Botany. — *Absorption and transport by the tentacles of Drosera capensis.* I. *Active transport of asparagine in the parenchyma cells of the tentacles.* By W. H. ARISZ.

(Communicated at the meeting of December 27, 1941.)

§ 1. *Introduction.*

OUDMAN (1936) showed that the tentacles of Drosera capensis are organs which can take up asparagine and caffeine with their glands and transport these substances by means of the pedicels to the leaf. He pointed out some differences in the absorption of asparagine and caffeine, which he attributed to the different transport-tracks for these substances. With caffeine we mainly observe a diffusion through the vacuole, with asparagine the transport would take place through the protoplasm, the protoplasm being much more permeable to caffeine than to asparagine. ARISZ and OUDMAN (1937) pointed out the different behaviour of these substances with regard to the causing of aggregation in the parenchyma cells of the pedicels. They connected the better transport of asparagine with the aggregating action of this substance, whereas caffeine does not cause aggregation but granulation.

From a research of ARISZ and OUDMAN (1938) on the absorption and the transport of asparagine through the leaves of Vallisneria it had appeared that asparagine-absorption is an active accumulation-process, which is dependent on a normal supply of oxygen. There is similarity between this process and the absorption of salts (HOAGLAND, STEWARD). This has given rise to investigating whether the absorption and the transport of asparagine by the tentacles of Drosera may be an analogous process. In this communication it will be shown that the transport of asparagine is carried out by the living parenchyma cells of the pedicels, and is dependent on the supply of oxygen; besides it is an accumulationprocess.

In making the analyses and experiments I have met with excellent help from Dr. J. OUDMAN, Miss J. VAN WEERDEN and Miss J. VAN DER SCHANS, for which I tender them my best thanks. In connection with the above the experiments have been divided into three series: series O in 1938, series W in 1938—1940 and series S in 1941.

§ 2. *Method.*

The experiments here discussed were made in the months of April to October 1938—1941 with plants of Drosera capensis grown from seeds. The plants were of various ages. Young plants have a higher content of N-compounds than old ones and absorb more asparagine too. Therefore we used material of the same age for an experiment as far as this was possible. The length of the leaves varies from 2.5 to 5 cms. The method used to examine the transport in the tentacles was already described before (ARISZ and OUDMAN 1937). The extremities of the marginal-tentacles on either side of the leaves are put between strips of agar. In the 2 % agar solution the substance which is to be taken up, is also present. If necessary other substances can be dissolved in the agar as well. The lamina the marginal tentacles of which are lying in the agar on both sides of the leaf, is for the rest entirely free from the agar-strips, so that transport to the leaf can only take place through the tentacles. The number of tentacles placed between the agar strips, amounts to about 150 with short leaves, with longer ones to 320 and more. Just as in previous experiments the variability in the behaviour of the leaves of various ages and of various plants was more or less eliminated by numbering the leaves of six plants from one to six and subsequently combining into one series one leaf of each plant, inserted on a different level. In this way 6 series are obtained

each containing 6 leaves. These show a fair correspondence in nitrogen-content. (See on this subject OUDMAN 1936, Table VI, p. 386). The substances of which the transport has been investigated are mainly the following: asparagine, glycocoll, alanine, leucine, urea, methyl-urea, thiourea, urotropine, caffeine, KH_2PO_4, K_2HPO_4, NH_4Cl and KNO_3. In this communication asparagine will be discussed in particular. The quantity taken up has been ascertained according to the micro-Kjeldahl method. The quantity of nitrogen is related to the fresh weight of the leaves.

All experiments were made in a room of a constant temperature at 25° C. in the dark.

