Citation:

Zijlstra, K., Kohlensäuretransport in Blättern, in:
KNAW, Proceedings, 11, 1908-1909, Amsterdam, 1909, pp. 649-668

Botany. — Prof. J. W. MOLL presents the dissertation of Mr. K. ZIJLSTRA, Assistant at the Botanical Laboratory, Groningen, entitled: *"Kohlensäuretransport in Blättern"*, Groningen, 1909, and with reference to this he communicates the following. [1])

In 1877 the speaker published the results[2]) of investigations, which proved, that the carbon dioxide, which is found in considerable quantity in soils containing much humus, and which is at the disposal of the roots, cannot lead to starch-formation in the leaves, when the latter are in a space, free from carbon dioxide; nor can this carbon dioxide appreciably accelerate starch-formation in the open air.

From the experiments, which led the speaker to this result, he further concluded, that a leaf or a portion of a leaf cannot form starch in a space devoid of carbon dioxide, even when parts, organically connected with, and bordering immediately on the portion in question, are placed in an atmosphere which is many times as rich in carbon dioxide as ordinary air. The experiment, which appeared to prove that starch cannot be formed even from carbon dioxide, which is offered to immediately adjoining parts, was the following.

A starch-free leaf was placed between the greased edges of two similar crystallizing dishes in such a way, that the apex was within the space enclosed by the crystallizing dishes, and the base was outside. The lower crystallizing dish contained potassium hydroxide solution; over the apparatus there was placed a bell-jar, containing air to which about 5 percent of carbon dioxide had been added.

After the leaf had been exposed to the light for some hours, the base was found to contain much starch, but in the upper portion, even right up to the edge of the crystallizing dish, which was 3 mm. thick, no starch whatever had been formed.

From this the speaker concluded that when carbon dioxide is abundantly present in any given part of a leaf, this can nevertheless not lead to starch-formation in an immediately adjoining portion, when the latter is in a space free from carbon dioxide.

This result was remarkable, for the starch-formation in the basal portion proved, that the carbon dioxide, offered to the leaf had indeed been taken up, and the presence of many intercellular spaces in the

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[1]) This dissertation will appear in one of the future numbers of the Recueil des Travaux Botaniques Néerlandais.

[2]) J. W. MOLL. Ueber den Ursprung des Kohlenstoffs der Pflanzen. Landwirthsch. Jahrb. VI. 1877. p 327—363.

mesophyll led to the supposition, that the transport of the carbon dioxide so taken up, need not be impossible.

A want of further experimental data made this question remain unsolved up to the present. The necessary data have now, however, been collected in the investigation of Mr. ZIJLSTRA, who has shown that the facts, previously observed by the speaker, had indeed been correctly described, but that other results may also be obtained, provided one works with different plants from those which the speaker happened to have used, or arranges the experiments in a different way. Mr. ZIJLSTRA was able to show that in the experiment described, starch-formation may sometimes indeed occur in the space free from carbon dioxide. The above conclusion as to the impossibility of starch-formation at the expense of carbon dioxide derived from the immediate vicinity, has therefore been found to be incorrect. The other results formerly obtained by the speaker, and especially the chief deduction, regarding the impossibility of starch-formation in the leaves at the expense of carbon dioxide, taken up by the roots from a soil rich in humus, were, however, completely confirmed and further elucidated by Mr. ZIJLSTRA's investigation. An explanation of the experiment described above, was also suggested and generally speaking Mr. ZIJLSTRA succeeded in solving pretty completely the question of the possibility and occurrence of carbon dioxide transport in leaves. How this was done the speaker wishes to communicate below.

Not unnaturally it seemed desirable to begin the investigation with a repetition of the above described experiments.

This was first done with the leaves of *Polygonum Bistorta* and of *Cucurbita Pepo* (experiment LIII and LIV)[1]), which were also employed by the speaker in his above-mentioned investigation. In these and in all later experiments Mr. ZIJLSTRA demonstrated the formation of starch by the so-called SACHS-SCHIMPER method, according to which the entire leaves, after decolorisation, are examined for starch content with the help of an iodine-chloralhydrate solution. This method was unknown in 1877, so that the speaker used microscopic sections, which is very cumbrous and gives less complete, albeit equally certain results.

The speaker limited himself to applying the starch reaction to sections of the apex of the leaf, which was in the space free from carbon dioxide, and to the base, which was in the air rich in carbon

[1]) The numbers of the experiments are here and in what follows the same as those in Mr. ZIJLSTRA's paper.

dioxide. He did not, however, examine the strip, 3 millimeters broad, which was in the grease between the edges of the crystallizing dishes. Now when Mr. ZIJLSTRA examined the entire leaves, it became apparent that starch-formation extended continuously from the leaf base upwards over part of this strip, in an area bounded by a sharply defined line, somewhat like a zig-zag, but that it nowhere extended so far, that starch had also been formed in the space, free from carbon dioxide. The speaker's observations were therefore confirmed, but were found to have been incomplete.

When, however, Mr. ZIJLSTRA repeated the experiment with a *Dahlia* leaf (exp. LV) the result was different. In this case also the starch-formation was found to extend into the greased strip, but in addition it extended here and there for some millimeters into the space freed from carbon dioxide. The border line of the starch reaction was here also somewhat zig-zag, but not everywhere equally sharp.

