Botany. — *Absorption and transport by the tentacles of Drosera capensis.* II. *The activation of the transport of different substances by oxygen.* By W. H. ARISZ.

(Communicated at the meeting of September 26, 1942.)

§ 1. *Introduction.*

In a previous paper it has been shown that the transport of asparagine by the tentacles of DROSERA is a process dependent on aerobic respiration. The tentacles take up asparagine even from an asparagine solution of very low concentration and transport it through their parenchyma cells to the lamina. This process may take place against a concentration-gradient. There does not seem to exist any fundamental difference between the process of absorption and that of transport of asparagine. In both cases the protoplasm accelerates a transfer of asparagine. Therefore we shall not make a difference between absorption- and transport-processes in this paper. It is essential to know whether this active transport is a special process, only obtaining for asparagine, or whether it also obtains for related substances as amino-acids, and how other organic and inorganic substances are transported. For there are many indications in literature that also other substances are taken up. This was already concluded by DARWIN from the aggregation and granulation caused by various substances in the parenchymacells of the pedicel.

ARISZ and OUDMAN (1937) have corroborated the observation of Miss KOK (1932) and of OUDMAN (1936), viz. that the absorption of caffeine much resembles a process of diffusion. In this communication it will be shown that the absorption of caffeine is not merely a process of diffusion after all, as it proceeds more strongly in the presence of oxygen than without oxygen. This activating influence of oxygen, which is particularly strong for aminoacids and asparagine, is found again in all substances examined in a greater or less degree. This also holds good for some inorganic substances.

The arrangement of the experiments and the method of research has already been described in the preceding publication. In these experiments I again had the valuable assistance of Miss J. VAN WEERDEN and Miss J. VAN DER SCHANS. The phosphate determinations have been made with ammonium-molybdate by using a colorimetric method, after destruction of the leaves with concentrated H_2SO_4 and HNO_3. Though in accuracy this determination is inferior to the Micro-Kjeldahl method, the values for the quantities of phosphates absorbed are sufficiently large for us to draw our conclusions. The accuracy of the experiments is mainly dependent on the uniformity of the plants examined in a certain experiment. This causes much more uniform results in one experiment than in another.

§ 2. *Influence of Oxygen on the absorption and the transport of organic substances.*

The influence of a decrease of the oxygen-pressure on the absorption of asparagine by the tentacles was already discussed in the preceding paper. In the same way the absorption of other organic substances has now been examined. In table I their data have been given. The following substances were examined: urea, methyl-urea, thio-urea, phenyl-urea, glycocoll, α alanine, leucine, phenylalanine, asparagine, urotropine and caffeine. All experiments were continued for 24 hours at a temp. of 25° C. The concentration in which the substances were administered in the agar, amounted to $^1/_{20}$ m in every case.

Various of these substances, as asparagine and the aminoacids cause a curving of the tentacles out of the agarstrips. In experiments with these substances we usually find already after 24 hours the tentacles curved out of the agar and their glands pressed against it. Though they are therefore still in touch with the agar, their uptake is reduced.

In a concentration of $^1/_{20}$ m. urea, thio-urea and caffeine cause no curving of the tentacles. In lower concentrations there may occur a curve in the case of caffeine. As was already shown in the previous communication addition of sucrose to the agar in a concentration of 0.35 m reduces the turgescence of the tentacles so much that an inflection is prevented. This method has also been used in many of these experiments to prevent curving of the tentacles from the agar. In table I the quantity of nitrogen taken up in

TABLE I. Absorption of organic substances in normal and anaerobic conditions. The concentration of all substances is 1/20 m. Duration of absorption 24 hours. 25° C. Sucrose 0.35 m has been added to the agar.

Substance	Nitrogen increase in ‰ of fresh weight		Anaerobic as % of normal	Mol. w.	Conc. of absorbed substance in leaves in millimol	
	Normal	Anaerobic			Normal	Anaerobic
Urea	0.87	0.21	24 %	60.05	31	7.5
Methyl-urea	0.31	0.13	42 %	74.06	11	4.6
Thio-urea	0.49	0.27	55 %	76.11	17.5	9.6
Phenyl-urea	0.43	0.28	65 %	136.08	15	10
Urotropine	0.69	0.45	65 %	140.13	12	8
Caffeine	0.93	0.64	69 %	194	17	11.4
Glycocoll	1.05	0.03	3 %	75.05	75	2.1
α alanine	0.90	0.04	4 %	89.06	64	2.8
Leucine	0.52	0.—	0 %	131.11	37	0.—
Asparagine	1.15	0.15	12 %	132.08	41	5.4

24 hours has been given, expressed in ‰ of fresh weight of the leaves, in normal circumstances, i.e. in the presence of oxygen in the air and in anaerobic conditions. Moreover the concentration of the absorbed substance in millimol present in the leaf at the end of the experiment has been given in the last two columns. It has been taken for granted that the substance taken up does not undergo any transformation [1]). On comparing these figures one should take into account that the amount of the absorption of a substance may differ a good deal in different experimental series, because plants of various sizes and various ages have been used. The experiments extend over some years and have been made in various times of the year, in consequence of which the condition and the reactivity of the plants have not been the same in all experiments. Besides in experiments in which curving of the tentacles has been prevented by the addition of sucrose to the agar, a comparatively greater quantity is taken up, so that experiments made in this way, are not to be compared with experiments without the addition of sucrose. Of course this does not obtain for experiments with urea, thio-urea and caffeine. As the tentacles do not curve out in these cases, it does not matter whether sucrose has been added or not. If an other concentration than $^1/_{20}$ m had been chosen, the absolute value of the quantities absorbed would have been altered for some substances, for others it would not in the same degree, and owing to this the relative proportion in which the substances are taken up, would have been modified too. It is therefore permitted to compare the values of table I for the absorption of *one and the same* experimental series under normal and under anaerobic conditions, but too great a value should not be attached to the difference in the amount of the uptake of *different* experimental series. Therefore we only produce the figures of the last two columns to give the reader a provisional impression of the relative amount in which various substances containing a different number of nitrogen atoms have been absorbed. From the table it appears that with all substances examined the absorption is

