Botany . — *Absorption and transport of asparagine in leaves of Vallisneria.* By W. H. ARISZ and J. OUDMAN [1]). (Communicated by Prof. J. C. SCHOUTE.)

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§ 1. *Introduction.*

In a previous publication (2) we have described experiments on the uptake and transport of asparagine and caffeine by pieces of leaves of Vallisneria spiralis. Pieces of leaves several cm in length absorbed the transport substance dissolved in blocks of 2 % agar which covered both sides of one extremity of the leaves over a length of 8 mm. At the end of the experiment the piece of leaf was cut into parts of 8 mm. The increase of nitrogen in each of these small pieces gives an impression of the increase of nitrogen due to absorption or transport. The first part of the leaf absorbs the transport substance at its outer surface and transports it to the cells situated under the epidermis. In the adjoining parts of the leaves only transport can take place.

The experiments showed that in Vallisneria leaves which consist practically exclusively of parenchyma cells asparagine was transported over a greater distance than caffeine. The strength of the asparagine transport was found to be dependent on the pretreatment of the leaves, on the temperature and on the length of the leaf pieces to which the transport takes place.

We have continued these experiments in order to prove that the uptake and transport of asparagine is a process of active accumulation by the living cells, and is quite different from the uptake of caffeine, this being a process of passive permeation. In a note (3) we have already communicated the phenomenon that by decreased oxygen pressure the absorption of asparagine is strongly inhibited, whilst the uptake of caffeine is unaffected.

In this publication we will discuss the uptake of asparagine both from agar blocks and from watery solutions of various concentrations. We shall then endeavour to determine the effect of the withdrawal of oxygen on the uptake, and finally the influence of an evaporation by the free part of the leaf on uptake and transport.

The data obtained point to the uptake and the transport of asparagine being a vital process. By this we understand a process which takes place at the expense of energy produced by the living cell.

So far we have spoken of uptake and transport as if it were possible

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to make a sharp difference between these two processes in the leaf of Vallisneria. We are aware that this is in point of fact impossible, and that the two processes, as far as we can judge at the moment, are essentially the same. A cell which borders on the outside solution, absorbs the asparagine on its surface and passes it on to other cells which are not themselves in contact with the outside liquid, this means that these cells take the transport substance up from the outside cells.

We shall therefore have to use the terms uptake and transport indifferently, whilst the transport process only becomes more prominent in those experiments in which part of the leaf does not come into contact with the outside solution.

§ 2. *Method of investigation.*

All the experiments were made with pieces of the leaf of Vallisneria spiralis. The individual variability of the leaves and of various parts of the same leaf was eliminated in the manner previously discussed. (See 2 fig. 1 on p. 441, 1937.) Whereas previously only longer pieces of leaf were used, experiments were now made also with short pieces of leaf, which were in contact with the outside liquid or with the agar containing asparagine over their entire length.

For this purpose the longer pieces of leaf were divided into pieces of 8 mm before the test. 12 such leaf-pieces formed a series. In one experiment we had in this way 15 or 18 of such series of 12 pieces of leaf at our disposal, which, owing to their having come from different parts of the leaves and also from different leaves, were completely comparable as regards their content of nitrogen. Usually 3 series of leaf-parts remained untreated, and served for the determination of the nitrogen present at the commencement of the experiments. The amount of nitrogen of these series differs in the microkjeldahl tests [1]) by at most 10 γ. The short leaf-parts were used in experiments in which more especially the uptake processes were investigated. In tests as to uptake from agar the entire leaf-part was laid between strips of agar with asparagine, the extremities being left free in order to leave the intercellular spaces as far as possible in contact with the surrounding air.

These small leaf-parts were also suitable for the investigation of the uptake from asparagine solutions; it was in this case only necessary to take steps to prevent the pieces of the various series from getting mixed up. With this object the 12 leaf-parts of a series were sewn up in stripes of tulle. This at the same time prevented the leaf-pieces from floating on the solution, and so ensured their being properly in contact with the solution on both sides. In this way it was possible to test several series in the same solution.

[1]) We have to thank Miss J. W. E. VAN WEERDEN for assistance with the KJELDAHL analyses.

Although aeration was later found to have little or no influence on the strength of the uptake, vessels of Jena glass with a bottom of sintered glass, through which air was blown, were used in all the experiments. The model of these vessels is given by ROSENFELS (6).

§ 3. *The absorption by the leaves from agar and from solutions.*

With the arrangement in air, glass boxes were used, in which the agar strips were placed on glass plates. Fig. 2, (2) p. 442, 1937, gives an illustration of the arrangement with long leaf-pieces; the arrangement with short leaf-parts is entirely identical with this. On the bottom of the glass box there was water, and the box was closed, so that the air was saturated with water vapour. The boxes were placed in a dark room at a temperature of 25° C.