Finally, when the experiment was performed with a leaf of *Pontederia cordata* (exp. LVI) starch-formation extended uniformly for 0.5 centimeters into the carbon dioxide free space. The limit of the starch reaction was in this case not zig-zag, but bent regularly and was not sharp, since the dark colour of the reaction disappeared towards the leaf apex by a gradual transition. STAHL's cobalt test showed, that the stomata of the apex were closed at the end of the experiment.

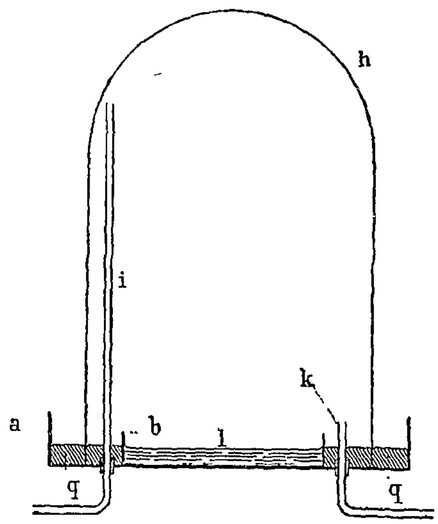

Figure 1.

The possibility now existed that in these experiments the greased join had not been absolutely tight, although it was not very probable that this fault should have revealed itself in the experiments with *Dahlia* and *Pontederia* and not in others. In order to exclude this possibility two pieces of apparatus were constructed, which, with the aid of mercury, permitted of a completely airtight separation being effected between the space free from, and that rich in carbon dioxide. Both pieces of apparatus were used in the investigation, but here the speaker will only describe the better of the two. (See fig. 1).

In a Petri dish *a*, of 15.5 cm. diameter a smaller one *b* of

9 cm. diameter was fastened down with resin and wax. Mercury was poured into the annular space *q* and concentrated caustic potash into the small Petri dish *l*. A glass bell-jar *h* of 3.2 litres' capacity was placed in the mercury, so that the air inside the jar was kept free from carbon dioxide. It was now possible to introduce the apex of a leaf, which had been freed from starch, into the carbon dioxide-free space through the mercury. The leaf base and the petiole thus remained outside the bell-jar *h*, and the petiole was always immersed in a dish of water in order to keep the leaf fresh throughout the experiment. A piece of metallic gauze was always placed over the small Petri dish *b* in order to prevent the leaf from coming into contact with the caustic potash. The base of the leaf could now be left at will in the open air with its unlimited supply of carbon dioxide at great dilution, or the base could be surrounded with an atmosphere richer in carbon dioxide. For the latter purpose the whole apparatus was placed on a tripod in a large porcelain dish, partly filled with water. A large glass bell-jar of 38 litres' capacity was placed over the apparatus containing the leaf, so that the space in the large bell-jar was cut off by the water in the porcelain dish. Into this space any desired quantity of carbon dioxide could be introduced. Finally attention may be drawn to the tubes *i* and *k*, which connected the interior of the small jar with the free atmosphere. This prevented differences of pressure between the interior and the exterior from raising the jar and establishing a communication between the outside and the inside. Moreover a stream of air, free from carbon dioxide, could be passed by these tubes through the inner jar. For this purpose the tube *k* was connected to an aspirator, and the tube *i* to absorption tubes for the carbon dioxide of the atmosphere.

With this apparatus a number of experiments were carried out, in some of which the base of the leaf was in the ordinary atmosphere, and in others in an atmosphere with much carbon dioxide. The portion of the leaves which was underneath the mercury had in all the experiments a length of 3 centimeters. These experiments led to the result, that not only in *Dahlia* and *Pontederia* leaves but also in all the other leaves investigated, a narrow strip of starch was formed in the space free from carbon dioxide at the border of the mercury. This strip was generally coloured jet black by iodine.

The following table summarizes a number of experiments performed in this manner.

In all these experiments the small starch strip was of course bordered on the side of the mercury by a straight line, but towards the apex

TABLE I.

Plant.	Number of exp.	The leaf base in:	Duration of exp.	Width of the starch strip.
Dahlia Yuaresii	I	5 % CO^2	5 hours	3 mm.
„ „	II	open air	5 „	2—3 „
„ *(Cactus) Thuringia*	XVI	2 % CO^2	5 „	3—4 „
Aster macrophyllus	V	open air	4 „	0.5 „
Sisymbrium Alliaria	VI	„ „	5 „	1 „
„ „	X	„ „	4 „	1.5 „
Polygonum Bistorta	VII	„ „	4 „	3 „
Aesculus Hippocastanum	VIII	„ „	4 „	0.5 „
„ *Pavia*	IX	„ „	3 „	0 5 „
Acer campestre	X	„ „	4 „	0.5 „
Sambucus nigra	XII	2½ % CO^2	5 „	2 „
„ „	XIII	„ „	5 „	3 „
Juglans regia	XIV	2 % „	6 „	1 „
Acorus Calamus	XV	„ „	7 „	2 „
Heliopsis laevis	XVII	2½ % „	6 „	1.5 „

of the leaf the border was a zig-zag line, which often coincided with the presence of veins in the leaf. In those places the border-line was sharply defined. In *Acorus* alone this line was also straight, without clearly depending on the veins; in this case moreover, the border line was not sharp, but towards the apex the reaction became weaker by imperceptible degrees and soon disappeared completely.

