy = -\frac{y}{a} dx.$$

The total increase is thus:

$$dy = \frac{c}{a} dx - \frac{y}{a} dx = \frac{c-y}{a} dx.$$

From this it follows that:

$$dx = a \frac{dy}{c-y} = -a \frac{d(c-y)}{c-y}.$$

By integration:

$$x = -a \ln (c-y) + k.$$

In order to determine the value of the integration constant k , one may keep in mind that in the beginning x and y were both = 0:

$$k = a \ln c$$

$$x = -a \ln (c-y) + a \ln c = a \ln \left(\frac{c}{c-y} \right).$$

One may now transpose y :

$$\frac{y}{c} = 1 - \frac{1}{e^{x/a}}.$$

In order to determine how much air it is necessary to pass through the system, in order that the concentration within the system has increased to 98 per cent of the introduced air, one may substitute:

$$\frac{y}{c} = \frac{98}{100} \text{ and one finds: } \frac{x}{a} = 3.91.$$

This tells us that 3.91 times the volume a must be passed through the system.

A constant consumption of CO_2 within the system does not alter the principle. It may be considered to decrease the amount of introduced CO_2 by a constant fraction b .

The increase in concentration then becomes

$$c \frac{(1-b)}{a} dx,$$

the y approaches asymptotically the final value $c(1-b)$ instead of c , as before.

Therefore one may simply substitute $c(1-b)$ for c in the formula.

We may now collect the discharged air in portions, each having a volume of $2a$. What will be the concentration of CO_2 in the first, second etc. portions?

To arrive at the answer the amount of CO_2 introduced into and withdrawn from the system is calculated.

Introduced cx ,

consumed bcx ,

present in the system ay ,

therefore the amount withdrawn from the system $cx - bcx - ay$.

For the first portion $x = 2a$. Remembering that

$$y = c(1-b) \left(1 - \frac{1}{e^{x/a}}\right),$$

the first portion must contain:

$$ac(1-b) \left(2 - 1 + \frac{1}{e^2}\right).$$

When $x = 4a$ the total amount of CO_2 discharged will be:

$$ac(1-b) \left(4 - 1 + \frac{1}{e^4}\right).$$

The second portion then will contain the above amount less the amount present in the first portion:

$$ac(1-b) \left(2 - \frac{1}{e^2} + \frac{1}{e^4}\right).$$

Similarly the third portion will contain the following amount of CO_2 :

$$ac(1-b) \left(2 - \frac{1}{e^4} + \frac{1}{e^6}\right).$$

The volume of each portion is $2a$. One may determine the CO_2 concentrations by dividing the above amounts by the volume $2a$:

$$\text{1st portion: } c(1-b) \frac{2 - 1 + \frac{1}{e^2}}{2} = c(1-b). 0.568.$$

$$\text{2nd portion: } c(1-b) \frac{2 - \frac{1}{e^2} + \frac{1}{e^4}}{2} = c(1-b). 0.941.$$

$$\text{3rd portion: } c(1-b) \frac{2 - \frac{1}{e^4} + \frac{1}{e^8}}{2} = c(1-b). 0.992.$$

The deviations from the final value $c(1-b)$ are in the first portion 43.2 per cent, in the second portion 5.9 per cent and in the third only 0.8 per cent.

According to this calculation the discharged air may be safely collected for analysis after $4 \times$ the volume of the system has been passed through.

It is thus obvious that the smaller the volume of the circulation system, the better. For this reason every effort was made to keep the volume of the assimilation vessel and the circulation pump at a minimum.

The calculations were now checked by experiments. For this purpose it was necessary to know the volume of the circulation system.

This was measured by closing the intake tube and by increasing the air pressure in the system through the discharge tube by means of a water column. The height of the water column, together with the decrease in the volume of the air resulting from the pressure, were measured and from these values the volume of the circulation system was calculated. It was found to amount to ± 17 cc.

In the control experiments portions of air of 35 cc each were collected and analysed for their CO_2 content. In the following table the results and the deviations from the theoretical final CO_2 value are given.

TABLE 4.

		per cent deviation		per cent deviation	calculated per cent deviation
CO ₂ in introduced air, in units of 0.001 per cent .	203	—	460	—	—
Same in 1st 35 cc. dis- charged air	120	41	246	47	43
Same in 2nd 35 cc. dis- charged air	188	7	409	11	6
Same in 3rd 35 cc. dis- charged air	199	2	446	3	1

It is evident that the results agree fairly well with the calculations. If one continues the experiment long enough, one may expect that the CO₂ concentration of the discharged air will in time equal that of the introduced air. This will, however, not be the case because of the minute CO₂ leakage in the circulation system, primarily through the rubber gasket with which the assimilation vessel is sealed.

At a CO₂ concentration of 450×0.001 per cent (I shall henceforth express CO₂ concentrations in terms of 0.001 per cent.) I found an average leakage of 10 cmm. per hour.

Assuming that the leakage is directly proportional to the CO₂ concentration in the circulation system, it may be calculated for any given concentration. For instance, at the concentration 200 the leakage calculated was 4.4 cmm. per hour and that determined experimentally 5 cmm. per hour.

Calculation of CO₂ assimilation.

By CO₂ assimilation is meant the CO₂ consumption under illumination plus the CO₂ evolution in the dark (respiration).

CO₂ consumption and evolution are calculated from the

difference between the quantities of CO_2 introduced into and discharged from the assimilation vessel and are expressed in cmm. per hour.

In the tables the columns CO_{2i} and CO_{2d} indicate the CO_2 concentration of the introduced and discharged air respectively in terms of 0.001 volume per cent, that is to say, *the number of cmm. CO_2 per 100 cc.* When the velocity of the air current amounts exactly to 100 cc. per hour, those columns indicate therefore also the number of cmm. CO_2 which are introduced and discharged per hour.

When the velocity of the air current is greater or less, the introduction and discharge of CO_2 is proportionately greater or less; for instance when the velocity amounts to 0.6×100 cc. = 60 cc. per hour, the introduction and discharge of CO_2 are computed by multiplication of the data in columns CO_{2i} and CO_{2d} by 0.6.

If a balance-sheet is made out for the CO_2 in the assimilation vessel, the following equation may be obtained:

Assimilation = introduction + respiration — leakage — discharge.

Usually a dark experiment with CO_2 free air was made previous to each set of experiments, in order to determine the respiration rate. In some cases, however, I introduced air, having the same CO_2 content as was used in the subsequent experiments. In these cases therefore I determined experimentally:

introduction + respiration — leakage.

Corrections.

1. *Corrections for leakage of CO_2 .*

As stated on page 213 each per cent CO_2 present within the assimilation vessel causes a loss of 22 cmm. CO_2 by leakage. This quantity must be added to the quantity of CO_2 discharged, to obtain the true amount of CO_2 which has left the assimilation vessel.

In experiments in which this CO_2 concentration amounted to no more than 100×0.001 per cent, this correction was neglected, as being within experimental errors.

2. *Corrections for growth.*

The algae are growing during the experiments, but only very slowly. However, since the duration of one series of experiments is often considerable (12 hours and more), it was considered desirable not to neglect this source of error.

As it was impossible to measure or to weigh the filaments at the beginning and the end of the experiment, the rapidity of growth was determined in the following way. Before and after each series of experiments the assimilation was determined under exactly the same conditions, viz. the same temperature and light intensity and an excess of CO_2 . The increase due to growth could be ascertained from the increase of CO_2 assimilation during this time and the growth per hour was computed therefrom.

Since many of the experiments covered a very long period the growth was not determined for each series of experiments, but only in certain instances. The rapidity of growth found on these occasions was used as a correction in other experiments. It is assumed that the increase due to growth is a rectilinear one.

The time at which the first determination of assimilation was made is taken as the zero point of time. The subsequent determinations were reduced to this point by subtracting the calculated percentage due to growth.

The determinations of the increase due to growth are assembled in table 5. At 20°C . the average increase amounted to 1.04 per cent per hour and at 12° amounted to 0.42 per cent per hour. The results from the different experiments show considerable deviations from each other. Apparently I did not control this factor entirely. But since only very small corrections are involved, this fact is of little importance.

In the experiments in which the growth was not determined, I applied the following growth corrections.

For 12° C.:	0.4	per	cent	per	hour.
„ 16° C.:	0.7	„	„	„	„
„ 20° C.:	1.0	„	„	„	„
„ 24° C.:	1.3	„	„	„	„

3. Corrections for the gradient of CO₂ in the assimilation vessel.

These corrections do not consider the assimilation itself, but the CO₂ concentration at which it takes place.

Although the velocity, with which the air is circulating through the assimilation vessel (± 5 L. per hour), is very great in comparison with the velocity of the supply and discharge current (40—60 cc. per hour) there must be nevertheless a small gradient of CO₂ within the assimilation vessel, when CO₂ is being consumed. This gradient is calculated as follows.

Five liters of air are passed through the assimilation vessel in one hour. In this hour a cmm. of CO₂ are consumed. The air, after being passed through the assimilation vessel, contains therefore a cmm. CO₂ per 5 L. or $\frac{a}{50} \times 0.001$ per cent CO₂ less than before.

The air is collected for analysis as it leaves the assimilation vessel. The difference between the CO₂ concentrations in this air and the *mean* CO₂ concentration in the assimilation vessel is half of the above mentioned amount, that is

$$\frac{a}{100} \times 0.001 \text{ per cent. } ^1).$$

¹⁾ It is assumed that there is a rectilinear decrease of CO₂ within the assimilation vessel. Although this will not be the case under all conditions, the errors, caused by this circumstance, are well within the experimental errors.

This correction was applied only in experiments concerning the influence of the CO_2 concentration.

The unit quantity of algae.

In order to obtain comparable results from different experiments, the resulting data have to be expressed in terms of a definite unit of cell material. Ordinarily 1 gm. dry weight is taken as a unit.

The quantities used in my experiments, however, were far too small to be weighed with the necessary accuracy. Therefore I have chosen another unit, to wit: the quantity, which assimilates 100 cmm. CO_2 per hour under certain, well defined conditions.

These conditions are: excess of CO_2 , illumination with 6 lamps (light intensity 6—18) and temperature 20°C . The temperature is a limiting factor under these conditions.

In the graphs, therefore, all curves coincide in the point indicating the above conditions (see fig. 12).

The unit of cell material, as defined above, contains 40 to 50 M. of filaments and has a weight of ± 1 mgm. when air dry.

After the different corrections have been made, the observed assimilation rates were converted to this unit by multiplying by a factor, which was different in each experiment. I have called this factor the *conversion factor*. The values converted in this manner are given in the last column of each table. The values, which have served to calculate the conversion factor are printed in italics in this column.

Calculation of light intensity.

I did not care to know the absolute light intensity of the different lamps, but only their relative intensities.

Measurement of the candlepower values by means of a visual method was not suitable to my purpose. For two intensities, which appear equal to the eye, may have different

assimilation values, because of differences in distribution of the light intensities over the spectrum.

Bolometer measurements of the total radiant energy are not suitable for the same reason. The only correct method is to use the algae themselves as a photometer. At the low intensity of one lamp, light is the limiting factor and the assimilation velocity is directly proportional to the light intensity (the validity of this assumption is proved in the next chapter). The intensity of one of the lamps (*viz.* lamp A) was arbitrarily taken as a unit and the intensity of the other lamps and also of the projection lamp are expressed in terms of this unit.