In these conditions the leaf-parts take up asparagine in the first 24 hours with a practically constant velocity. In the succeeding 24 hours the uptake first comes to a standstill and changes after about 36 hours into the exosmosis of the asparagine first taken up. It is therefore impossible to make tests with leaf-parts between agar in air which last longer than 24 hours. Such leaf-parts still look turgescent and normal after 24 hours. They are also still able to assimilate. It seems probable that the well-oxygenated air is in the long run not a desirable environment for the leaves of this aquatic plant, which is accustomed to the much lower O_2 tension of water. There is, however, no objection to experiments which do not last longer than 24 hours being carried out in air.

For the sake of comparison we investigated the uptake from solutions of asparagine. We did not find so strong an exosmosis in any of these tests as with the tests in air. In no case was a decrease of the nitrogen content to be demonstrated within 72 hours, although this was so in some cases after 96 hours.

§ 4. *The strength of the uptake from solutions of asparagine of different concentrations.*

We shall not discuss here in detail the analysis of the uptake process. For the question whether the absorption process of asparagine is a vital process we considered it of importance to determine how much asparagine can be taken up by the leaf-parts from solutions. Since the tests in air cannot last longer than 24 hours, the obvious course was to investigate the uptake from solutions of asparagine in water of different concentrations. More especially the uptake from very weak solutions was of importance, in order to determine whether, as former investigators (cf. STILES) had found for the uptake of salts, relatively greater amounts were taken up from weaker solutions than from stronger ones.

The results of various experiments differed in respect of the amount of the absorption. Older leaves, especially, were found to take up more than younger ones. In the same leaf, also, the older top portion takes up

a relatively greater amount than the younger base. It is easy to understand that such differences occur; the course of the absorption curves also shows differences. In spite of all these differences, however, there is agreement in the point that interests us here, that relatively larger amounts are taken up from weak solutions than from stronger ones.

We will report one of the various experiments which we made on this point. In table 1 the absorption from solutions of $^1/_{20}$ mol., $^1/_{160}$ mol., and $^1/_{1280}$ mol. asparagine are compared. In column 2 is shown the concentration

TABLE 1.

Absorption of asparagine from solutions of different concentrations in 72 hours. 25° C.

1 Concentr. asparagine in mol.	2 Concentr. asparagine in %	3 Absorption by 12 leaf-pieces in γ nitrogen	4 Absorption in γ asparagine	5 Absorbed in % of fresh weight
1/20	0.66	150	707	0.66
1/160	0.082	121	570	0.53
1/1280	0.01	71	335	0.31

of the asparagine solutions in percentages. Besides that, the increase in nitrogen of 1 series of 12 leaf pieces after 72 hours. In the fourth column how much asparagine this amounts to and in column 5 the asparagine absorption expressed in percentages of the fresh weight of leaf.

If it be assumed that the asparagine goes into the cell-sap, it would be present after 72 hours in a concentration which would be higher than that of the outside solution (cf. column 5 with 2). For the $^1/_{20}$ mol. solution there is no difference, but for the $^1/_{160}$ mol. solution it is already 6 times as large, and for the $^1/_{1280}$ mol. solution it is 31 times as large. The result of this test is therefore that *the asparagine is taken up in comparatively much greater amounts from weak solutions than from stronger ones.*

§ 5. *Influence of withdrawal of oxygen on the uptake of asparagine and caffeine.*

We have already described a part of these tests, but it is necessary to go into this in greater detail. We have investigated the effect of the withdrawal of oxygen both with long leaf pieces, which were able to take up the asparagine from agar with one extremity, and with short leaf-parts which were placed entirely in an asparagine solution. The two series of experiments are complementary to one another.

The tests were carried out in a MCINTOSH and FILDES anaerobic jar. In some tests only purified nitrogen gas was led through the jar for one

hour. In order to obtain a complete withdrawal of oxygen, nitrogen gas was first passed through for a short time, and then hydrogen gas. The oxygen was bound to the hydrogen by means of a palladium catalysator, the jar being entirely free from oxygen after about half an hour. The withdrawal of the oxygen was determined by means of reduced aqueous methyleneblue.

Table 2 relates to an experiment with long leaf-pieces in air free from oxygen. The test shows convincingly that the uptake is considerably

TABLE 2.

Transport of *asparagine* 0.05 mol at 25° C. Time of transport 22 hours.
A. Amount of nitrogen in γ present in untreated leaves.
B. Increase of nitrogen in γ in leaves kept in an anaerobic jar during transport of asparagine in air without any oxygen.
C. The same as in B for leaves kept in normal air.