Since in these experiments the apex of the leaf was in a space free from carbon dioxide we must assume, that the starch strip had been formed at the expense of the carbon dioxide, which had been transported from the portions of the leaf nearer the base.

The most plausible assumption now seemed to be the following: that the carbon dioxide which had been absorbed by the leaf base, had been conducted through the parts of the leaf under the mercury, and having arrived in the part of the leaf exposed to the light, had there given rise to the formation of starch. In order to test the validity of this assumption, comparative experiments were undertaken, in which the one leaf was placed with its base in air containing

2—3 % of carbon dioxide, and the other in ordinary air, containing therefore very little carbon dioxide.

If the hypothesis just brought forward were correct, one might expect that owing to a more copious supply of carbon dioxide, a wider strip of starch would be formed in the former case than in the latter. It did not seem probable that the increased supply of carbon dioxide would manifest itself by a stronger iodine reaction, for, as we have seen, in most of the experiments described above, the reaction, if it appeared at all, was as strong as possible.

These comparative experiments were carried out with two sets of apparatus of the kind described, both of which were provided with a large bell-jar, including therefore the one in which the leaf-base was in ordinary air. This was done in order to maintain as far as possible the same temperature in the two smaller jars, for it was found that in these experiments the temperature had a great influence on starch-formation.

The result of these experiments varied considerably in different plants. In some cases it could be definitely shown, that carbon dioxide, supplied to the base, had influenced the manufacture of starch in the upper part of the leaf, but this could not be demonstrated for other plants.

The speaker first considers the experiments with a positive result, summarized in the following table:

TABLE II.

Name.	Number of experiment.	Duration of experiment.	Width of the starch strip in the CO_2 free space.	
			Base in 2 % CO_2.	Base in ordinary air.
Pontederia montevidensis	XLVIII	7 hours	6 mm.	2 mm.
Eichhornia speciosa	XLIX	7 „	1 cm.	2 „
„ „	L	$5\frac{1}{2}$ „	1.5 „	1 „
Eucomis punctata	LI	9 „	1 „	3 „

The first two experiments were carried out with leaves which were as nearly as possible equal, and the last two each with the halves of one and the same leaf.

All these experiments very clearly demonstrate the influence of the increased supply of carbon dioxide by the formation of wider strips of starch.

In the above experiments the leaf base in the space with much carbon dioxide was exposed to the light and accordingly this part of the leaf was full of starch at the end of the experiment. We might now expect that on darkening the leaf-base, more carbon dioxide would remain available for transport to the apex, and that a wider strip of starch would be formed than in the former case. If this expectation were realized, it would be an additional proof, that carbon dioxide is transported from the base to the apex. An experiment, carried out with the two longitudinal halves of the same leaf of *Eichhornia* (exp. LII) indeed gave proof of this. Here both bases were kept in air with 2 °/₀ carbon dioxide, but one was darkened, the other not. In the latter case less carbon dioxide remained available for transport on account of the consumption of carbon dioxide for starch-formation in the base, and in the course of 4 hours a strip of starch , 2—5 mm. wide, was formed in the upper part of the control half, as compared with one 5—8 mm. wide in the other half of the leaf, of which the base had been darkened.

In these experiments the strip of starch never had anything like a sharp edge on its upper side, nor was there any connexion between the limit of the reaction and the veins. The reaction simply became weaker and weaker at the edge of the strip and soon stopped completely.

A series of experiments with other leaves yielded, however, a totally different result. Here the strip of starch in the two leaves experimented on was always equally wide, no matter whether the leaf base had been placed in ordinary air or in air with a high carbon dioxide content.

In some of these experiments leaves of the same plant individual were taken, in each case as nearly as possible equal. The subjoined table summarizes them.

TABLE III.

Plant.	Number of exp	Duration of exp.	Width of the starch strip in both leaves.
Sambucus nigra	XIX	5 hours	3 mm.
Juglans regia	XX	6 „	1 „
Acorus Calamus	XXI	7 „	2 „
Zea Mays	XXII	6 „	1.5 „
Hordeum vulgare	XXIII	7 „	1 „

The negative result thus obtained, could now, after the experience with the influence of darkening on the leaf base of *Eichhornia* in experiment LII, be attributed to the fact, that the carbon dioxide absorbed by the base, had been wholly used up for starch-formation on the spot. Experiments were therefore also made, in which the leaf bases were darkened with black paper right up to the edge of the mercury, and therefore remained free from starch. But these experiments also gave exactly the same result; the strips of starch were equally wide in the leaves with bases in air containing much carbon dioxide, as in those with their bases in ordinary air.

A survey of these experiments is given in the subjoined table:

TABLE IV.

Plant.	Number of exp.	Duration of exp.	Width of starch strip in both leaves.
Triticum vulgare	XXIV	6 hours	1.5 mm.
Zea Mays	XXV	6 „	2 5 „
Dahlia Yuaresii	XXVI	5 »	4 „
Aesculus Pavia	XXVII	4 „	< 0.5 „
Tradescantia virginiana	XXVIII	4 „	1 „

In order to eliminate more thoroughly possible inequalities between the leaves compared than could be done by careful choice, a few experiments were also performed, in which the longitudinal halves of the same leaf were used for comparison, and were darkened at the base. These experiments yielded the same result, as given below:

TABLE V.