§ 3. *Transport by the parenchyma cells of the pedicel.*

The tentacles of Drosera possess an ellipsoidical gland, in which there are a number of gland-cells. (HOMÈS 1929). The function of these gland-cells is evidently in the first place secreting the viscid fluid which always covers the tentacle-glands. On stimulation of the tentacles or on stimulating the leaf a strong and sometimes prolonged secretion takes place (DARWIN 1875). The gland is likewise capable of taking up a great number of substances. Where the pedicel is covered with a cuticle, water and dissolved substances cannot penetrate it. This becomes evident when from a tentacle both gland and pedicel are brought into a caffeine-solution. The caffeine that has penetrated immediately precipitates in the vacuole. From this very sensitive reaction it appears that caffeine only penetrates into the gland and this substance cannot diffuse through the outer wall of the pedicel. Only into those places of the pedicel, where there are so-called papillae, the caffeine enters. After the substances have penetrated through the gland, they must be carried through the pedicel to the leaf. The length of the pedicels of the marginal-tentacles amounts to 3 to 4 mms. In the pedicel one or two spiral vessels occur. When the caffeine ist taken up, we see from the occurrence of the precipitate in the vacuole, how the caffeine passes on from cell to cell and starts in every cell from the apical end. From this KOK rightly concluded that caffeine is transported through the vacuole and not through the cell-wall or the protoplasm. In her experiments on tentacles without glands, transport through the spiral vessel would have been possible, because on removing the tentacle-gland the spiral vessels were opened. The course of the transport through the parenchyma-cells, however, proves, that hardly any transport of caffeine takes place through the spiral vessel.

On the analogy of this ARISZ and OUDMAN have also assumed for the transport of asparagine that this would take place in the parenchyma cells and not in the spiral vessel.

Now the question arises whether it will be possible to prove that the parenchyma cells of the pedicel actually transport asparagine. The transport might be brought about in a different way. For instance the tentacle-gland might be an organ that takes up substances from outside and then gives them up again to the liquid in the spiral vessel. The transport of the dissolved substance might then take place either by means of active secretion of the tentacle gland inwards or by means of suction, resulting from a suction pressure which may arise in the leaf for instance through transpiration. Though in the transport experiments of OUDMAN (1936) and ARISZ and OUDMAN (1937) this possibility was taken into account and the leaves were put in closed glass boxes, the air of which was saturated with water vapour, yet it seemed desirable to obtain more data on this matter. Therefore two series of experiments have been made: one in which the transpiration by the leaf was increased by putting the leaves in dry air, and a second series in which sugar was added to the agar, so that water was osmotically absorbed from the tentacles. This arrangement caused in the tentacles a flow of water in exactly the opposite direction to that in which the transport of the absorbed substances towards the leaf had to take place. The result of the experiments leaves no doubt but the transport of asparagine is entirely independent of currents in the tentacles which are caused by the transpiration of the leaf or by absorption of water from the tentacle by osmotically acting substances in the agar.

Three series of experiments have been taken: Series A, experiments in which the leaves were in a dry atmosphere; series B experiments in which through addition of sucrose to the agar water absorption from the tentacles took place and series C experiments in which the leaf was exposed to greater transpiration and sugar was also added to the agar.

Series A. Increase of the transpiration of the lamina. The dry air was obtained by bringing a saturated solution of Na_2SO_4, $(NH_4)_2SO_4$ or Na_2CO_3 into the closed glass boxes in which the Drosera leaves were. The relative humidity of the air above these liquids is 93 % (20° C.), 81.1 % (25° C.), 92 % (18.5° C.) respectively. From the experiments mentioned in table I it may be concluded that with intact tentacles the uptake

TABLE I. Influence of increased transpiration of the leaves on the uptake by the tentacles. In experiments 4 and 5 sugar 0.35 mol. is added to the agar.