The distance from the filaments of the small lamps to the glass wall of the water bath remained the same in all experiments, *viz.* 3 cm. In all experiments in which light was the limiting factor, the algae were confined to the middle third of the assimilation vessel. Although the distance from the algae was not quite the same for the different lamps, this distance remained the same for each individual lamp in all experiments.

The distance from the filament of the projection lamp to the glass wall of the water bath was 75 cm. in the experiment mentioned above, and 12 cm. in the other experiments. The relation between this distance and the light intensity in the assimilation vessel is shown by the following calculation. (see fig. 11).

When the glass wall of the water bath is situated at B and the assimilation vessel at C, the light of a lamp, which is placed at A_1 , travels through air over the distance a_1 and through water over the distance b . The thickness of the two glass walls may here be neglected. A ray of light projected from the lamp at an angle α , attains a diameter $c_1 + d$ in the assimilation vessel.

If the lamp is now moved to A_2 , the same ray of light will now have a diameter in the assimilation vessel of

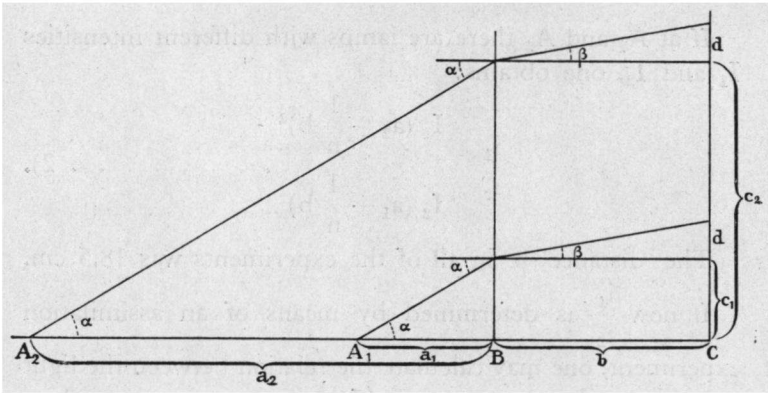


Fig. 11.

$c_2 + d$. The intensities of light in the vessel in these two cases (i_1 and i_2) are inversely proportional to the squares of these diameters:

$$\frac{i_1}{i_2} = \frac{(c_2 + d)^2}{(c_1 + d)^2}$$

Taking $c_1 = a_1 \operatorname{tg} \alpha$ and $c_2 = a_2 \operatorname{tg} \alpha$
 $d = b \operatorname{tg} \beta$

It follows from this, that:

$$\frac{i_1}{i_2} = \frac{\left(a_2 + \frac{\operatorname{tg} \beta}{\operatorname{tg} \alpha} b\right)^2}{\left(a_1 + \frac{\operatorname{tg} \beta}{\operatorname{tg} \alpha} b\right)^2}$$

At small angles of incidence $\frac{\operatorname{tg} \beta}{\operatorname{tg} \alpha}$ is approximately equal to $\frac{\sin \beta}{\sin \alpha}$, or the inverse value of the refraction constant n of water ($n = 1.33$)

$$\frac{i_1}{i_2} = \frac{\left(a_2 + \frac{1}{n} b\right)^2}{\left(a_1 + \frac{1}{n} b\right)^2} \dots \dots \dots 1).$$

If at A_1 and A_2 there are lamps with different intensities I_1 and I_2 , one obtains:

$$\frac{i_1}{i_2} = \frac{I_1 (a_2 + \frac{1}{n} b)^2}{I_2 (a_1 + \frac{1}{n} b)^2} \dots \dots \dots 2).$$

The distance b in all of the experiments was 18.5 cm.

If now $\frac{i_1}{i_2}$ is determined by means of an assimilation experiment, one may calculate the relation between the light intensities of the two lamps $\left(\frac{I_1}{I_2}\right)$.

The results of the series of experiments, in which the light intensity of each lamp was measured separately, are given in table 6. From the values obtained I calculated the light intensity of the projection lamp to be 31.0 times that of lamp A.

In the following experiments the distance a for the projection lamp was 12 cm, from which an intensity of 13.2 in the assimilation vessel was calculated following formula 1.

Further sources of error.

The partial drying of the algae remains still to be mentioned as a source of error. Although a gas washing bottle was inserted in the circulation system, it sometimes happened that the film of solution, on which the algae were resting, was partially evaporated. I observed in such cases a considerable decline in assimilating power; the experiments were thus rendered worthless.

A small number of bacteria were present in the cultures; this number was probably not always the same. To this circumstance I ascribe the fact, that the relation of respiration to assimilation was not always identical.

However, since the respiration value was always deter-

mined previously, I am of the opinion that the presence of bacteria did not constitute a source of error.

CHAPTER VI.

EXPERIMENTS AND RESULTS.

In the following experiments the relation was determined successively between the light intensity, temperature and CO_2 concentration on one hand and the assimilation velocity

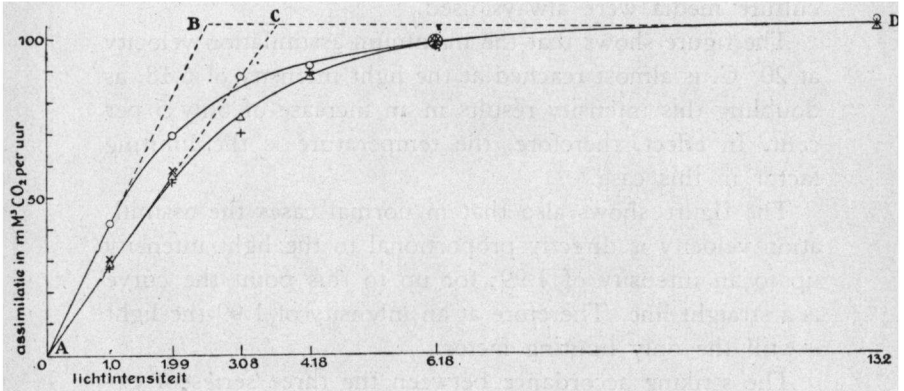


Fig. 12.

on the other hand. In this way a number of corresponding curves was obtained.

The influence of the light intensity.

The light intensity was varied by illuminating with 1, 2, 3, 4 or 6 lamps or with the projection lamp. In these experiments the temperature was 20° C. The CO_2 was kept in excess, that is to say in such a concentration, that it exerted no influence upon the assimilation rate. It was found from several preliminary experiments, that this was the case at CO_2 concentrations above 40×0.001 per cent.

The results are graphically represented in fig. 12, in which

the light intensity is taken as the abscissa and the assimilation velocity as the ordinate.

The signs +, Δ and \times represent three series of experiments with algae, which were cultivated upon fresh culture media (see tables 7, 8 and 9 respectively). The sign O represents a series of experiments made with algae upon an old, exhausted nutrient solution. As the results differ in these two cases, it is evident, that the algae must be cultivated under constant conditions in order to obtain comparable results. As a precaution in this direction algae on fresh culture media were always used.

The figure shows that the maximum assimilation velocity at 20° C. is almost reached at the light intensity of 6.18, as doubling this intensity results in an increase of only 5 per cent. In effect, therefore, the temperature is the limiting factor in this case.

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

The striking accordance between the three series of experiments indicate a great uniformity in the material. It is a corroboration of my view that assimilation may be taken as a measure for the quantity of material.

The influence of the temperature.

The assimilation was determined at a high light intensity (viz. 6.18) at 12°, 16°, 20° and 24° C. consecutively.

The results of the experiments (given in the tables 11 and 12) are represented in fig. 13 by the signs O and Δ .

Between 12° and 20° C. an almost constant $Q_{10} = 1.87$ is evidenced by these determinations.

Not to much reliance may be placed upon the determinations at 24° C. The S shaped bending of the curve possibly

may be explained by the assumption that at this high assimilation rate the light intensity exerts a certain depressing influence.

Also the "time factor" may enter into play. This part of the curve is therefore represented only by a dotted line.

At a low light intensity (viz. 1.00) the assimilation velocity

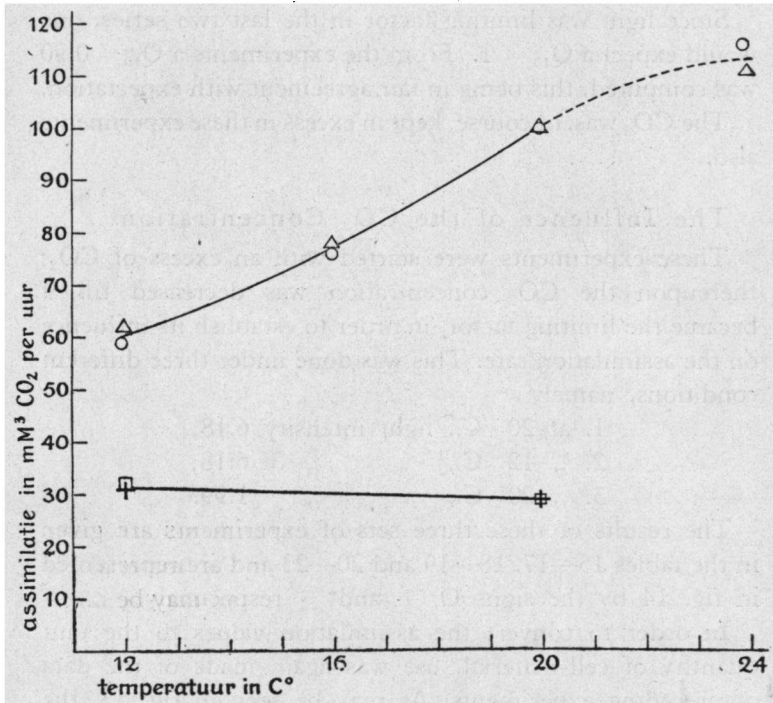


Fig. 13.

was also determined at different temperatures, namely at 12° and 20°C . (see the tables 13 and 14 and fig. 13, signs + and □).

In order to convert the assimilation rates, found in these experiments, to the unit quantity of material previously mentioned, I made use of the results given in the previous paragraph (see fig. 12).

Those experiments showed, namely, that the unit quantity in question assimilates 29 cmm CO_2 per hour at 20°C . and light intensity 1.00.

The assimilation velocities under these conditions are therefore taken as 29 in tables 13 and 14 (printed in italics) and the remaining values are calculated accordingly.

Since light was limiting factor in the last two series, one would expect a $Q_{10} = 1$. From the experiments a $Q_{10} = 0.90$ was computed, this being in fair agreement with expectation.

The CO_2 was, of course, kept in excess in these experiments also.

The Influence of the CO_2 Concentration.

These experiments were started with an excess of CO_2 ; thereupon the CO_2 concentration was decreased till it became the limiting factor, in order to establish its influence on the assimilation rate. This was done under three different conditions, namely

1. at 20°C ., light intensity 6.18;
2. „ 12°C ., „ „ 6.18;
3. „ 20°C ., „ „ 1.99.

The results of these three sets of experiments are given in the tables 15—17, 18—19 and 20—23 and are represented in fig. 14 by the signs O, + and Δ respectively.

In order to convert the assimilation values to the unit quantity of cell-material, use was again made of the data of preceding experiments. As may be seen in fig. 13, the unit quantity assimilates on an average 61 cmm. CO_2 at 12°C . and a light intensity 6.18. In fig. 12 it is shown that the same unit quantity assimilates 57 cmm. CO_2 at 20°C . and a light intensity 1.99.

The assimilation velocities, observed under these conditions and at the highest CO_2 concentration found in the series, were therefore taken as 61 and 57 respectively (printed in italics).