	A	B	C
	Nitrogen content of untreated leaves	Increase of nitrogen in air without O_2	Increase of nitrogen in normal air
First part of 8 mm in contact with agar 2 %, containing asparagine in B and C	156	22	122
Second part of 8 mm	154	6	48
Third part of 8 mm	154	0	18
Fourth part of 8 mm	152	0	10
Fifth part of 8 mm	156	0	0

reduced when oxygen is absent. When only nitrogen was passed through the absorption was also greatly reduced. In these tests there was, of course, practically no transport.

In order to show with certainty that transport to parts of the leaf which were not in contact with the outside solution can also be impeded by lack of oxygen, it would be conceivable to go to work in the following manner. One extremity of the leaf is first allowed to take up asparagine under normal conditions, and the leaf is then placed in an environment free from oxygen. If a displacement of the asparagine in the leaf is then still possible, the transport cannot be dependent on oxygen.

It was impossible, however, to demonstrate such a displacement of asparagine to any considerable extent even in ordinary air. As we have already stated, pieces of leaf are no longer in a normal condition after remaining 24 hours in air. We therefore did not continue these experiments in this direction.

In the second place we endeavoured to determine the effect of the

withdrawal of oxygen with absorption from asparagine solutions. For this purpose a $^1/_{20}$ mol. asparagine solution was placed in a glass beaker in an anaerobic jar, and pure nitrogen was passed through the solution for more than 1 hour. The leaf-parts sewn up in tulle were then put into the oxygen-free solution, nitrogen was again passed through, and the remaining oxygen in the air was catalytically bound with added hydrogen. In water free from oxygen only 16 γ N was found to have been taken up after 48 hours, a quantity which scarcely exceeds the limit of error, whilst in the control 78 γ had been taken up.

For the sake of comparison with the effect of withdrawal of oxygen on the absorption of asparagine the effect on the uptake of caffeine was also investigated. Table 3 shows the results of this. In order to determine

TABLE 3.

Transport of *caffeine* 0.05 mol. at 25° C. Time of transport 22 hours.

	A	B	C
	Nitrogen content of untreated leaves	Increase of nitrogen in air without O_2	Increase of nitrogen in normal air
First part of 8 mm in contact with agar 2 %, containing caffeine in B and C	166	241	237
Second part of 8 mm	168	83	91
Third part of 8 mm	164	0	0
Fourth part of 8 mm	168	0	0
Fifth part of 8 mm	160	0	0

the value of this result, however, it is necessary to know the uptake of caffeine under normal conditions in the time. From the course of the curve for uptake from a caffeine solution of $^1/_{20}$ mol. it is seen that, in contrast with asparagine, the caffeine absorption takes place very quickly. The absorption from agar has a similar course.

When we suppose that it takes half an hour before the action of the withdrawal of oxygen has become effective, a large part of the amount of caffeine will already have been taken up. Great differences are therefore not to be expected with this method. The lack of any difference certainly points to the caffeine uptake not being influenced, but it was judged necessary to extend the experiments and to allow the withdrawal of oxygen to be effective during the entire process of uptake. It was therefore desirable to investigate the uptake in such a way that the leaf-pieces remained during the whole experiment in a space free from oxygen. For this purpose a few series of short leaf-parts, wrapped in wet tulle, were put

into the anaerobic jar. A beaker with caffeine solution without O_2 was also put into the jar. After the jar had been freed from oxygen, it was held obliquely, so that the caffeine solution was brought to the leaf-pieces on the bottom of the jar. Under these conditions 198 γ nitrogen had been taken up after 1 hour in an environment absolutely free from oxygen, whilst the control series under ordinary circumstances had taken up 193 γ (average of 3 determinations). This sufficiently proves that *withdrawal of oxygen has no inhibitory effect on the uptake of caffeine.*

§ 6. *Transport and uptake under the influence of evaporation.*

Although the foregoing experiments on transport all took place in a space saturated with water vapour, it was judged desirable to determine whether the free portion of the leaf might not give off water to the environment by evaporation, and whether, as a result, a suction stream was produced in the leaf, which was the cause of the accelerated transport of the asparagine.

It is conceivable that a suction stream of this kind passes round the living cells, either through the cell walls or possibly along the large intercellular canals.

The arrangement of these experiments was the same as that of all the tests with long leaf-pieces.

In the first experiment (cf. table 4) the transport was investigated in

TABLE 4.

Influence of transpiration on absorption of 0.05 mol. asparagine; time of transport 23 hours.
I. The free part of the leaf-pieces between strips of agar 2 %.
II. The free part of the leaf-pieces in air above saturated sodiumsulphate.
III. Above ammonium sulphate.