	Absorption	Nitrogen increase in γ		Nitrogen increase in $^0/_{00}$ fresh weight	
		humid air	dry air	humid air	dry air
1	24 hours 1/20 mol. asparagine	254	286	0.90	0.97
2	24 hours 1/20 mol. asparagine	332	220	0.75	0.70
3	24 hours 1/20 mol. asparagine	358	352	1.05	1.04
4	24 hours 1/20 mol. asparagine	241	210	0.84	0.81
5	24 hours 1/20 mol. asparagine	604	638	1.77	1.75

and the transport of asparagine and caffeine is little or not affected by an increased transpiration of the lamina. In an atmosphere saturated with water vapour an equally strong absorption takes place as in an atmosphere of 89—90 % humidity. In these experiments two difficulties present themselves. The first difficulty shows when the quantity of N taken up is related to the fresh weight of the leaf. If the series of leaves are weighed before the beginning of the experiment, the mucilage of the tentacle-glands would be included, unless it is first removed from the glands by washing. The quantity of secreted fluid is not slight in proportion to the fresh weight of the leaf. So 2 series of 6 leaves appeared to weigh 489 and 522 mg., secretion included; after washing and removal of the secretion 317 and 326 mg. resp.; so that the secretion amounted to 172 and 196 mg.; that is more than 50 % of the fresh weight of the leaves. As washing of the secretion before the experiment would injure the tentacles too much, it is necessary to determine the fresh weight at the end of the absorption period. If the absorption takes place in an environment in which during the experiment there is a loss of water from the leaves, relating the nitrogen content on the fresh weight could give rise to an error. The absorbed quantity of N expressed in fresh weight per thousand will be found too high in this case. In these cases it is preferable to compare the absolute values of N taken up per series. As each series possesses an equal number of leaves, this method is useful in such cases, though it is not particularly accurate. If we take this into account, it is evident that an increase of absorption owing to transpiration of the leaf is out of the question, either with the absorption of caffeine, or with that of asparagine.

In these experiments a second difficulty arises, i.e. that the tentacles under the influence of asparagine curve from the agar, especially in a humid environment. Owing to this the absorption of the asparagine from the agar is not so great. To meet this difficulty a series of experiments C has been made, in which transpiration of the leaves also took place, but inflection of the tentacles was prevented.

Series B. In order to get a flow of water in the tentacles in the direction of the leaf to the tentacle-gland sugar was dissolved in the agar. Owing to the water absorption from the tentacles and from the leaf, the increase in N-content will become too large, if it is calculated as the difference in N-content at the beginning of the experiment, related on the fresh weight at the beginning of the experiment, and the N-content at the end of

the experiment related on the final fresh weight. Therefore the absolute values N, in γ per series have also been given in Table II. From the data obtained it appears, that

TABLE II. Influence of adding sugar to the agar with the asparagine on the uptake by the tentacles.

Absorption	Sugar conc. in agar	Nitrogen increase in γ		Nitrogen increase in $^0/_{00}$ fresh weight	
24 hours 1/20 mol. asparagine	none	306		0.70	
	0.2 mol	274		0.84	
	0.3 mol	372		1.32	
	0.4 mol	398		1.35	
24 hours 1/20 mol. asparagine	none	200		0.74	
	0.3 mol	212		0.87	
	0.45 mol	310		1.26	
	0.6 mol	312		1.34	
		1	2	1	2
24 hours 1/20 mol. asparagine (two experiments)	none	202		0.76	
	0.45 mol	220	496	1.17	1.46
	0.60 mol	280	514	1.37	1.84
	0.75 mol	198	406	1.11	1.83
	0.90 mol	176	236	1.12	1.51

sugar-concentrations higher than 0.3 mol. prevent the tentacles from curving out off the agar. These get less turgescent on account of the loss of water and cannot make a curvature. So at the same time this provides a method of preventing the inflection of tentacles. As far as it was possible to ascertain this by experiments, the strength of the absorption and the transport of asparagine does not alter in the least through a sugar-concentration of 0.3—0.4 mol. On the contrary, because the tentacles remain in closer contact with the agar, the absorption is much increased. In the highest sugar-concentrations, however, the absorption does decrease. A plasmolysis of the parenchyma cells of the pedicel does not show in these circumstances even in high sugar-concentrations. Nor is this to be expected, as the sugar-solution cannot pass through the impermeable outer wall. It is rather a shrivelling of the whole pedicel that takes place, which is shown in the slighter turgor of the tentacles, owing to which inflections cannot arise and especially the parts close below the gland clearly show folds of the wall. In spite of the fact that a flow of water is brought about in the tentacles towards the gland, yet absorption and transport takes place towards the leaf.