Plant.	Number of exp.	Duration of exp.	Width of starch strip in both halves of leaves
Dahlia (Cactus) Thuringia	XXIX	5 hours	not noted
Heliopsis laevis	XXX	6 „	1.5 mm.

Finally two experiments may be mentioned, which were performed under conditions, similar to those of the last named, but in which the caustic potash was omitted in the smaller bell-jar. In all the previous experiments the carbon dioxide of the leaf apex would, if it were at all possible diffuse into the surrounding space, which remained permanently free from carbon dioxide. This, however, did evidently not take place so fast, but that in all experiments starch was formed near the mercury.

Nevertheless the possibility was not excluded, that a wider strip of starch might appear, if the carbon dioxide absorbing potash were absent.

Such experiments were accordingly undertaken, and in order to make it possible that there should even be some accumulation of transported carbon dioxide, the inner bell-jar of the apparatus, which hitherto had been of a capacity of 3.2 litres, was replaced by one of only 0.8 litres' capacity.

In both experiments two halves of the same leaf were taken in each case. The two bases were placed in 3 % carbon dioxide, one being exposed to the light and the other darkened. The two halves gave exactly the same result and the starch strips were not wider than in all the previous experiments, as is shown by the following summary:

TABLE VI.

Plant.	Number of exp.	Duration of exp.	Width of starch strip in both halves of leaves.
Dahlia (Cactus) Thuringia	XXXI	4 hours	3—4 mm.
Heliopsis laevis	XXXII	4 „	0.5—1 „

It is obvious from all these experiments that with a number of leaves from widely different *Monocotyledons* and *Dicotyledons* a totally different result is obtained from that of the experiments described first. Carbon dioxide, even when abundantly supplied to the leaf base of these plants, cannot bring about the formation, at about 3 cm. distance in the apex of the leaf, of a wider strip of starch, than would have been formed without the addition of this carbon dioxide. Attention may further be drawn to a difference, which again clearly showed itself in these experiments, between the reticulate *Dicotyledons* and the parallel-veined *Monocotyledons* as regards the edge of the starch strips on the side nearest the apex. In all leaves with reticulate venation these edges were zig-zag and in most places sharply defined, owing to the presence of small veins. In the leaves of *Grasses*, of *Acorus* and of *Tradescantia* on the other hand there was a gradual transition to the starch-free apical portion, without any relation to veins, and there was no zig-zag border:

It would certainly be going too far to deduce directly from these experiments, that the carbon dioxide, which is supplied to the base of these leaves, cannot at all contribute to starch-formation in the apex. Nevertheless the results obtained gave a clear indication as to further experiment, intended to show, beyond dispute, if possible, what was the state of affairs in these leaves.

The way in which such experiments should be performed was now clear.

The base of the leaf must be deprived of any supply of carbon dioxide, and the question was whether, as might almost have been expected from the above, a strip of starch would be formed above the mercury. If this were to take place, and in the same way as before, the proof would have been given, that the part of the leaf, placed under the mercury, itself produced the carbon dioxide, required for starch-formation in the adjoining part, which was exposed to light.

The experiments which supplied an answer to this question were made by Mr. ZIJLSTRA in various ways.

In the first place an experiment was made with the apparatus described above, but without the large bell-jar. A small leaf ot *Dahlia Yuarezii* (exp. XXXIII) was placed in the apparatus in the ordinary way, but the base and the petiole were immersed in water which had been poured on the mercury outside the small jar. In the space free from carbon dioxide the apex now produced a strip of starch, similar in all respects to that in the experiments previously described, and in addition, a starch strip was formed under water in that part of the base which adjoined the mercury.

Similar experiments were also made with a simpler apparatus, which moreover permitted of the water, in which the leaf base was placed, being kept quite free from carbon dioxide.

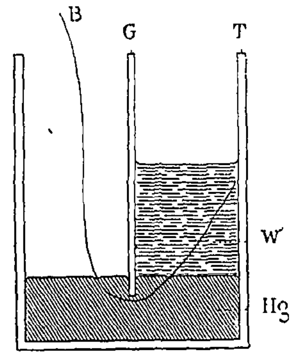

Figure 2.

Fig 2 gives a representation of this little apparatus. It consisted of a rectangular glass box, measuring 9 by 4.5 cm., and 5 cm. high, which is represented in the figure in section, the wall being indicated by *T*. With resin and wax a vertical plate of glass was fixed longitudinally but it did not reach to the bottom. The box was filled with mercury to slightly above the lower edge of the glass plate *G*. The leaf *B* was introduced underneath the vertical plate, so that its base was on the right side of the figure. There a layer of boiled water *W* was poured on the mercury, so that the base was completely submerged. The apparatus was now placed under a bell-jar with air, free from carbon dioxide, and the whole was exposed to the light.