In this manner the effects of light and temperature were studied in that part of the curve, in which CO_2 is the limiting factor. As may be seen in fig. 14, this part is a straight line almost to the "transition point"; in this part of the curve temperature and light intensity are apparently without any influence on the assimilation velocity (as far as the accuracy

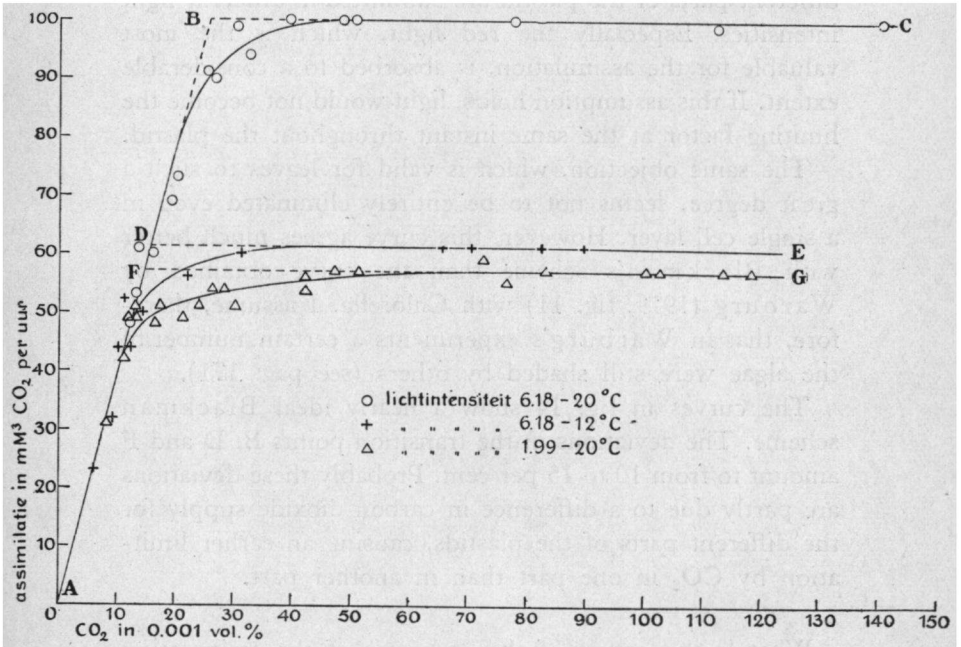


Fig. 14.

of the experiments permit a conclusion). For here (on the line AF) the curves for high and low light intensity as well as for high and low temperature coincide exactly.

Discussion of the results.

When the results are examined in connection with the discussions in Chapter I and II, it is evident that Blackman's formula gives a fairly good description of the assi-

milation behaviour of the single cell. In figures 12 and 14 the "ideal Blackman curves" are represented by dotted lines. The greatest deviations are found in fig. 12, where they amount to 30 and 25 per cent respectively at the transition points B and C. I might ascribe these to the fact, that even in a single chloroplast much light is absorbed and the different parts of the plastid are submitted to different light intensities. Especially the red light, which is the most valuable for the assimilation, is absorbed to a considerable extent. If this assumption holds, light would not become the limiting factor at the same instant throughout the plastid.

The same objection, which is valid for leaves to such a great degree, seems not to be entirely eliminated even in a single cell layer. However, this curve agrees much better with Blackman's scheme than the curve obtained by Warburg (1919, fig. 11) with *Chlorella*. I assume, therefore, that in Warburg's experiments a certain number of the algae were still shaded by others (see pag. 171).

The curves in fig. 14 show a nearly ideal Blackman scheme. The deviations in the transition points B, D and F amount to from 10 to 15 per cent. Probably these deviations are partly due to a difference in carbon dioxide supply for the different parts of the plastids, causing an earlier limitation by CO_2 in one part than in another part.

What is the nature of the processes of the assimilation chain which may alternately control the rate of assimilation?

When *temperature* is the limiting factor, the properties of the assimilation process are such as would be expected in a dark chemical process, although the $Q_{10} = 1.87$ is rather low (see pag. 153).

Similarly, when *light intensity* is the limiting factor, the properties of the assimilation process are to be considered as agreeing with the properties of a photochemical process (see pag. 153).

But what is the situation when the CO_2 concentration is the limiting factor?

Evidence will here be presented that notwithstanding the small dimensions of the object, the assimilation velocity under these conditions is governed by a *diffusion process*.

It was maintained on page 153 that, if all CO_2 entering the chloroplast is immediately consumed and the partial CO_2 pressure in the plastid is practically zero, there must exist a rectilinear relation between the CO_2 concentration in the medium and the assimilation rate.

Such a rectilinear relationship appears in fact in the line A F D B in fig. 14.

In this part of the curve the light has no influence, as is to be expected in a diffusion process.

However, one would be inclined to expect an influence of the temperature as a Q_{10} of from 1.2 to 1.3 is generally stated for diffusion processes.

On second thought this consideration is not correct. We may consider the cell wall and the protoplasm, through which the CO_2 has to diffuse, as a water-like medium. Before penetrating into the cell the CO_2 has to dissolve in the outermost layer of the cell wall.

It may be taken for granted, that in this outermost layer the CO_2 concentration is in equilibrium with that of the adjacent air¹⁾.

Now the solubility of CO_2 in water decreases with increasing temperature. The so called absorption coefficient is

¹⁾ Romell (1926 b) does not agree with this conception and is of opinion that the surface boundary constitutes a resistance against diffusion. He applies (although with reservations) the so called "invasion coefficients", experimentally determined by Bohr. Since Krogh (1919) has proved, however, that the apparent "invasion coefficient", as determined in experiments, is in reality caused by the resistance in diffusion through a very thin film of liquid, it may be considered superfluous to take Romell's conception into further consideration.

± 1.1 . at 12° C. and ± 0.9 at 20° C. Therefore, with this temperature increase of 8° C the concentration in the outermost layer will be reduced to $9/11$, causing also the same decrease in the concentration gradient. The diffusion velocity is increased by a factor 1.3 at each temperature increase of 10° C., at an increase of 8° C. therefore by a factor $(1.3)^{8/10}$

When taking these two factors together it is clear that the total diffusion process will be increased by a factor $(1.3)^{8/10} \times 9/11 = \pm 1$. This explains the $Q_{10} = 1$, as found in the experiments.

The diffusion resistance.

Is the process in question really one of diffusion?

Assuming that it is a diffusion process, the diffusion resistance is known from the data of the experiments. When the thickness of a water film, which would have the same diffusion resistance, is calculated, this thickness ought to be of the same order of magnitude as the length of the diffusion path in the cell.

In order to calculate this, it was necessary to know the total surface of the cells, available for the penetration by CO_2 . This was determined approximately in the following way.

The total quantity of algae used in an assimilation experiment, is washed into a small flask containing a hot 10 per cent gelatine solution. This solution is made up to 100 gm. Subsequently it is thoroughly stirred, so that a very equal distribution of the filaments in the solution is accomplished. The filaments usually break into small pieces.

Several cc. of this hot solution are poured into a glass dish and allowed to congeal. A very small cube is cut from this mass and carefully weighed on a glass microscope slide to an accuracy of 0.1 mgm. Subsequently a cover glass is placed over it and the mount is warmed carefully over a small flame, so that the gelatine spreads as a thin film under the cover glass.

Under the microscope this film is systematically examined with the aid of a mechanical stage. By means of a camera lucida each filament is drawn in its relative size on a sheet of paper. The enlargement obtained in this way is determined by means of an objective-micrometer.

The total length of the lines drawn on the paper is measured by means of a curvimeter and the total length of the filaments present in the small block of gelatine calculated therefrom. If it is assumed, that the distribution is equal throughout the gelatine, the number of meters of filament, used in the experiment, may be computed.

Since it is important to know the total length of the filaments, contained in the unit quantity of algae, the calculated total length is multiplied by the „conversion factor” derived from the last determination of each series of experiments under consideration. In this manner the number of meters of filaments contained in a quantity of algae, which assimilate 100 cmm. CO₂ at a light intensity of 6.18, a temperature of 20° C. and an excess of CO₂, is found.

The results of two duplicate determinations are given in table 24.

TABLE 24.

No.	Assimilation experiment from table	Gelatine weighed	Length measured	Total length	Conversion factor	Length of the unit quantity of algae
1a	19	18.8 m.gm.	21.2 mm.	112.9 m.	61	41.7 m.
b		15.5 „	14.6 „	92.5 „	165	34.2 „
2a	14	26.6 „	45.2 „	170.4 „	29	59.4 „
b		28.6 „	39.1 „	136.6 „	83	47.6 „
Mean						45.7 m.

Assuming that the cylindrical filaments are projecting half-way above the film of solution on which they rest,

the surface available for penetration by CO_2 may be calculated to be 6 qcm.

In the formula for the diffusion velocity:

$$S = \frac{k q}{l} (C_1 - C_2)$$

all required values are now known.

For the sake of simplicity I perform the calculation for a temperature of 15°C ., since the absorption coefficient of CO_2 in water happens to be 1 at this temperature and the outermost surface film of the water has therefore the same CO_2 concentration (C_1) as the adjacent air.

S indicates the quantity of CO_2 which diffuses during one day, expressed in arbitrary units (I shall use as a unit 1 cmm.).

$(C_1 - C_2)$ is the difference in concentration between the beginning and the end of the diffusion path, expressed in the same units per cc. According to our assumption $C_2 = 0$.

l indicates the length of the diffusion path in cm.

The values S and C_1 are to be found in fig. 14, for the line AB is valid for all temperatures and, therefore, also for 15°C . It is shown that at a CO_2 concentration of 10×0.001 per cent. ($C_1 = 0.1$) the diffusion velocity amounts to 40 cmm. per hour or 960 cmm. per day ($S = 960$).

When we substitute values for the diffusion constant of CO_2 in water at 15°C . ($k = 1.4$ qcm/day) and for the cross section of the diffusion path ($q = 6$ qcm.) the thickness l of the water film may be calculated to be 8μ .

As has been said previously the cells have a diameter of about 8μ . The diameter of the chloroplasts amounts to as much as 4μ at some places. As it is very probable that the diffusion of CO_2 in the cell wall and the protoplasm proceeds slower than in water, the agreement in order of magnitude may be considered satisfactory.

The hypothesis, that a diffusion process is concerned here, I consider to be proved, as it has fulfilled four different

criteria, namely the influence of CO_2 concentration, light intensity, temperature and the order of magnitude of diffusion resistance.

Warburg's opinion, that diffusion resistance might be neglected in *Chlorella* cells, I believe to be incorrect, especially so, since in Warburg's experiments CO_2 became limiting at about the same concentration as in mine.

When CO_2 is the limiting factor Warburg finds, it is true, an extremely high temperature coefficient ($Q_{10} = 5$); however, he records only one determination of this coefficient in his paper (1919, table IV No. 1). A possible injurious influence of the rather alkaline buffer mixture might have had something to do with this phenomenon.

According to my previous discussion, the diffusion velocity is directly proportional to the CO_2 concentration outside the cell, as long as this concentration remains zero inside the cell. Assuming that the process in question is one of diffusion, it may conversely be said that, as long as a rectilinear relationship exists, the CO_2 concentration inside the chloroplast remains practically zero and as soon as the rectilinear relationship ceases, the concentration within the chloroplast is increased to above zero.

Now it may be seen in fig. 14 that the deviations from the straight line A B occur only when the maximum assimilation rate is nearly reached. At a CO_2 pressure within the chloroplast, in equilibrium with a concentration of 0.001 per cent. in the air, the assimilation process is already working almost at full speed.