Series C. In these experiments (table I exp. 4 and 5) there is a sugar-concentration of 0.35 mol. in the agar, while the lamina lies either in humid air above water or in dry air above saturated $(NH_4)_2SO_4$. A curving-out of the tentacles from the agar cannot take place under the circumstances, while through the transpiration of the leaf a tension is developed by suction, which carries water towards the leaf through the tentacle. Under these circumstances there was not found any influence of the water-flow through the tentacle on the transport of asparagine and caffeine either.

§ 4. *Influence of withdrawal of oxygen on the uptake of asparagine.*

Seeing that the absorption of asparagine by the leaves of Vallisneria spiralis is a process that takes place in the presence of oxygen (ARISZ and OUDMAN 1938) it seemed desirable to ascertain whether the uptake of asparagine by the Drosera-tentacles is also a process dependent on the presence of oxygen. From a number of experiments, comprised in table III it appears that this is actually the case. Without an exception the absorption is slighter when oxygen is absent. The withdrawal of oxygen was

obtained by conducting into a McIntosh and Fildes anaerobic jar first purified N-gas and next combining the oxygen still present to hydrogen-gas by means of a palladium-catalysator. In one of the experiments the oxygen was probably not completely removed, in the remaining the transport was considerably checked and amounts to only 0 to 14 % of the transport in aerobic conditions.

In entire correspondence with this OUDMAN (1936) found, that aethernarcosis checks the uptake of asparagine. From table III experiment 6 it appears that also under the influence of 1/300 mol. KCN, which was added to the agar-strips, the absorption is greatly inhibited.

These experiments prove satisfactorily that absorption and transport of asparagine are sensitive to decrease of the oxygen-pressure and inhibited by KCN and aether-narcosis.

Now it seemed important to find out, whether tentacles of which the glands had been removed, were still capable of taking up asparagine and if even then this process would be sensitive to the withdrawal of oxygen. In this way it must be possible to prove that transport of asparagine through the pedicels is an active process, for which the presence of oxygen in the living cells is required.

OUDMAN has already ascertained whether tentacles from which the glands have been cut-off, still take up asparagine. For tentacles without glands he found an uptake of 73 % of those with glands.

In table III (exp. 7, 8 and 9) a summary has been given of some experiments. From

TABLE III. Influence of oxygen withdrawal and KCN on the uptake of asparagine. In exp. 7, 8 and 9 the glands have been cut off before the beginning of the experiment.

	Absorption	Nitrogen increase in $^0/_{00}$ fresh weight		Anaerobic as $^0/_0$ of normal
		normal	anaerobic	
1	24 hours 1/10 mol.	1.45	0.21	14.5%
2	24 hours 1/10 mol.	1.71	0.44	25.7%
3	24 hours 1/20 mol.	0.83	0.—	0 %
4	48 hours 1/20 mol.	1.60	0.06	4 %
5	24 hours 1/20 mol. with 0.35 mol sugar in agar	1.15	0.15	12.2%
			KCN $\frac{1}{300}$	
6	24 hours 1/20 mol.	1.14	0.26	22.8%
	tentacles without glands		anaerobic	
7	24 hours 1/20 mol.	0.79	0.41	
8	24 hours 1/20 mol.	0.51	0.10	
9	24 hours 1/20 mol. with 0.35 mol sugar in agar	0.41	0.20	

the figures it appears that also in tentacles without glands the transport is stronger in the presence of oxygen. It is true the percentage that has been taken up anaerobically is higher, but that is not to be wondered at. In the first place these tentacles are in an abnormal condition owing to the injury caused on cutting off the glands and besides the spiral vessel has been opened, so that a free diffusion and flow may take place in it. As also from agar to which 0.35 mol. sucrose has been added, more is taken up aerobically than anaerobically, it is evident that the transport takes place in the parenchyma cells of the pedicels.