After some hours starch had been formed in this apparatus in *Dahlia Yuarezii* (exp. XXXIV) and in *Populus pyramidalis* (exp. XXXV); there was a strip of starch in the apical portion along the mercury, quite like that of the previous experiments; there was a similar strip in the basal portion under water, also along the edge of the mercury; and finally a narrow strip of starch had appeared in the mercury itself, at the place where the leaf had been in contact with the lower edge of the vertical glass plate and had therefore received light.

The carbon dioxide, which in this case had been used up in starch-formation, could only have been derived from the portions of the leaf under the mercury and could scarcely be anything but carbon dioxide of respiration from these parts.

When this had once become evident, a still simpler arrangement naturally suggested itself. For this purpose pieces of leaves, free from starch, were placed on a layer of mercury, were partially covered with black paper, and finally pressed under the mercury by means of a glass crystallising dish with flat bottom. The light could now reach those parts of the leaves which were not covered by black paper, through the bottom of the glass dish.

Such experiments were made with *Dahlia Yuarezii* (exp. XXXVI, XXXVII and XLI), *Sambucus nigra* (exp. XXXVIII), *Syringa vulgaris* (exp. XXXIX) and *Tilia platyphyllos* (exp. XL). In all these experiments, which lasted about 5 hours, strips of starch were formed in the ordinary way at the border of the black paper in the lighted portions of the leaves; the border of the starch on the side opposite the paper was again sharply defined in many places by veins.

We may hence assume, that in all the experiments of the second group, in which the result was negative, starch had been formed exclusively at the expense of the carbon dioxide, resulting from the respiration of the parts of the leaf under the mercury, which carbon dioxide had been transported a certain distance to those parts, which were exposed to light.

In this case the supply of carbon dioxide from outside was prevented by the mercury, which also kept off the light. The respiratory carbon dioxide could therefore not be immediately reduced on the spot, but could spread and thus reach the lighted portions of the leaf in fairly large quantity. It should further be noted, that the epidermis of the darkened leaf-fragment was closed hermetically by the mercury, so that the carbon dioxide formed could not escape from the leaf, but had to move sideways.

The question now arose whether this closing of the epidermis was a necessary condition for the success of the experiments. From the nature of things this might be considered probable, for otherwise the respiratory carbon dioxide would follow the line of least resistance through the stomata and epidermis. Experiments made with this object in view have completely confirmed this opinion. A leaf of *Dahlia Yuarezii* was introduced into the apparatus first described, with its apex in the small bell-jar without caustic potash. One longitudinal half of the apex was uncovered, but to the other half a strip of black paper was fastened, by which a transverse strip of this half was darkened; this strip measured 17 mm. from the mercury in the direction of the tip (exp. XLII). In the same leaf it was therefore possible to compare: a lighted portion adjoining a darkened one, the epidermis of which was shut off by the mercury; and a lighted portion adjoining one darkened by paper, the epidermis of which was therefore not shut off. A starch zone was indeed formed along the edge of the mercury, but not along that of the black paper. When the experiment was repeated (exp. XLIII), with the portion of the leaf under the paper smeared with a mixture of cocoa-butter and wax (according to STAHL), the result was quite different. This mixture is known to close the epidermis almost completely to carbon dioxide, and the well-known starch zone now also appeared at the edge of the paper.

The use of cocoa-wax finally led to some experiments which may be called extremely simple, and which once more confirmed the result obtained.

Leaves of *Aesculus Pavia* (exp. XLIV) and of *Juglans regia* (exp. XLV), free from starch, were completely covered with cocoa-wax, in which condition, according to STAHL's experiments, extremely little or no starch is formed during exposure to light in the open air. The leaves were, however, exposed to the light by Mr. ZIJLSTRA after they had been partially covered with black paper or tin-foil, and now, as was to be expected, black borders of starch were formed along the edges of the paper and of the tin-foil.

A rather pretty modification of these experiments was finally obtained by using variegated leaves, of which the colourless portions were quite white, and did not therefore contain any carotin, by means of which carbon dioxide might be decomposed, even in the absence of chlorophyll. It is further necessary that such leaves, in order to be suitable for the experiments in question, should possess in their colourless parts a well-developed parenchyma, capable of producing by respiration a proper amount of carbon dioxide. If such leaves, after having been freed from starch, are smeared with cocoa-wax to prevent the escape of the carbon dioxide formed by respiration and are exposed to the light, borders of starch are developed in the green portions, at the edge of the colourless patches, in the same way as in the earlier experiments, but without any portion of the leaf having been darkened. Such experiments were performed with leaves of *Cornus tartarica* and *Elaeagnus Frederici* (exp. XLVI) and the best result was obtained with *Pelargonium zonale* (*Mad. Salleroi*) (exp. XLVII).

It appears from the above, that in all the leaves investigated a transport of carbon dioxide was possible to a greater or lesser extent, and that this might lead to starch-formation in the portions of the leaf exposed to the light. The experiments were arranged in such a manner, that this starch-formation generally showed itself in more or less broad strips of the leaf. But the carbon dioxide which led to the formation of these starch strips was found to be of dual origin.

In the majority of the leaves investigated, and in all the experiments of the various tables, except those of table 2, we had to assume, that the starch strips were exclusively formed at the expense of the respiratory carbon dioxide, which had been formed in the neighbouring darkened portions, hermetically shut off by mercury or by cocoa-wax.