We are forced to the conclusion, that at the spot, at which the reaction subsequent to the diffusion process takes place, almost an equal amount CO_2 is present at this pressure as at much higher CO_2 pressures.

The assimilating agent (either chlorophyll or an enzyme) possesses an enormous affinity for CO_2 , so that it is able to saturate itself almost entirely with CO_2 at a pressure

corresponding to a concentration of 0.001 per cent. CO_2 in the air or $\frac{1}{100.000}$ atm.

In the last chapter I shall return to this subject.

CHAPTER VII.

THE TRANSITION POINT AND THE ASSIMILATION NUMBER. THE ASSIMILATION QUOTIENT.

The Significance of the transition point.

I have defined the transition point as the point, at which two factors exert the same influence on the assimilation process. It is the point, at which two processes would have the same velocity if they were allowed to proceed unhampered.

The transition point indicates the proportion of the capacities of two processes and likewise the proportion of the factors present in the plant, which govern those processes.

The transition point from CO_2 limitation to temperature limitation, for instance, defines not only a diffusion velocity as compared with a reaction velocity, but may also be considered to be a measure of the proportion of a structural factor (diffusion resistance) to a chemical agent (Willstätter's „enzymic factor“).

In a leaf, for instance, the CO_2 will penetrate with much more difficulty than in an *Hormidium* cell, due to differences in structure. In the presence of equal amounts of „enzymic factor“ the transition point will be found at a much higher CO_2 concentration in the case of the leaf than in the case of the *Hormidium* cell. That is to say, the line A B in fig. 14 will make a much wider angle with the ordinate.

Likewise the transition point from limitation by light to limitation by temperature indicates not only the proportion between the velocities of a photochemical and a dark

chemical reaction, but also the proportion between the quantities of a photochemical and a dark chemical agent.

I might therefore attribute to the transition point a fundamental significance. This significance, however, exists only when the external factors influencing the processes in question are completely and quantitatively defined.

Such a definition is applicable in the case of the diffusion process and the dark chemical reaction, since the rates of these two processes are entirely fixed by the concentration of the CO_2 and by the temperature respectively.

With the photochemical process this is not the case, since it is determined by the light intensity. To define the velocity of this process in terms of intensities of light, one ought to know:

1. the intensity of the light in absolute units;
2. the energy distribution over the spectrum;
3. the distribution of light intensity within the cell.

It is obvious that such a definition is completely impossible. However, it is possible to determine the proportion between the *velocity* of the dark chemical reaction and the *quantity* of the photochemical agent (chlorophyll) present in the plant, although it is thus a comparison between two values having quite different natures. This proportion is represented by Willstätter's assimilation number (1918) the definition of which is expressed as follows:

$$\frac{\text{Assimilation of } \text{CO}_2 \text{ in grams per hour}}{\text{chlorophyll content in grams.}}$$

Of course this number must be determined under conditions, in which temperature is the limiting factor. This involves the tacit assumption, that the chlorophyll has the same activity in all plants. Since Willstätter has stated the identity of chlorophyll in all plants, the correctness of this assumption may be considered probable.

Determination of the assimilation number.

In order to determine the chlorophyll content Willstätter made use of a colorimetric method. The standard solution required for that purpose has to be freshly prepared each time by saponification of a definite quantity of pure chlorophyll.

Now pure chlorophyll is not available to most investigators and the preparation of the pure substance is by no means easy. A second great disadvantage is the instability of the standard solution¹⁾.

Weigert (1916), however, has elaborated a spectrophotometrical method, by means of which chlorophyll concentrations can be determined without the use of a standard solution.

The principle of this method may be briefly pointed out. When in a certain narrow region of wave length $\lambda + d\lambda$ the law of Lambert-Beer is applicable (for instance when dilution does not alter by dissociation the color of the diluted substance) the absorption of light by the colored substance may be expressed by the following formula, as given by Bunsen:

$$\epsilon_{\lambda} = \left(\log \frac{I_0}{I} \right)_{\lambda} = k_{\lambda} c d.$$

In this formula ϵ_{λ} indicates the so called extinction, I_0 the intensity of the incident light, I the intensity of the light transmitted by the colored substance (the absorption by the solvent not included), c the concentration in gm. per cc, d the thickness of the absorbing layer in cm. and k_{λ} a constant, specific for each different substance and each different wave length.

After ϵ has been determined for a number of wave lengths

¹⁾ Shortly before the conclusion of my experiments Guthrie (1928) published an article in which a stable, inorganic standard solution is described.

in the visible spectrum for a solution with a concentration, c , an extinction curve may be constructed by plotting the different wave lengths on the abscissa and the corresponding extinctions on the ordinate. Then a curve is obtained which shows one or more maxima, corresponding to the absorption bands of the substance.

When similar extinction curves are constructed for the concentrations $2c$ and $4c$, it may be seen at once from the formula that at each λ the ordinates increase in height by 2 times and 4 times respectively; the curves take on a different shape with the maxima extended in the direction of the ordinate.

If, however, instead of ϵ the logarithm of ϵ is plotted, multiplications are converted to additions and one obtains the curves for $2c$ and $4c$ by a displacement of the entire curve parallel to the ordinate by the amounts $\log 2$ and $\log 4$ respectively.

Such a curve, the shape of which is independent of concentration, characterises completely a given colored substance and is therefore called by Weigert a "typische Farbkurve".

If this curve is known for the unit of concentration and one afterward determines the curve for an unknown concentration, the latter may be calculated by determining the distance by which it must be displaced, in order to make it coincide with the curve of the known concentration.

Weigert determined the "typische Farbkurve" for a mixture of the chlorophylls a and b in their natural proportions, which he obtained from Willstätter. This curve is shown at A in fig. 15. It is computed for a layer 1 cm. in thickness at a concentration of 1 gm. per cc.

The curve B , drawn at an arbitrary distance below the first, was obtained by Weigert for an extract of spinach leaves in 85 per cent acetone. If the one is superimposed upon the other (this is facilitated by the use of transparent

paper) it may be seen that the curves coincide at wave lengths above 550 $\mu\mu$.

Below this wave length the carotin and xanthophyll present in the leaf extract begin to show considerable ab-

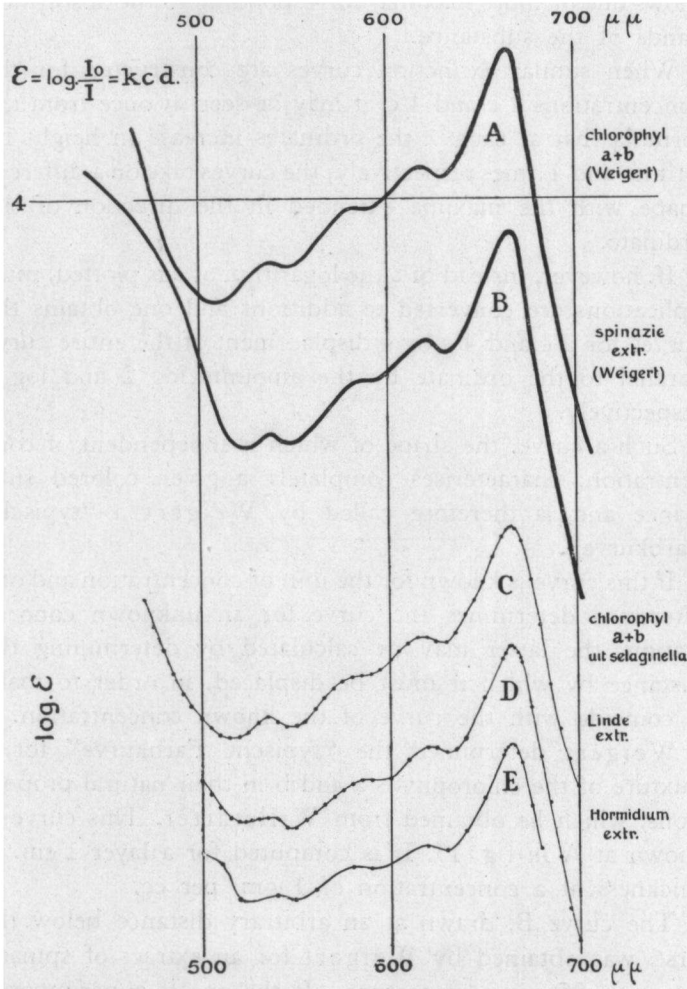


Fig. 15.

sorption, with the result, that the curve for the leaf extract is much higher in the blue region of the spectrum.

Even though the chlorophyll has not been separated from its accompanying yellow materials, it is possible to determine its concentration.

In this way I proceeded to determine the chlorophyll content of my algae. I first tried to do this with an apparatus consisting of a van Cittert monochromator and Moll thermopile. It appeared, however, that the wave length range of the light to be measured could not be reduced to a sufficient extent, without considerable changes in the arrangement and I was compelled to use another instrument. I must not neglect to acknowledge my indebtedness to Prof. Dr. L. S. Ornstein and Dr. P. H. van Cittert for the pains which they took to familiarise me with the technique of spectrophotometry.

The instrument, with which the chlorophyll determinations were finally made, was a "Color Analyser" of Keuffel and Esser Co.

I wish at this point to thank Prof. Dr. N. Schoorl for his kind permission to use this excellent instrument. I also wish to thank Prof. Dr. T. M. Kolthoff for his help.

As for the construction of the apparatus the reader is referred to a short description by van Tusschenbroek (1927). I will mention here only a few particulars. Two identical beams of light pass through two tubes 10 cm. long, one of which contains the solution, the other the pure solvent. Subsequently the light passes through a photometer in which are two rapidly rotating sector discs, which intermittantly allow the light to pass through. This light, however, appears to the eye to be continuous. The percentage, which is permitted to pass through, may be altered by varying the angles of the sectors. The proportions of light in the two beams may thus be changed at will by means of a graduated drum.

The beams of light subsequently pass through a spectrometer, which may be adjusted for any desired wave length.

The two halves of the optical field in the spectrometer may be adjusted to an equal brightness by turning the above mentioned drum. The amount of light transmitted by the dissolved material may be read directly in per cent.

Thus one reads: $\frac{I}{I_0} \times 100$.

In this manner I determined the "typische Farbkurve" for several solutions of chlorophyll in 85 per cent acetone.

The value $\frac{I}{I_0} \times 100$ was obtained by averaging at least five duplicate determinations. These curves are plotted in fig. 15 at arbitrary distances below each other.

Figure 15, C represents a curve for a mixture of chlorophylls a and b, prepared from *Selaginella* according to Willstätters instructions and kindly placed at my disposal by Miss J. Koning. It is evident that this curve agrees closely in its shape with curve A, except in the blue region, where the first curve is considerably higher. This may be ascribed to the carotinoids still present. It thus appears that such a curve is a good check on the purity of the preparation.

The two lower curves represent the absorption of two chlorophyll extracts, the first from *Tilia* leaves and the second from a quantity of *Hormidium*. For this purpose the fresh material was extracted with 85 per cent acetone. This was accomplished by grinding it in a mortar with emory powder or infusorial earth with the addition of a little calcium carbonate to neutralise the organic acids. The solution was subsequently filtered.

The two last mentioned curves show much greater deviations in the blue region from Weigert's chlorophyll curve than the former curve C, as was to be expected.

In order to determine the concentrations, it seemed to me to be desirable to measure the absorption in the maximal region of the red absorption band (663—665 $\mu\mu$), which lies so high that one need have no fear of error due to absorption by other materials.