§ 5. *Absorption of asparagine is an accumulation-process.*

Now that it has been shown that the transport of asparagine must take place by the

living tentacle-cells and is dependent on aerobic respiration, it is important to know whether this process like the taking up of asparagine by the leaf cells of Vallisneria is an accumulation process, in which the substance is accumulated against a concentration gradient. OUDMAN has shown by experiments in which the tentacles were cut off before the analysis, that the absorbed asparagine does not remain in the tentacles but also arrives in the lamina of the leaf. Where the asparagine taken up is found in the leaf, has not further been ascertained. It has, however, appeared from OUDMAN's research, that in the leaf there occurs no formation of protein from asparagine. In how much other conversions of asparagine take place is unknown. For the present we will presume that for the first 24 hours the asparagine in the leaf continues unaltered and is equally distributed over all leaf-cells. Though this supposition will not be quite correct, as the asparagine concentration will probably be higher nearer to the marginal tentacles, yet it enables us to get an impression of how the concentration of asparagine would be, if it were present in solution in the cell-sap of the leaf cells. On the question whether the asparagine taken up arrives in the cell-sap completely or partly, we have no data. On the ground of what is known about the absorption by other tissues, however, it is likely that a considerable part of the asparagine is present in solution in the vacuole.

Table IV gives an impression of the strength of the accumulation. In weaker concen-

TABLE IV. Accumulation of asparagine.

Mol. asparagine conc. in agar strips	Nitrogen in $^0/_{00}$ of the fres weight	Mol. asparagine conc. in leaves	Accumulation factor
0.05	1.47	0.0525	1
0.0125	1.90	0.0679	5
0.003125	1.50	0.0536	17
0.000781	0.80	0.0286	37
0.000195	0.20	0.0071	37

trations the accumulation-factor is greater and amounts in the case of 1/1280 mol. and 1/5120 mol. asparagine to about 37. With this it is satisfactorily shown that the transport of asparagine may take place against a concentration gradient of this substance.

§ 6. *Summary and Discussion.*

From the preceding it appears that the tentacles of Drosera capensis are capable of transporting asparagine through the pedicels. As already shown by OUDMAN this process has a high temperature-coefficient. It has been shown that this transport is dependent on a proper oxygen-supply to the living tentacle-cells. By narcosis and by KCN it is inhibited. Accumulation occurs, so that transport can take place against a concentration gradient. The tentacle-gland has no specific function in the absorption. Tentacles, of which the glands have been removed, are also capable of taking up asparagine actively. Transport through the spiral vessels of the pedicel does not figure in it. Neither by sucking water from the leaf towards the gland, by bringing it into agar to which osmotically working sucrose has been added, nor by increasing the transpiration of the leaf and sucking water towards the leaf through the tentacles, absorption or transport can be noticeably accelerated or retarded.

Accordingly it has been proved that the transport of asparagine in the tentacles is a transport-process in parenchyma cells, which is not dependent on a concentration gradient of the substance transported and therefore cannot be a diffusion process. MASON and MASKELL's theory about activated diffusion does not obtain for this process. It is, however, closely connected with what is known about the absorption and the transport of salts in the cortex of the root. We shall go further into the nature of the process

in a following communication in connection with results obtained about the transport of other substances.