In the experiments with water plants, mentioned in table 2, the respiratory carbon dioxide must no doubt have also contributed to the formation of starch borders. These experiments did show, however, that another source of carbon dioxide also cooperated, namely the supply of carbon dioxide, which had been added to the air surrounding the leaf-base, which was absorbed by this base, and which was transported through the 3 cm. long portion of the leaf under the mercury into the space free from carbon dioxide, and was assimilated there.

In other words: in most leaves, of *Monocotyledons* as well as of *Dicotyledons*, only a very limited transportation of carbon dioxide is possible. But in these leaves one has an excellent method for the study of this transportation, by utilizing the respiratory carbon dioxide of the adjoining parts.

In a few parallel-veined leaves of water plants on the other hand, a much wider transportation is possible, which can be demonstrated with the relatively rough apparatus employed.

The question now arose, how these two varieties of carbon dioxide transportation must be imagined and on what the difference of the two categories of leaves referred to, depended.

Mr. ZIJLSTRA succeeded in giving a complete account of these phenomena by the study of the anatomical structures of the various leaves used in the experiments.

The speaker begins with those cases, constituting the majority, in which starch-formation only took place at the expense of the respiratory carbon dioxide.

The carbon dioxide of respiration, produced by the living cells, will of course diffuse into the intercellular spaces, and as these are connected up for longer or shorter distances, we may indeed assume that a transportation of carbon dioxide will in the first place take place by diffusion along this route.

Further we must consider, that the veins generally have far fewer intercellular spaces than the parenchyma; indeed, they may have none at all.

In this connexion a fact deserves notice, to which repeated attention has already been drawn in the above, namely, that the starch strips were generally sharply defined by veins on the side opposite to the carbon dioxide supply, so that it gave the impression, as if these formed a barrier across which the starch-formation could not extend. The edge of the starch strip was therefore frequently toothed in an irregular manner. In the *Dahlia* leaf, which is coarsely reticulate, the strips of starch came out largest in all experiments; especially in those places where the veins happened to be a little more remote from the border-line between carbon dioxide production and carbon dioxide consumption, the starch had spread furthest, and then there was often no sharp delimitation. In leaves with very fine meshes between the veins, such as those of *Aesculus* and *Acer*, the starch zones were correspondingly narrow.

In parallel-veined *Monocotyledonous* leaves on the other hand, as for instance in the experiments with *Acorus*, *Zea*, *Hordeum*, *Triticum* and *Tradescantia*, there was no relation between the edge of the starch strip and the small transverse veins. On the contrary, in these leaves the starch strip was generally seen to be straight on the side facing the apex of the leaf and not sharply defined; it faded away gradually, albeit fairly rapidly.

These observations led to an anatomical investigation of the various leaves used in the experiments, especially with a view to answering the question, to what extent their veins, in the absence of intercellular spaces, formed barriers, across which the carbon dioxide could not move at all, or only very slowly. Should this really prove to be the case with the leaves employed, then the simplest interpretation of the observed facts would be, that the carbon dioxide can indeed be readily distributed through the intercellular spaces by diffusion, but that this distribution can be limited by the wholly or partially closed tissue of the veins. In that case one would come to the conclusion, that such leaves are divided up by smaller and larger veins into areas, within which carbon dioxide transportation can readily take place. The passage from any one such area to another is difficult however, or quite impossible. The distance across which carbon dioxide can be transported in such a leaf will therefore depend very largely on the average size of the transport areas in the leaf.

The anatomical investigation showed, in the first place, that in the case of net-veined *Dicotyledonous* leaves, the conception which has been worked out above, completely explains the phenomena observed. Mr. ZIJLSTRA indeed found, that in these leaves veins, which take up the whole thickness of the leaf, are devoid of intercellular spaces. A leaf such as that of *Dahlia*, in which similar transverse veins occur only at comparatively long intervals, will have large transport areas, and will be able to form relatively wide strips of starch. On the other hand in leaves like those of *Acer* and *Aesculus*, in which numerous vein branches occur close together, and take up the whole thickness of the leaf, we can only expect to find narrow starch strips such as indeed occur.

The transport areas, even in the *Dahlia* leaf, which is in this respect in the most favourable condition among reticulate leaves, are nevertheless very small, certainly much smaller than 3 cm. in diameter, as simple inspection of the leaf shows. It is therefore evident, that in the first apparatus, in which the part of the leaf under the mercury measured 3 cm., these leaves were bound to give negative results, as regards the conduction of carbon dioxide supplied to the leaf base.

In all experiments with these leaves, an idea of the carbon dioxide transportation could, however, be obtained from the starch-formation which took place at the expense of respiratory carbon dioxide, derived from other parts of the same transport area. It is also evident that in these leaves the edges of the starch strips must often follow the irregular course of the veins and must suddenly cease at the veins. Only in those cases, in which only a small portion of the leaf area was in the dark and so could produce but little carbon dioxide, it might be expected that the large lighted portion could not fill itself completely with starch, and that the edge of the starch strip would not be sharp. Places in which this could be observed, were indeed pretty frequent in *Dahlia* leaves.