	Increase of nitrogen in γ		
	I	II	III
First part of 8 mm in contact with agar containing asparagine	104	96	118
Second part of 8 mm	40	58	72
Third part of 8 mm	14	24	24
Fourth part of 8 mm	6	16	12
Fifth part of 8 mm	0	0	0
Total amount	164	194	226

the case of leaves, with which the evaporation was quite out of the question, owing to the free portion being placed between 2 % agar, whilst direct contact of these agar strips with the agar which contained asparagine and was attached to one extremity, was carefully avoided. In the other

series of this experiment evaporation of the free portion was promoted by putting on the bottom of a glass box a saturated solution of sodium sulphate, above which a vapour tension prevails of 93 % at 20° C, and on that of another box a saturated solution of ammonium sulphate, above which a vapour tension of 81 % prevails at 20° C.

This experiment shows in the first place that the transport also takes place with complete saturation of the free portion of the leaf and secondly that, under the influence of the evaporation of the free portion, the transport is accelerated especially towards the second piece of the leaf. More especially with the low vapour tension had the leaf plainly lost its turgidity.

With a second test half of the free portion of leaf was covered with vaseline, to prevent an evaporation of this part. One extremity of 8 mm was therefore in contact with asparagine containing agar, then followed a piece of 16 mm, covered with vaseline, so that only 16 mm of the leaf could evaporate strongly. With this test (cf. table 5) it is very evident

TABLE 5.

Influence of transpiration on absorption and transport of 0.05 mol. asparagine. Time of transport 18 hours.
The first 8 mm of the leaf-pieces in contact with agar containing asparagine. The next 16 mm covered with vaseline. The rest free in the air.
I. In air saturated with water.
II. In air above sodium sulphate.
III. In air above ammonium sulphate.

	Increase of nitrogen in γ		
	I	II	III
First part of 8 mm in contact with agar containing asparagine	124	110	120
Second part of 8 mm covered with vaseline	50	42	42
Third part of 8 mm covered with vaseline	22	26	38
Fourth part of 8 mm free in air	18	34	56
Fifth part of 8 mm free in air	14	16	18
Total amount	228	228	274

that the fourth piece of the leaf, that is, the zone bordering on the part covered with vaseline, takes up more asparagine under the influence of the stronger evaporation. A suction stream may therefore really have an effect on the transport of substances, such as asparagine.

As it was found that practically no transport of asparagine takes place in an environment free from oxygen, it was interesting to try to ascertain whether an asparagine transport was also possible in an environment free

from oxygen, under the influence of a suction force caused by stronger evaporation.

In three parallel tests (cf. table 6) the normal process of uptake and transport was compared with that in case of oxygen withdrawal, in an

TABLE 6.

Influence of transpiration on absorption and transport of 0.05 mol. asparagine in air without oxygen. 25° C. Time of transport 22 hours.

I. Normal uptake in air above water.
II. Uptake in air above sodium sulphate without oxygen.
III. Uptake in air above water without oxygen.

	Increase of nitrogen in γ		
	I	II	III
First part of 8 mm in contact with agar containing asparagine	114	20	18
Second part of 8 mm	70	12	10
Third part of 8 mm	48	0	0
Fourth part of 8 mm	34	0	0
Fifth part of 8 mm	22	0	0

environment saturated with water vapour and above sodium sulphate. It was found that in both experiments in an environment free from oxygen only an extremely small uptake and transport took place. From this it may be concluded that the increased transport under the influence of a suction stream cannot come about if the living cells are not able to take up asparagine. A non-vital uptake of asparagine is therefore impossible even under the influence of a suction stream in the Vallisneria leaf.

§ 7. *Summary of results.*

The most important results of these experiments with leaves of Vallisneria are:

1. that the absorption of asparagine from weak solutions is relatively stronger than out of more concentrated ones;

2. that in an environment free from oxygen the uptake of asparagine both from solutions and from agar is greatly impeded, whilst the uptake of caffeine proceeds unchanged;

3. that a suction force, brought about by evaporation of the free portion of the leaf, accelerates the uptake and transport;

4. that in an environment free from oxygen, even in a leaf that is under the influence of a suction force of this kind, the uptake of asparagine is inhibited.

The different behaviour of caffeine and asparagine with withdrawal of oxygen points, in our opinion, to a fundamental difference between

the uptake processes of these two substances. The asparagine uptake is a process dependent on the respiration, whilst the caffeine uptake proceeds even when the cell has an anaerobic respiration. This agrees with the fact that we previously found, both in the case of Vallisneria and with the tentacles of Drosera, that the transport of asparagine takes place more rapidly and over a greater distance than that of caffeine.

The process of asparagine uptake is something like that of salts, as has been made known by the investigations of STEWARD and HOAGLAND. COLLANDER, also, has repeatedly pointed out the importance of these active absorption processes (adenoide Tätigkeit), and found a similar influence inter alia with the uptake of sulphonic-acid dyes (4). We wish, however, to postpone a detailed discussion of the question whether all these processes are really based on the same mechanism until we have more data at our disposal regarding the uptake of Vallisneria leaves.

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