The data obtained on the strength of the transport enable us to calculate the strength of the transport in the pedicels. When 6 leaves wih 1200 tentacles take up 300 γ nitrogen in 24 hours, $\frac{1}{4}\gamma$ N. is transported per tentacle, i.e. $\frac{33}{28}\gamma$ asparagine. The diameter of a tentacle just below the gland amounts to about 0.04 mm. So $\frac{33}{28}\gamma$ asparagine is transported through a surface of 0.00126 mm² in 24 hours, i.e. 0.039 mg. asparagine per mm² per hour. If the transport takes place through the protoplasm, this figure rises considerably. Reliable data on the transport in parenchyma cells in root, stalk or leaf are not known to me. So we come to comparing the transport in the tentacles with that in the sieve tubes. For transport in the stalk (MÜNCH) 10.7—63.3 mg. per mm² per hour was found, for transport of assimilates from a beanleaf (BIRCH–HIRSCHFELD) 5 mg. per mm² per hour, for supply of assimilates to fruit (MÜNCH) 4.7—6 mg. per mm² per hour, for the rate of transport of sugars in the stalk in cotton (MASON and MASKELL) 2.3 mg per mm² per hour. From this it appears that the rate of transport in the sieve-tubes is more than 100 times faster than the transport in the parenchyma cells of the tentacles. Therefore it appears on comparison with the transport in the sieve-tubes that the rate of the latter is much greater. For the present there is no reason to assume that the transport in the sieve-tube would be a process that is analogous with the active transport in the Drosera tentacles.

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Hydrodynamics. — *On the influence of the concentration of a suspension upon the sedimentation velocity* (in particular for a suspension of spherical particles) *). By J. M. BURGERS. (Mededeeling N⁰. 42 uit het Laboratorium voor Aero- en Hydrodynamica der Technische Hoogeschool te Delft).

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15. With the aid of the results obtained in sections **11.—14.** we will now attempt to calculate the influence which a given particle experiences from all the surrounding particles, in a field extending indefinitely in all directions and everywhere possessing the same average number of particles per unit volume. It will be seen in **17.** that a difficulty still remains in the problem, in so far as there occurs an integral, the value of which depends upon the way the integration is carried out. By prescribing a certain definite way a particular value is obtained, which to the author would appear the one best adapted for the present purpose, but the problem cannot yet be considered as being wholly settled.

We begin with the summation of the velocities induced in a particle A in consequence of the presence of the other particles ("particles B"). These particles can be taken together in groups, each group being situated at some definite distance r_i from A; the number of particles per group being n_i. The contribution by each group will be calculated upon the assumption that we may use the mean value of (54) over a surface $r = constant$. Restoring the factor $F/8\pi\eta$ the total amount becomes:

$$\delta u_1 = \Sigma u_i = -\frac{5Fa^3}{24\pi\eta}\sum\frac{n_i}{r_i^4} \quad . \quad . \quad . \quad . \quad . \quad . \quad (55)$$

In working out the sum it is not necessary to proceed far: from a certain distance r_m onward it is sufficiently accurate to make use of the integral:

$$n\int_{r_m}^{\infty} 4\pi r^2\, dr\,(1/r^4) = 4\pi n/r_m \quad . \quad . \quad . \quad . \quad . \quad . \quad (56)$$

where n is the average number of particles per unit of volume. The distance r_m is defined by:

$$n\,.\,(4\pi r_m^3/3) = 1 + \Sigma n_i \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (57)$$

the summation extending just as far as we take separate terms in (55).

For purposes of comparison we write:

$$\delta u_1 = -\lambda_1 n s u_0\,. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (58a)$$

where $s = 4\pi a^3/3$, $u_0 = F/6\pi\eta a$ (compare 30c), and:

$$\lambda_1 = \frac{15a}{4r_m} + \frac{15a}{16\pi n}\sum\frac{n_i}{r_i^4} \quad . \quad . \quad . \quad . \quad . \quad . \quad (58b)$$

16. The evaluation of (58*b*) is possible only when we possess a statistical theory of the distribution of the particles in the neighbourhood of a given one. As it is not a part of our task to develop such a theory here, we shall restrict to the consideration of a few typical cases.

We might assume in the first place that the surrounding particles may take all positions

*) Continued from these Proceedings **44**, 1941, p. 1184.