An important question now arose, as to the condition of the various parallel-veined leaves, which, in the experiments described above, also formed rather narrow and not very sharply defined starch strips. As regards the grass leaves of *Hordeum, Triticum* and *Zea Mays*, the transverse veins, which connect the longitudinal veins, are here indeed insignificant; the vascular bundles by no means fill up the whole thickness of the leaf and much parenchyma remains above and below. Investigation showed, however, that in transverse section the intercellular spaces of this latter parenchyma are very narrow, although they extend pretty far longitudinally. When passing through this parenchyma above and below the veins, the carbon dioxide is therefore checked very much more than in the general parenchyma between the veins, and its course must be much less rapid, although it is not stopped completely. In accordance with this, only narrow starch strips were formed in these leaves in the limited duration of the experiments, which lasted generally for 6, or at most for 7 hours. In other words, the carbon dioxide transportation was very limited, so that a transference of carbon dioxide across a greater interval than 3 cm., in the apparatus first described, was an impossibility. The transverse anastomoses of the veins did not however, sharply define the starch strips.

The leaves of *Acorus* and *Tradescantia* behaved similarly in the experiments performed. In the green parenchyma of *Acorus* only small intercellular spaces occur and here some veins moreover take up the whole thickness of the leaf, the colourless central parenchyma of the leaf contains it is true many large spaces which extend longitudinally, but at frequent intervals they are shut off by transverse cell-layers, diaphragms without intercellular spaces. Lastly *Tradescantia* has a very spongy assimilating tissue, but in it many vein-anastomoses occur, which only have minute intercellular spaces. In these cases too therefore the agreement between the anatomical structure and the experimental result was sufficient to warrant the acceptance of the above view.

Finally the question arose how the intercellular spaces are distributed in the leaves of *Pontederia, Eucomis* and *Eichhornia*, which, as is evident from the experiments of table 2, are much better adapted for carbon dioxide transportation, so that in the apparatus employed this gas could he carried from the leaf base to the apex.

The anatomical investigation of these leaves yielded the following result. The leaf of *Eucomis* is parallel-veined, the whole of the leaf-parenchyma is very spongy, and the longitudinal as well as the transverse veins are very insignificant, so that carbon dioxide can everywhere pass freely. There are still better gas passages in the curved-veined leaves of *Eichhornia* and *Pontederia*. In both these species the parenchyma contains air-channels, running continuously from the base to the apex and taking up from one third to one half of the area of a transverse section. In these channels there are, it is true, diaphragms of one cell thick, but these are themselves also provided with many wide intercellular spaces.

It is therefore merely a matter of course, that in these leaves carbon dioxide can be carried over much larger distances; indeed, it is clear that this carriage could extend from the lowest part of the base to the very tip, given a sufficient duration and suitable arrangement of the experiments.

It is likewise quite natural, that no veins form a sharp boundary to the starch strips on the side towards the leaf apex.

These observations therefore also completely confirmed the view, that the above conception of carbon dioxide transport in leaves is the correct one.

At the same time it will be clear that fundamentally this representation is the same for all the leaves examined. In net-veined leaves the transport areas are small and very sharply defined; in the parallel-veined leaves of *Grasses, Acorus* and *Tradescantia* they are small but less sharply defined; in the leaves of *Eichhornia, Eucomis* and *Pontederia* the whole leaf is one transport area. If it were possible to make experiments with the two first-named categories of leaves in an apparatus of the first type described, but of much smaller size, positive results as regards carbon dioxide transportation would then be obtained as readily as has now been the case with leaves of the third category only.

Lastly there is the question over what distance the carbon dioxide transport can extend in various leaves.

We have seen that in *Eucomis, Pontederia* and *Eichhornia* carbon dioxide can be transported through a piece of leaf 3 cm. long under mercury and then even 1.5 cm. farther through the apical portion, which was placed in air, free from carbon dioxide and of which the stomata were closed. As has already been said, we may assume that this distance is by no means the maximum one, but that it might be increased at will, on condition that the duration of the experiment were also increased as much as possible.

In all the other leaves, however, it was found that the carbon dioxide could not reach the apex through the 3 cm. long portion. With some of these leaves experiments were now made in order to determine the maximum distance, through which carbon dioxide could be transported during the course of the experiment. These experiments were arranged as follows. The leaves, freed from starch, were

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completely covered with cocoa-wax and then, here and there, with strips of paper of various widths; between these strips portions of the leaf remained exposed to the light.

Borders of starch were then formed along both sides of the black strips of paper, since the respiratory carbon dioxide, formed under the paper, escaped on both sides. The width of these starch borders was slight in the case of the narrowest strips of paper, because beneath these but little carbon dioxide was formed; it increased with the width of the strips and of course reached its maximum as soon as the half-width of the black strip of paper corresponded more or less to the maximum distance through which carbon dioxide could pass during the time of the experiment. The half-width of that strip of paper, at which the starch borders just reached their maximum width, was therefore a measure of the distance through which the carbon dioxide in the leaf could pass under the given conditions.

Such experiments were first made with parallel-veined leaves, in which the carbon dioxide transport was only limited by the small dimensions of the intercellular spaces. The result was, that in *Triticum* (exp. LVII) the carbon dioxide could be transported in 6 hours over at least 2.5 cm., in *Acorus* (exp. LVIII) in 6 hours over rather more than 1 cm., in *Tradescantia* (exp. LIX) in 5 hours over less than 1.5 cm. There is every reason for the assumption, that in these leaves, in experiments of longer duration, somewhat higher values might have been obtained.