The maxima in all my curves proved to be somewhat lower than in Weigert's curve for pure chlorophyll. This is the case also in Weigert's spinach extract curve. These deviations, which seem to be very slight in a $\log \epsilon$ curve, prove upon calculation to represent concentration differences of from 10 to 20 per cent.

As to the cause of these deviations I am unable to decide. I think it likely that the relative proportion of chlorophyll a to chlorophyll b has an influence, for the maximum determined is that of chlorophyll a. It is not impossible, however, that Weigert's curves are more correct than mine.

From curve A is derived the absorption constant which was used as a standard for my concentration determination:

$$\log k_{\lambda} = 4.78 \quad (\lambda = 664 - 655 \mu\mu).$$

To establish the reliability of my determination I made several preliminary experiments. For this purpose I made from a given leaf extract two dilutions, the concentrations of which had to each other a definite proportion $\frac{C_1}{C_2}$. I then determined the absorption in an arbitrary region of the spectrum. The results are given in tables 25, 26 and 27.

The average error of the determinations of $\frac{C_1}{C_2}$ arrived at in this way were $2\frac{1}{2}$ and 4 per cent respectively. The amounts of transmitted light in these experiments were quite large, in many cases more than 50 per cent. Now according to Weigert the determinations are most accurate when ± 10 per cent of the light is transmitted. Indeed it

may be seen that the results in table 27 are much more accurate, having an error of only 0.2 per cent. This error will, however, be below the average.

Subsequently I proceeded to make determinations of the assimilation number. The assimilation rate was first determined in the usual manner, the algae were then air dried, scraped from the glass and ground in a small mortar with 1 gm. of infusorial earth, a little calcium carbonate and with enough 85 per cent acetone to form a thin paste.

On the perforated bottom of a small Büchner funnel a filterpaper was placed and covered with a thin layer of infusorial earth. A quantity of 85 per cent acetone was sucked through it until the percolating fluid was quite clear. Then the chlorophyll extract was filtered and collected in a suction tube. Additional amounts of the pure solvent were added several times, until the percolating fluid was colorless. In a volumetric flask enough of the pure solvent was added to bring the volume to 25 cc. and subsequently the chlorophyll concentration was determined spectrophotometrically. From this concentration was calculated the amount present in 25 cc. of the solution, in other words the total chlorophyll content of the algae used in the experiment. The results of these determinations and also the calculated assimilation numbers are given in table 28.

As an average I found an assimilation number of 6.75. This agrees with the determinations of Willstätter, who finds for normal leaves at 25° C. assimilation numbers between 5 and 12.

As may be seen from the table, the deviations of the separate determinations from each other are rather small. While the amounts of carbon dioxide (cmm. per hour) and chlorophyll (thousandths m.grams and less) are extremely small, the average deviation from the mean value is only ± 3.6 per cent of this value. In view of this fact I regret especially, that I had no opportunity to test the value of the

absorption coefficient, which I derived from Weigert's curve.

The chlorophyll content of the unit quantity of cell material I calculated to be 27.1×10^{-6} gm.

Determination of the assimilation quotient.

At the conclusion of my experiments I made a few determinations of the assimilation quotient: $\frac{O_2}{CO_2}$.

In order to determine this value for assimilation only, as distinct from respiration, I made first a dark experiment, in which CO_2 containing air was passed through the assimilation vessel. The CO_2 and O_2 content of the discharged air were determined. Thereafter the same procedure was followed under illumination. From the increase in O_2 and the decrease in CO_2 in the discharged air the assimilation quotient was computed.

The results are represented in the tables 29 and 30, in which the concentrations of CO_2 and likewise those of O_2 are given in terms of 0.001 per cent. As will be seen, I found the values of 1.09 and 1.13 for the assimilation quotient.

This is in contradiction to the results of Maquenne and Demoussy (1913) and of Willstätter and Stoll (1918), who have always found the proportion $\frac{O_2}{CO_2}$ to be 1 for the true assimilation process.

Now *Hormidium* is an organism the first visible assimilation product of which is oil. If in the course of the assimilation process no sugars are first formed but a direct synthesis of oil takes place, a greater value than 1 might be expected.

I ascribe, however, no conclusive value to my own determinations. They should have to be repeated with the careful elimination of all possible sources of error. The reason

for mentioning these results is mainly to demonstrate that my method is also applicable to investigations of this problem. Moreover, the above results may draw the further attention of other investigators to this subject, which is certainly in need of more complete investigation.

CHAPTER VIII.

THEORETICAL CONSIDERATIONS.

The Rule of Chain Processes.

We have considered the peculiarities of the assimilation process as a special case of the general rule, which applies to consecutive reactions: the *slowest* reaction in the chain determines the rate, at which the end-product is formed.

The above statement is a bit paradoxical, although the intended meaning is clear. If there is no accumulation of intermediate products, all reactions in the chain are proceeding at an equal rate. One may therefore, define the „slowest” reaction as the one which would proceed at the slowest rate if all were free to progress unhindered.

It seems to me that this rule for chain reactions is itself a special case of a much more general rule, which one meets very frequently.

I would formulate this rule as follows: When a current, whatever its nature, encounters successively a number of resistances, the speed of the current is determined by the greatest of these resistances. This principle is found to be applicable regardless of whether it has to deal with a current of electricity or of matter; or whether the movement takes place by means of convection or diffusion, or whether it is intermolecular in nature, as in chemical reactions.

In all of these cases the rule applies only approximately and holds only when one resistance is considerably larger than the others. If two or more resistances are of the same

order of magnitude the deviations from this rule may be quite large. In such cases a quantitative expression of the intensity or speed of the current may become very complicated. I shall, however, discuss here some simple cases.

When under influence of an electromotive force E an electric current flows through two conductors, having resistances R_1 and R_2 respectively, which are connected in series, the intensity of the current I is, according to Ohm's law:

$$I = \frac{E}{R_1 + R_2} \dots \dots \dots (3).$$

If one assigns to E and to R_2 a constant value, for example 1 Volt and 1 Ohm respectively, while R_1 is varied from ∞ to 0, one may then calculate the intensity of the current I (in Ampères).

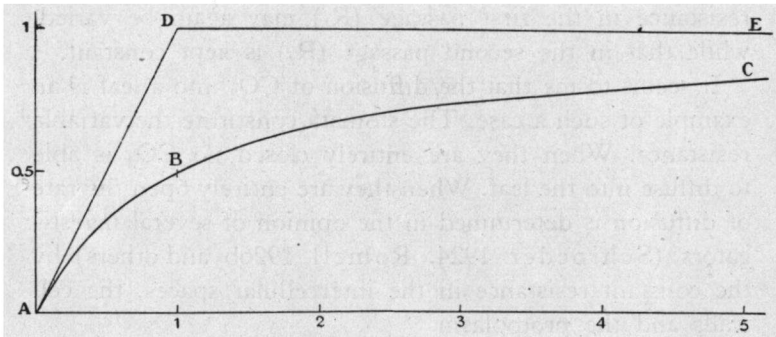


Fig. 16.

The varying values of I are graphically represented in fig. 16. The intensity of the current I is taken as the ordinate and the reciprocal of the resistance of the first conductor (i.e. conductivity) $\frac{1}{R_1}$ as the abscissa. If this conductivity is increased, the intensity of the current increases, but in decreasing proportions as $\frac{1}{R_1}$ becomes larger (A B C).

If instead of an electric current we now consider a stream

of water, which flows successively through two tubes of equal diameter, the same calculation applies. One merely substitutes for the number of Ampères the number of cc. per unit of time, for the electromotive force the hydrostatic pressure gradient and for the resistances R_1 and R_2 the lengths of the two tubes l_1 and l_2 , multiplied by a certain constant. If the length l_1 is now varied while l_2 remains constant we may calculate the speed of the current (assuming the applicability of Poiseuille's law) and we obtain a curve of exactly the same shape.

A third instance in which this rule applies is the case of two successive diffusion processes. Here we may substitute for E the difference in concentrations at the beginning and at the end of the diffusion path ($C_1 - C_2$). The diffusion resistance in the first passage (R_1) may again be varied, while that in the second passage (R_2) is kept constant.

It seems to me that the diffusion of CO_2 into a leaf is an example of such a case. The stomata constitute the variable resistance. When they are entirely closed no CO_2 is able to diffuse into the leaf. When they are entirely open the rate of diffusion is determined in the opinion of several investigators (Schroeder 1924, Romell 1926b and others) by the constant resistance in the intercellular spaces, the cell walls and the protoplasm.

In all these examples, as illustrated in fig. 16, the above rule for the intensity or speed of the current applies. In the region AB it is determined primarily by the resistance R_1 and in the region BC primarily by the resistance R_2 . Were Blackman's formula applicable to this case, the rate of flow would be determined exclusively by the greater resistance, the smaller would have no influence whatsoever, in which event the velocity of the current would be represented by the curve ADE .

As may be seen in the figure the calculated curve deviates at the transition point B by exactly 50 per cent from the ideal Blackman-curve.

If the assimilation process is analogous to this simple example, one would have to expect here also deviations of 50 per cent at the transition point.

Romell's criticism of Blackman's formulation is based on entirely similar considerations. He uses the same formula (3) in a somewhat different form (l.c. pag. 752). He has devised a model, which represents visually the interaction of the different factors. It consists of a series of connected batteries, generating an electric current, which drives a motor. The battery jars may be filled to a greater or lesser height, which in turn influences the energy production of the motor. Therefore, in this model also several consecutive resistances are involved, which together determine the current intensity.

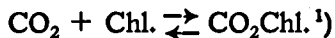
In principle Romell is certainly right in assuming that all factors are always simultaneously exerting an influence upon the assimilation process and that from a theoretical standpoint the ideal Blackman curve is untenable.

From what has been said it is obvious that *it is easy enough to explain deviations* from the ideal Blackman-curve. It is not so easy to explain *why in the experiments the observed deviations are so slight*, the more so, since they may still be ascribed in part to the fact, that the light and CO₂ supply is not entirely uniform in all parts of the chloroplasts. Had this not been the case the observed deviations would probably have been still smaller.

I will try to point out by means of an example the direction in which the explanation may be sought.

I shall take as an example a diffusion process, in which CO₂ diffuses into the plant, followed by a union of CO₂ with chlorophyll and the photochemical transformation of the compound thus formed.

In regard to this union, I assume, as already remarked on page 153, that a dissociation equilibrium exists between carbonic acid and chlorophyll.



I shall now apply to this equilibrium the law of mass action. I am aware that I am not quite justified in doing so. The law of mass action does not apply exactly to reactions in a surface boundary (heterogeneous reactions), like those which are being considered here; the quantitative relationships are somewhat different in such cases. It seems to me that Freundlich's formula for the absorption isotherm is more nearly applicable. The following is therefore to be regarded as a semi-quantitative expression.

The above equilibrium may be expressed as follows:

$$\frac{[\text{CO}_2] \cdot [\text{Chl}]}{[\text{CO}_2\text{Chl}]} = k,$$

in which k represents the dissociation constant. If we now represent the total quantity of chlorophyll present as 1, then:

$$\frac{[\text{CO}_2] \cdot [1 - \text{CO}_2\text{Chl}]}{[\text{CO}_2\text{Chl}]} = k.$$

The quantity of chlorophyll united with CO_2 is therefore:

$$[\text{CO}_2\text{Chl}] = \frac{[\text{CO}_2]}{[k + \text{CO}_2]}$$

In fig. 17 the abscissa represents the CO_2 concentration, the ordinate the concentration of carbonic acid-chlorophyll. To the dissociation constant k the values 1, 0.1 and 0.01 respectively are assigned. One thus obtains the dissociation curves a, b and c which have the form of an hyperbola.

Now if it is assumed that the dissociation equilibrium establishes itself instantaneously, the quantity of the CO_2 -Chlorophyll is always determined by the CO_2 concentration in the chloroplast. At a definite constant light intensity we may assume that the speed of the photochemical process is directly proportional to the quantity of CO_2 -chlorophyll.

¹⁾ The question whether CO_2 or H_2CO_3 is combined with the chlorophyll may be omitted from this discussion.

The ordinate in the diagram, therefore, represents not only the concentration of CO_2 -chlorophyll, but also the reaction rate of the photochemical process, as related to the CO_2 concentration in the chloroplast (represented by the abscissa).

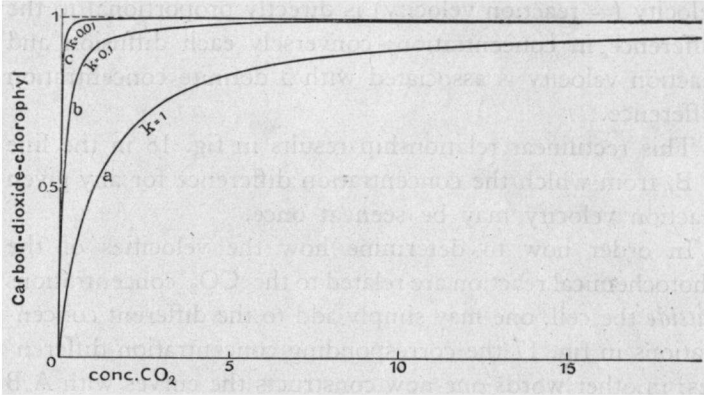


Fig. 17.

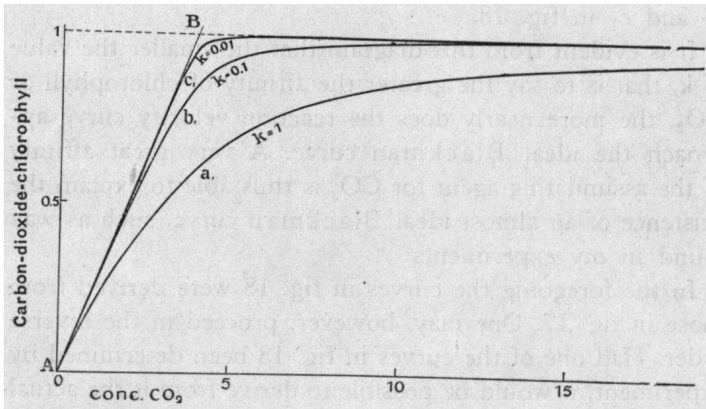


Fig. 18.

Although it is not possible to determine directly the amounts of CO_2 -chlorophyll, one may arrive at them *by using as a measure the reaction velocities*. It should be possible in this way to discover the dissociation curve.

What has just been said applies to the CO_2 concentration within the chloroplast. However, this is not determined; we determine only the CO_2 concentration outside the cell, which is always higher. As we have already seen, the diffusion velocity (= reaction velocity) is directly proportional to the difference in concentration; conversely each diffusion and reaction velocity is associated with a definite concentration difference.

This rectilinear relationship results in fig. 18 in the line A B, from which the concentration difference for any given reaction velocity may be seen at once.

In order now to determine how the velocities of the photochemical reaction are related to the CO_2 concentrations *outside* the cell, one may simply add to the different concentrations in fig. 17 the corresponding concentration differences; in other words one now constructs the curves with A B in fig. 18 as the ordinate. One obtains thus the curves a_1 , b_1 and c_1 in fig. 18.

It is evident from this diagram that the smaller the value of k , that is to say the greater the affinity of chlorophyll or CO_2 , the more nearly does the reaction velocity curve approach the ideal Blackman curve. A very great affinity of the assimilating agent for CO_2 is thus able to explain the existence of an almost ideal Blackman curve, such as was found in my experiments.

In the foregoing the curves in fig. 18 were derived from those in fig. 17. One may, however, proceed in the reverse order. Had one of the curves in fig. 18 been determined by experiment, it would be possible to derive from it the actual dissociation curve, the intervening diffusion resistance notwithstanding.

Before I began my experiments I had hoped to be able to determine the dissociation curve of CO_2 -chlorophyll, but I did not succeed in doing so. It is certain, however, that the point at which the assimilatory agent is only half saturated with CO_2 is below 10^{-5} atm. CO_2 pressure.

Were the ascending portion of the dissociation curve situated within the region of measurable CO_2 pressure, it would be possible to determine whether the carbonic acid actually combined with the chlorophyll, as Willstätter believes, thus actually participating in the photochemical process, or whether the relationship is otherwise.

In order to visualise this, one may imagine a condition, in which there is no resistance to diffusion — the condition represented in fig. 17. As has been mentioned the ordinate here represents not only the quantity of CO_2 -chlorophyll, but also the reaction velocity of the photochemical process.

A decrease in the CO_2 concentration would thus retard the photochemical process and would therefore have an effect only when light is the limiting factor.

On the other hand, if CO_2 undergoes first a dark chemical transformation (as was assumed in Warburg's acceptor-theory), we may imagine that a dissociable compound is formed with an enzyme, in the manner outlined above for chlorophyll. In such an event a decrease in the CO_2 concentration would retard the dark chemical process, thus having an effect only when temperature is the limiting factor.

Now these phenomena occur at such low CO_2 concentrations, that I was not able to detect which of the two possibilities is the correct one. Whether it is chlorophyll or an „enzyme”, which has such an enormous affinity for CO_2 , has not yet been established.

I believed at first, however, to have found an indication which points in the direction of the first possibility. If one scrutinizes fig. 14 carefully, it may be seen that the deviations from the ideal Blackman-curve are somewhat greater in the curve A F G (transition from CO_2 to light as the limiting factor) than in the curves A D E and A B C (transition from CO_2 to temperature as the limiting factor). This fact would argue for the correctness of Willstätter's theory.

Another explanation, however, is more probable, namely,

the unequal distribution of light intensity in the chloroplast. As was the case in fig. 12, this circumstance may here again be the cause of a greater deviation from the ideal Blackman curve.

Therefore it is here impossible to arrive at a definite conclusion. Indeed I doubt whether it is possible to determine CO_2 with sufficient accuracy to solve the problem in this way, especially since there is always the difficulty of diffusion resistance.

Since it is evident that the dissociation curve could not be determined directly, one should like to be able to displace the curve artificially in the direction of higher CO_2 concentrations. It seems to me that this should be possible with the aid of surface-active substances (narcotics) having the property of accumulating in the surface boundary and expelling from it the substances there present.

Indeed this principle was made use of in Warburg's experiments with urethanes, from which it appeared, that the reaction takes place in a surface and that the reacting substances (thus probably the CO_2) might be partly or entirely displaced from the surface.

In other words the dissociation curve is lowered. The question now arises as to how this takes place.

If the curve a in fig. 19 represents the dissociation curve under normal circumstances, assuming $k = 0.1$, a certain quantity of urethane should be able, for example, to lower the curve to half its level throughout its length (curve b). This would indicate that carbon dioxide had been excluded from half of the chlorophyll molecules present, that these had therefore been rendered inactive, while the other half continued to be available.

The curve might, however, be displaced to the right instead of downward (curve c). This would indicate that all of the chlorophyll remained available, but that, by the addition of urethane, the dissociation constant k had in-

creased considerably (the curve has been constructed for $k = 10$).

In the latter case the ascending portion of the curve should come to lie within the region of measurable CO_2 concentrations and it would then be possible to demonstrate experimentally whether or not the CO_2 participates in the photochemical process.

Warburg presents data which give some indication that this mode of attack is feasible. He states that at the very lowest CO_2 concentrations under which condition CO_2

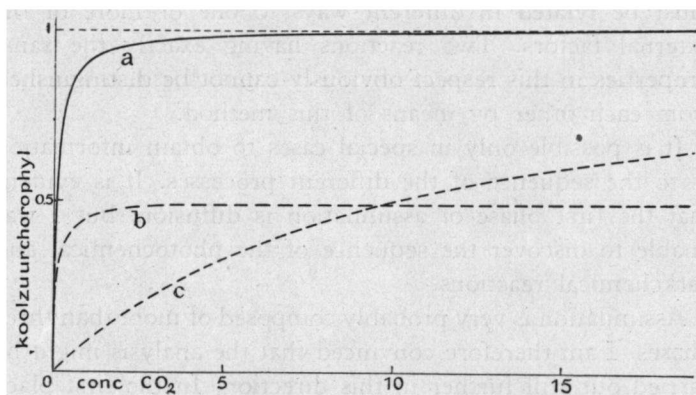


Fig. 19.

is limiting factor, small amounts of urethane have a pronounced inhibiting effect (1920 table 8).

This effect might be explained by means of curve c, but not by curve b.

Lack of time prevented the undertaking of experiments in this direction.

Possibilities of the method.

The method which I have used may be characterised as follows. One by one the separate reactions of the chain process are made the „slowest” by varying the external

conditions and the properties of each separate reaction may then be studied. As has already been shown, it is thus possible to investigate different phases of the process, each sharply separated from the others, without disturbing the organism.

It is self-evident, that only reactions which proceed slowly may be studied in this manner. Spontaneous reactions, as for example ionization, escape this mode of investigation.

If two reactions are to be distinguished from each other, they must have different properties, that is to say that they must be related in different ways to one or more of the external factors. Two reactions having exactly the same properties in this respect obviously cannot be distinguished from each other by means of this method.

It is possible only in special cases to obtain information as to the sequence of the different processes. It is evident that the first phase of assimilation is diffusion, but I was unable to discover the sequence of the photochemical and darkchemical reactions.

Assimilation is very probably composed of more than three phases. I am therefore convinced that the analysis might be carried out still further in this direction. In the first place the dark chemical process will have to be separated into its component parts.

An indication is seen in the results of Warburg, who found a very great change in Q_{10} with increasing temperatures, which points toward the complex nature of the reaction. It seems to me that, when the temperature-curve for assimilation has been determined exactly (for instance from 0° to 25° C.) it might be possible to detect abrupt changes in the temperature coefficient, as Crozier (1924) believes to be the case with many types of life-processes. Such an abrupt change would indicate the existence of two chemical reactions, each with its own Q_{10} .

The environmental factors, the effects of which may be

made use of in such studies, are not confined to CO_2 concentration, light intensity and temperature. Hydrocyanic acid and urethanes as used by Warburg belong to the same category, but only if their effect is reversible and produces no permanent change in the organism.

As other possible tools I should like to mention ammonium salts, to which Benecke ascribes a specific effect upon the assimilation process, and the altering of water vapour pressure, which I believe to have observed as exerting a marked effect upon the assimilation rate. Changing the water vapour pressure seems to me to be well suited to this purpose because it may be regarded as a „natural” influence. An organism like *Hormidium* is able to withstand drought without harm and complications, such as the closure of the stomata in leaves, are avoided in this case. The reader is referred to Stiles (1925) for a survey of the researches on this subject.

Finally it would be possible to try to alter the constitution of the cell by growing the organism under different conditions, as for example by withholding one or more of the chemical nutrients. An attempt in this direction was made by Briggs (1923). I refer also to Stiles (l.c. pag. 115).