In net-veined *Dicotyledonous* leaves the case is somewhat different, for, as we have seen, in consequence of the absence of intercellular spaces from veins of a certain order, the carbon dioxide transportation is strictly limited to definite areas, the size of which may be very different in different leaves. If these areas are minute we observe with the narrowest strips of darkening paper also the maximum width of the starch borders, the absolute dimensions of which are likewise minute.

This was the case in *Juglans, Aesculus* and *Tilia,* in which the distance of the transport could not be accurately determined, but certainly did not exceed 2—3 mm.

In *Dahlia* and in *Sambucus* the transport areas referred to are relatively very large, the starch borders along the narrowest strips of paper are narrowest, and they increase to a certain maximum width along the wider strips. Here, however, this method cannot give very accurate results. For if a transport area is darkened over its greater part, then much carbon dioxide is indeed formed in it, but this can only cause starch-formation in a small part. Conversely, if only a small part of the area is darkened, the starch-formation can be observed at a relatively large distance, but then too little carbon dioxide is formed in the small darkened portion to give rise to starch-formation in the more distant parts. With this reservation an experiment may be mentioned, which was made with the leaf of *Dahlia* (*Cactus*) *Thuringia* (exp. LX), with the result, that the carbon dioxide can here be carried over through at least 0.5 cm.

In summarizing his communication, the speaker points out that Mr. ZIJLSTRA has shown that transport of carbon dioxide is possible in all the leaves examined, and that it takes place through the intercellular spaces. The transport is completely dependent on the size and extent of these spaces in the leaf.

In some parallel-veined leaves, such as those of *Eichhornia, Pontederia* and *Eucomis,* the intercellular spaces are very wide and extend in an uninterrupted series throughout the whole length of the leaf. By the use of suitable apparatus the leaf base can be made to absorb carbon dioxide, which moving on through the intercellular spaces by diffusion can give rise to starch-formation a comparatively long way off in the leaf apex, when the latter is exposed to light.

In the great majority of leaves however, carbon dioxide transport cannot be shown with the relatively crude apparatus employed. In such leaves the carbon dioxide transport can be studied by another method, which utilizes the fact that respiratory carbon dioxide, which is formed in a darkened and shut off portion of the leaf, can diffuse from there through the intercellular spaces to neighbouring lighted portions of the leaf and can there cause starch-formation.

Such leaves possess limited transport areas, which are formed by spacious and connected intercellular spaces, and which are either connected at their margins through much narrower intercellular spaces, greatly retarding carbon dioxide transport (*Grasses, Acorus, Tradescantia*) or the areas are completely cut off by veins, which have no intercellular spaces (net-veined *Dicotyledonous* leaves). In these leaves with limited transport areas a carriage of carbon dioxide over a distance of from 2—3 mm. to at most 2.5 cm. is possible.

Finally the speaker draws special attention to the impossibility of carbon dioxide transport being of any advantage to the plant in nature, in the first place, because this transport is so extremely limited in the majority of cases, and in the second place especially because for transport it is necessary that the conducting part should not itself assimilate, and also, that the epidermis should be impervious to carbon dioxide. These conditions will doubtless never be fulfilled in land plants, in water plants perhaps very exceptionally.

It has therefore been established by Mr. ZIJLSTRA's investigations, that the speaker was wrong when, in his above cited paper, he came to the conclusion, that a leaf or leaf fragment cannot form starch in a space free from carbon dioxide, when parts organically connected with it, or even immediately adjoining it, are placed in an atmosphere very rich in carbon dioxide. Mr. ZIJLSTRA's results are however, in complete agreement with the main result, formerly obtained by the speaker, according to which the carbon dioxide of the soil, even if it should be absorbed by the roots, cannot appreciably contribute to the synthesis of organic matter in the leaf.

Groningen, January 29th, 1909.

Microbiology. — *"Investigations on the subject of disinfection".* By Prof. C. EIJKMAN.

Last year I communicated results of experiments [1]) from which it appeared that the resistance against high temperature of bacteria of the same pure culture is individually very different. While for example the majority die off in a few minutes, some may remain alive after $^1/_4$, $^1/_2$ hour, etc. If the times are noted on the absciss and the corresponding numbers of survivors are drawn to it as ordinates, we get as "curve of survivors" a line which in general has the form of a _. In a slow process, as it occurs when the mortal temperature is taken relatively low, the first part of the curve shows itself clearly as an horizontal line and therefore represents a latent stage of incubation. Notwithstanding this the period within which the first half dies off, is much shorter than the following, in which the second half passes away.

In a quick process, as is observed when the temperature is far above the physiological limit, the duration of the incubation will become so brief that it easily escapes notice. In connection with the inevitable circumstance that the number of observations in this kind of experiments cannot be increased arbitrarily, but is confined within a rather definite period, the curve may, instead of the _ form, assume the shape of a _.

The latter has also come to light in investigations published the other day by MADSEN & NYMAN, [2]) which differed from mine in so

1) Biochem. Zeitschrift, Bnd. XI, Hft. 1—3, Festband Dr. H. J. HAMBURGER gewidmet.

2) Z. f. Hyg. u. Inf. Kr. Bnd. LVII.