In one or more of these ways it may be possible to gain more knowledge concerning the nature of the separate processes, which constitute assimilation and the agents, by which each of them is controlled.

SUMMARY.

The carbon dioxide assimilation is a chain reaction, consisting of at least three consecutive processes viz. a diffusion process, a photochemical process and a dark chemical process. This may be derived from Blackman's principle of limiting factors and from a number of other phenomena. Each of the processes in question may individually determine the velocity of the assimilation.

According to several investigators considerable deviations from Blackman's scheme are to be observed. This must be ascribed almost entirely to the fact, that the different chloroplasts in a leaf or a plant are subject to different conditions in regard to light and carbon dioxide.

A method was elaborated, in which this objection was evaded. A single cell layer of the filamentous terrestrial alga *Hormidium* was used as the subject in these studies.

The carbon dioxide concentration of the air introduced into the assimilation vessel was regulated by means of a buffer solution of carbonate and bicarbonate. The consumption of CO_2 was determined by gas analysis.

In a number of experiments the influence of light intensity, temperature and CO_2 concentration was studied. The results agree fairly well with Blackman's formulation.

The deviations from the „ideal Blackman curve“ are very slight.

At temperatures between 12°C . and 20°C . a $Q_{10} = 1.87$ was found under conditions, in which temperature was the limiting factor, while a $Q_{10} = \pm 1$ was found when light was the limiting factor.

When the concentration of carbon dioxide is the limiting factor, the assimilation velocity, even in very small, unicellular organisms, is always determined by the diffusion process.

The process, subsequent to the diffusion process, is caused by an agent (probably chlorophyll), which has an extremely high affinity for carbon dioxide. Even at a carbon dioxide pressure of $\frac{1}{100.000}$ atm. it is already almost entirely saturated.

The assimilation number $\frac{\text{gm CO}_2 \text{ per hour}}{\text{gm chlorophyll}}$ was determined for *Hormidium*. For this purpose the chlorophyll

content of the algae was measured spectrophotometrically. The assimilation number at 20° C. proved to be 6.75.

The assimilation quotient $\frac{O_2}{CO_2}$ in *Hormidium* is probably greater than 1, which may be related to the fact, that the first visible assimilation product in this organism is an oil.

The investigations here reported were carried out in the Botanical Laboratory at Utrecht. I wish to express my sincere thanks to Prof. Dr. F. A. F. C. Went, not only for his aid and helpful criticism, but also for his encouragement throughout the course of these studies.

TABLES.

TABLE 2.

13—2—'27.

Determination of the rate with which CO_2 is absorbed and emanated by rubber, in cc. per hour. Temp. 20°C .

A. 10 gm. section bicycle inner tubing, thickness 1 mm., surface area $2 \times 86,5 \text{ cm}^2$.

B. 19 gm. vacuum tube, thickness of wall 4 mm., total surface area 62 cm^2 .

1. Absorption in 100 per cent. CO_2 .

2. Emanation in 0 per cent. CO_2 .

Number of minutes after beginning of experiment	A		B	
	1	2	1	2
5	280	185	33	45
10	190	100	30	30
15	130	85	30	24
20	105	60	30	17
25	85	45	31	16
30	60	36	30	14
35	50	30	32	—
40	40	24	31	—
45	30	20	30	—
50	20	16	29	—
55	15	13	28	—
60	9	9	27	—
65	7	7	26	—
70	5	6	26	—
75	4	4	25	—
80	—	—	24	—
100	—	—	19	—
120	—	—	16	—

TABLE 3.

CO₂ content of air in 0,001 per cent in equilibrium with solutions of Na₂CO₃ and NaHCO₃ at 30° C.

Na ₂ CO ₃ in grammol per L.	0.325	0.259	0.182	0.126				
NaHCO ₃ in grammol per L.	0.175	0.241	0.318	0.374				
	Date	Analyses	Date	Analyses	Date	Analyses	Date	Analyses
	29-8-'27	90 } 92 }	23-1-'28	203 } 202 }	23-1-'28	498 } 497 }	5-1-'28	1005 } 1006 }
	30-8-'27	92 } 93 }		205 } 203 }		499 } 496 }		1005 } 1004 }
	1-12-'27	86 } 90 } 90 }		211 } 208 }		496 } 497 }		1011 } 1010 }
	15-12-'27	85 } 89 }	10-2-'28	206 } 209 }		500 } 496 }		1007 } 1006 }
		92 } 92 }		202 } 200 }		496 } 495 }		1004 } 1001 }
	5-1-'28	92 } 92 }		196 } 196 }				
		88 } 86 }		204 } 205 }				
		92 } 90 }		203 } 201 }				
Average.....		90		204		497		1006
Calculated ±		71		169		419		856

TABLE 5.

Data concerning rate of growth, assembled from different tables.

Date	From table	Temperature	Light-intensity	Per cent. growth per hour	Average
21-1-'28	16	20° C	6.18	1.38	} 1.04
15-1-'28	20	20° C	1.99	1.32	
17-2-'28	22	20° C	1.99	0.60	
21-2-'28	23	20° C	1.99	0.68	
11-1-'28	6	20° C	1.00	1.22	
8-2-'28	18	12° C	6.18	0.50	} 0.42
11-2-'28	19	12° C	6.18	0.34	

TABLE 6.

11-1-'28.

Measurement of the light intensity of the lamps by means of assimilation rates. Temp. 20° C. Growth corrections calculated from Nos. 2 and 8 (1.22 per cent per hour).

No.	Hour	Lamp.	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Relative light-intensities
					Ana-lyses	Ave-rage			
1	1.00	None.	100	204	220 220	220	—	—	—
2	2.20	A	100	204	144 141	142	78	0	1.00
3	3.35	B	100	204	133 133	133	87	1.5	1.10
4	5.10	C	100	204	139 136 138	138	82	3.5	1.02
5	6.40	D	100	204	140 138	139	81	5.5	0.99
6	10.25	E	100	204	127 127	127	93	10.0	1.09
7	11.35	F	100	204	134 136	135	85	11.5	0.98
8	12.45	A	100	204	132 133	132	88	13.0	1.00
9	2.05	Proj. lamp dist. 75 cm.	100	204	120 120	120	100	14.5	1.12

TABLE 7. (Fig. 12).

20—12—'27.

Influence of the light intensity. Temp. 20° C.

Conversion factor $\frac{100}{273}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
					Ana-lyses	Ave-rage				
1	2.00	0	100	460	476 479	478	10	—	—	—
2	3.45	1.00	100	460	401 404	403	9	76	0	28
3	5.15	1.99	100	460	326 328	327	7	154	1.5	56
4	9.00	3.08	100	460	264 264	264	6	218	5	76
5	10.55	4.18	100	460	222 221	222	5	261	7	89
6	12.30	6.18	100	460	187 185	186	4	298	9	100
7	2.15	13.2	100	460	171 172	171	3	314	10.5	104

TABLE 8. (fig. 12).

23—12—'27.

Influence of the light intensity. Temp. 20° C.

Conversion factor $\frac{100}{142}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
					Ana-lyses	Ave-rage				
1	2.50	0	120	210	220 219	220	3	—	—	—
2	4.20	1.00	120	210	187 186	187	2	41	0	29
3	5.35	1.99	120	210	154 152	153	2	81	1	57
4	12.35	3.08	120	210	129 131	130	1	110	8	71
5	1.55	4.18	120	210	106 104	105	1	140	9.5	91
6	3.15	6.18	120	210	93 89	91	1	157	11	100
7	4.40	13.2	120	210	82	82	1	168	12	106

TABLE 9. (fig. 12).

13—1—'28.

Influence of the light intensity. Temp. 20° C.

Conversion factor $\frac{100}{227}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
					Ana-lyses	Ave-rage				
1	12.00	0	100	460	482					
					481	481	11	—	—	—
					480					
2	3.00	1.00	100	460	411					
					413	412	10	70	0	31
3	4.30	1.99	100	460	349					
					343	348	8	136	1.5	59
					347					
4	6.10	6.18	100	460	251					
					252	252	6	234	3	100
					252					

TABLE 10. (fig. 12).

21—12—'27.

Influence of the light intensity. Temp. 20° C.
Exhausted culture medium.

Conversion factor $\frac{100}{170}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
					Ana-lyses	Ave-rage				
1	4.00	0	100	460	479 479	479	10	—	—	—
2	5.55	1.00	100	460	409 409	409	9	71	0	42
3	7.45	1.99	100	460	360 360	360	8	121	2	70
4	9.30	3.19	100	460	324 326	325	7	157	3.5	89
5	11.50	4.18	100	460	317 315	316	7	166	6	92
6	1.20	6.18	100	460	299 299	299	7	183	7.5	100
7	2.50	13.2	100	460	289 287	288	7	194	9	105

TABLE 11. (fig. 13).

24—1—'28.

Influence of the temperature. Light intensity 6.18.

 Q_{10} of respiration assumed to be 1.8.Conversion factor $\frac{100}{329}$.

No.	Hour	Light	Temp.	Air current rate	CO ₂ i	CO ₂ d		Respiration		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
						Ana-lyses	Ave- rage	Deter- mined	Calcu- lated				
1	11.40	0	11.95°	100	0	28 28	28	28	—	0	—	—	—
2	1.40	6.18	11.95°	100	497	285 287	286	—	28	6	233	0	59
3	3.50	6.18	16.0°	100	497	223 224	224	—	35	5	303	1	76
4	6.15	6.18	20.0°	100	497	133 133	133	—	45	3	406	3.5	100
5	7.30	6.18	23.9°	100	497	76 75	76	—	57	2	476	5	116

TABLE 12 (fig. 13).

9—2—'28.

Influence of the temperature. Light intensity 6.18.

Conversion factor $\frac{100}{411}$.

No.	Hour	Light	Temp.	Air current rate	CO ₂ i	CO ₂ d		Respiration		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
						Ana-lyses	Ave-rage	Deter-mined	Calcu-lated				
1	12.30	0	12°	100	0	35 36	36	37	—	1	—	—	—
2	2.30	6.18	12°	120	497	311 308	310	—	37	7	254	0	62
3	3.50	6.18	16°	120	497	258 258	258	—	42	6	323	1	78
4	5.00	6.18	20°	120	497	184 187 184	185	—	49	4	419	2	100
5	6.10	6.18	24°	120	497	146 145	146	—	56	3	474	4	111
6	7.15	6.18	24°	100	0	54 55	55	56	—	1	—	—	—

TABLE 13. (fig. 13).

27—1—'28.

Influence of the temperature. Light intensity 1.00.

Conversion factor $\frac{29}{105}$.

No.	Hour	Light	Temp.	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim.
						Ana-lyses	Ave-rage				
1	3.25	0	12°	120	204	231 230	231	5	—	—	—
2	4.40	1.00	12°	120	204	139 139	139	3	112	0	31
3	5.50	1.00	20°	120	204	151 151	151	3	106	1	29
4	7.00	0	20°	120	204	240 236	238	5	—	—	—

TABLE 14. (fig. 13).

14—2—'28.

Influence of the temperature. Light intensity 1.00.

Conversion factor $\frac{29}{82}$.

No.	Hour	Light	Temp.	Air current rate	CO ₂ i	CO ₂ d		Leakage corr.	Assimilation	Growth corr. per cent	Corrected assim
						Ana-lyses	Ave-rage				
1	1.40	0	12°	100	0	33 31	32	0	—	—	—
2	3.20	1.00	12°	100	209	148 148	148	3	90	0	32
3	4.50	1.00	20°	100	209	160 159	160	3	83	1.5	29
4	6.05	0	20°	100	0	37 36	37	0	—	—	—

TABLE 15. (fig. 14).

18—1—'28.

Influence of the CO₂ concentration. Temp. 20° C.
Light intensity 6.18.

Conversion factor $\frac{100}{225}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	12.00	0	100	0	28 29	29	—	—	—	—
2	1.35	6.18	150	204	76 74	75	223	0	77	100
3	4.45	6.18	100	204	23 23	23	210	3	25	91
4	6.05	6.18	110	204	30 27 32	30	220	4.5	32	94
5	7.15	6.18	50	204	12 11	12	125	5.5	13	53
6	10.20	6.18	80	204	18 17	18	178	9	20	73

TABLE 16. (fig. 14).

21—1—'28.

Influence of the CO₂ concentration. Temp. 20° C.

Light intensity 6.18.

Conversion factor $\frac{100}{266}$.Growth correction calculated from Nos. 3 and 6
(1.38 per cent per hour).

TABEL 16.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave- rage				
1	1.15	0	100	0	35	35	—	—	—	—
					35					
2	3.15	6.18	80	204	18	18	184	0	19	69
					19					
3	4.25	6.18	150	204	47	47	270	1.5	50	100
					47					
4	5.50	6.18	50	204	11	11	132	3.5	12	48
					11					
5	8.25	6.18	140	204	27	27	283	7	30	99
					27					
6	9.30	6.18	160	204	46	45	289	8.5	48	100
					44					

TABLE 17. (fig. 14).

2—3—'28.

Influence of the CO₂ concentration. Temp. 20° C.
Light intensity 6,18.

Conversion factor $\frac{100}{127}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	11.30	0	100	0	29 29	29	—	—	—	—
2	2.15	6.18	140	209	139 138	139	127	0	140	100
3	3.30	6.18	100	209	112 110	111	127	1	112	99
4	5.45	6.18	60	209	37 39	38	132	3.5	39	100
5	8.25	6.18	50	209	25 25	25	121	6	26	90
6	11.15	6.18	70	90	11 12	12	84	9	13	61

TABLE 18. (fig. 14).

8—2—'28.

Influence of the CO₂ concentration. Temp. 12° C.

Light intensity 6.18.

Conversion factor $\frac{61}{169}$.Growth correction calculated from Nos. 2 and 7
(0.50 per cent per hour).

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	12.30	0	100	0	28 30	29	—	—	—	—
2	2.30	6.18	120	204	88 86	87	169	0	89	61
3	4.00	6.18	80	204	30 28	29	169	1	31	60
4	5.40	6.18	70	204	20 19	20	158	1.5	22	56
5	8.15	6.18	50	204	11 10	11	126	3	12	44
6	10.15	6.18	60	204	13 13	13	144	4	14	50
7	1.00	6.18	120	204	80 80	80	178	5	82	61

TABLE 19. (fig. 14).

11-2-'28.

Influence of the CO₂ concentration. Temp. 12° C.
Light intensity 6.18.

Conversion factor $\frac{61}{160}$

Growth correction calculated from Nos. 2 and 7
(0.34 per cent per hour).

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	2.50	0	100	0	19 19	19	—	—	—	—
2	4.15	6.18	100	209	67 69	68	160	0	70	61
3	5.30	6.18	120	90	9 9	9	116	0.5	10	45
4	8.00	6.18	50	90	4 6	5	61	1	6	23
5	9.30	6.18	80	90	7 8	8	85	1.5	9	32
6	12.10	6.18	150	90	11 9 11	10	139	2.5	11	52
7	1.35	6.18	100	209	64 62	63	165	3	65	61

TABLE 20. (fig. 14).

15—1—'28.

Influence of the CO₂ concentration. Temp. 20° C.
Light intensity 1.99.

Conversion factor $\frac{57}{162}$.

Growth correction calculated from Nos. 2 and 7
(1.32 per cent per hour).

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	1.00	0	100	0	36 34	35				
2	2.50	1.99	120	204	99 97	98	162	0	100	57
3	4.05	1.99	100	204	69 70	70	169	1.5	72	59
4	6.00	1.99	80	204	40 40	40	166	4	42	56
5	8.30	1.99	60	204	11 11	11	151	8	13	49
6	10.50	1.99	50	204	11 9	10	132	10	11	42
7	12.40	1.99	120	204	81 81	81	183	13	83	57

TABLE 21. (fig. 14).

20—1—'28.

Influence of the CO₂ concentration. Temp. 20° C.
Light intensity 1.99.

Conversion factor $\frac{57}{145}$.

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave-rage				
1	12.10	0	100	0	30 31	31				
2	4.00	1.99	60	204	25 24	25	138	0	26	54
3	5.20	1.99	120	90	12 10 11	11	126	1.5	12	49
4	6.25	1.99	130	90	12 11 12	11	132	2.5	13	51
5	7.15	1.99	60	90	6 9 6 8	7	81	3.5	8	31
6	10.15	1.99	70	204	41 39	40	146	6	42	54
7	11.55	1.99	120	204	100 100	100	156	8	102	57

TABLE 22. (fig. 14).

17—2—'28.

Influence of the CO₂ concentration. Temp. 20° C.
Light intensity 1.99.

Conversion factor $\frac{57}{146}$.

Growth correction calculated from Nos. 2 and 6
(0.60 per cent per hour).

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave- rage				
1	1.00	0	100	0	35 35	35	—	—	—	—
2	4.40	1.99	120	209	117 117	117	146	0	118	57
3	6.30	1.99	80	209	74 75	75	142	1	76	55
4	9.00	1.99	55	209	28 29	29	134	2.5	30	51
5	11.00	1.99	50	209	19 20	20	130	3.5	21	49
6	12.40	1.99	120	209	112 110	111	153	5	113	57

TABLE 23. (fig. 14).

21—2—'28.

Influence of the CO₂ concentration. Temp. 20° C.

Light intensity 1.99.

Conversion factor $\frac{57}{95}$.Growth correction calculated from Nos. 2 and 7
(0.68 per cent per hour).

No.	Hour	Light	Air current rate	CO ₂ i	CO ₂ d		Assimilation	Growth corr. per cent	Corrected CO ₂ conc.	Corrected assim.
					Ana-lyses	Ave- rage				
1	2.50	0	100	0	35 34	35	—	—	—	—
2	4.30	1.99	150	90	50 50	50	95	0	51	57
3	5.50	1.99	60	90	14 16	15	80	1	16	48
4	9.05	1.99	70	90	15 16	16	87	3	17	51
5	11.00	1.99	80	90	23 23	23	89	4.5	24	51
6	12.30	1.99	90	90	28 26	27	92	5.5	28	52
7	1.45	1.99	150	90	46 46	46	101	6.5	47	57

TABLE 25.

5-3-'28.

Spectrophotometric determination of concentrations of leaf
extracts in solutions 1 and 2, proportion of

$$\text{concentrations } \frac{C_1}{C_2} = 2.$$

λ in $\mu\mu$	Solution No.	$\frac{I}{I_0} \times 100$	$\epsilon = \log \frac{I_0}{I}$	$\frac{C_1}{C_2}$ calculated
645	1	15.0	0.824	2.00
	2	38.8	0.411	
640	1	22.8	0.642	2.03
	2	48.2	0.317	
635	1	28.6	0.544	2.10
	2	55.0	0.260	
630	1	30.8	0.510	2.03
	2	56.0	0.252	
625	1	29.8	0.526	2.04
	2	55.2	0.258	
620	1	27.8	0.556	2.03
	2	53.2	0.274	
615	1	26.5	0.577	2.03
	2	52.0	0.284	
610	1	27.3	0.564	2.04
	2	53.0	0.276	
605	1	29.8	0.526	2.10
	2	56.2	0.250	
600	1	35.5	0.450	2.07
	2	62.0	0.218	
			Average 2.05 Deviation + 2.5 p. cent.	

TABLE 26.

8-3-'28.

Spectrophotometric determination of concentrations of leaf
extracts in solutions 3 and 4, proportion of

$$\text{concentrations } \frac{C_3}{C_4} = 2.$$

λ in $\mu\mu$	Solution No.	$\frac{I}{I_0} \times 100$	$\epsilon = \log \frac{I_0}{I}$	$\frac{C_3}{C_4}$ calculated
650	3	11.3	0.949	2.02
	4	34.0	0.469	
645	3	17.7	0.753	2.04
	4	42.8	0.369	
640	3	23.6	0.627	2.13
	4	50.8	0.294	
635	3	30.8	0.511	2.10
	4	57.2	0.243	
630	3	34.8	0.458	2.09
	4	60.4	0.219	
625	3	34.7	0.460	2.07
	4	59.8	0.223	
620	3	33.8	0.470	2.08
	4	59.4	0.226	
			Average 2.08	
			Deviation + 4 p. cent.	

TABLE 27. 24-9-'27.
Spectrophotometric determination of concentrations of leaf
extracts in solutions 5 and 6, proportion of
concentrations $\frac{C_5}{C_6} = 1.5$.

λ in $\mu\mu$	Solution No.	$\frac{I}{I_0} \times 100$	$\epsilon = \log. \frac{I_0}{I}$	$\frac{C_5}{C_6}$ calculated
666	5	4.95	1.314	1.469
	6	13.00	0.895	
664	5	4.25	1.380	1.497
	6	12.20	0.922	
662	5	4.55	1.350	1.524
	6	13.25	0.886	
Average 1.497				
Deviation — 0.2 p. cent.				

TABLE 28.

Determination of the assimilation number.
Temp. 20° C. Light intensity 6.18.
Chlorophyll dissolved in 25 cc. of 85 per cent acetone.
Thickness of layer $d = 10$ cm.

No.	Date	Assimilation in cmm. CO ₂ per hour	$\frac{I}{I_0} \times 100$ (λ 664—665)	$\frac{I_0}{I}$ $\epsilon = \log. \frac{I_0}{I}$	m.gm. chlorophyll.	Assimilation number	Per cent deviation from average
1	21-2-'28	191	5.19	1.285	0.0533	6.56	- 2.8
2	23-2-'28	130	16.37	0.786	0.0326	7.29	+ 8.0
3	23-2-'28	183	6.37	1.196	0.0496	6.75	0
4	28-2-'28	139	12.52	0.902	0.0374	6.81	+ 0.9
5	28-2-'28	161	7.57	1.121	0.0465	6.34	- 6.1
Average 6.75 \pm 3.6							

TABLE 29. 5—3—'28.
 Determination of the assimilation quotient. Temp. 20° C.

No.	Light	Air current rate	CO ₂ i	CO ₂ d		O ₂ d	
				Analyses	Average	Analyses	Average
1	0	100	1006	1031	1028	20560	20549
				1033		20540	
				1021		20547	
2	6.18	100	1006	553	549	21068	21072
				545		21077	
				CO ₂ = 479		O ₂ = 523	

$$\frac{O_2}{CO_2} = 1.09$$

TABLE 30. 8—3—'28.
 Determination of the assimilation quotient. Temp. 20° C.

No.	Light	Air current rate	CO ₂ i	CO ₂ d		O ₂ d	
				Analyses	Average	Analyses	Average
1	0	100	1006	1037	1035	20579	20568
				1034		20560	
				1035		20565	
2	6.18	100	1006	564	559	21100	21105
				555		21120	
				558		21096	
CO ₂ = 476				O ₂ = 537			

$$\frac{O_2}{CO_2} = 1.13$$

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