[1]) This presupposition seems permitted, as OUDMAN could not show any protein-sythesis in the leaves after feeding.

diminished by decrease of oxygen. In order to get an impression about the extent to which withdrawal of oxygen affects the process of absorption, the quantity of nitrogen absorbed anaerobically, expressed in percents of that which is absorbed in normal conditions, has been given in the fourth column. This value enables us to compare the influence of oxygen withdrawal on the absorption for various substances. When the values obtained for amino-acids are compared with those obtained for asparagine in the previous paper (ARISZ, I) it appears that these substances behave in the same way and that on removal of oxygen, transport is as good as impossible. In how far a slight transport takes place anaerobically cannot be decided, as the values found are within the limits of error of the determinations. It is surprising that also urea, methyl-urea, thio-urea, phenyl-urea and caffeine are transported in greater quantities in aerobic than in anaerobic conditions. For caffeine we find that anaerobically about 70 % of the quantity absorbed in normal conditions is taken up. So in this case even without the influence of aerobic respiration a rather strong absorption takes place.

From these data the general conclusion must be drawn, that the acceleration caused in the transport by aerobic respiration, is a process which occurs in the transport of all substances examined. As long as only the influence was known which decrease of oxygen-pressure has on the absorption and the transport of asparagine, we might get the impression that there existed a sharp distinction between an active transport in the case of asparagine, which is dependent on aerobic respiration and a passive transport in the case of caffeine which would be based on diffusion. If, however, we consider the results of a decrease of oxygen-pressure on the uptake of different substances, it is clear that such a sharp contrast does not exist between actively and passively transported substances. There is a gradual transition from asparagine and amino-acids to urea, methyl-urea, thio-urea, phenyl-urea and caffeine. The activation cannot but be based on an influence which originates from the protoplasm. As a measure of activation we shall use

$$100\left(1 - \frac{\text{anaerobic absorption}}{\text{aerobic absorption}}\right).$$

In table 2 the values for the activation of the various substances examined have been given. The special behaviour of asparagine and the amino-acids is clearly visible, in which transport is activated for nearly 100 %. Next urea, methyl-urea, thio-urea, phenyl-urea, urotropine and caffeine follow, the activation of the transport growing smaller and smaller in this series, until the transport rather resembles an ordinary diffusion-process.

TABLE II. Activation of the transport of different substances. The activation is expressed as $100\left(1 - \frac{\text{anaerobic transport}}{\text{aerobic transport}}\right)$.

	Activation		Actiyation
Glycocoll	97	Methyl-urea	58
Alanine	96	Thio-urea	45
Leucine	100	Phenyl-urea	35
Asparagine	88	Urotropine	35
Urea	76	Caffeine	31

On the basis of the data now obtained the behaviour of caffeine deviates somewhat from what has been found before.

Miss KOK examined the diffusion of caffeine in the pedicel by ascertaining after different periods, in how far granulation had occurred in the vacuole of the cells. She found that the distance covered in the transport is proportional to the square root of the time, as according to STEFAN may be expected with a diffusion process. In her opinion the transport-route for caffeine is mainly the vacuole. The acceleration which according to

the experiments discussed above appears to occur under the influence of respiration in the transport of this substance, must, however, be based on an influence executed by the protoplasm. We shall have to assume that this acceleration affects the transport during the passage of the caffeine through the protoplasm from one vacuole to the adjoining one. Miss KOK states that the resistance offered by the protoplasm to the transport is 160 times larger than that of the vacuole (KOK 1932, p. 104). In her experiments of short duration diffusion must have been relatively strong, whereas the activated transport was relatively slighter than in experiments mentioned here, which lasted 24 hours. In her short experiments Miss KOK found no influence of aether-narcosis, whereas OUDMAN with an experiment that lasted 18 hours, found a feeble inhibition of the transport of caffeine by aether-narcosis. With narcosis the transport amounted to 79.6 % of the normal one. This observation can be understood, now that it has been shown that also in the transport of caffeine protoplasmic activation cooperates.

After all the uptake of caffeine is chiefly a diffusionprocess. From experiments with different concentrations of caffeine it appears that in 24 hours no typical accumulation is found. The difference with the accumulation of asparagine becomes obvious in comparing table III with table IV of the first publication of this series (ARISZ, I).

TABLE III. Absorption of caffeine from different concentrations. 25° C.

m conc. of caffeine in agar	Nitrogen-increase in ‰ of fresh weight of leaves	m conc. of caffeine in leaves	Accumulation-factor
0.05	0.81	0.0145	0.29
0.0125	0.38	0.0068	0.54
0.003125	0.17	0.0030	0.96
0.000781	0.		

The data of tables 1 and 2 give rise to the question whether there is any connection between the nature of the substance and the strength of the transport. For the permeation of substances through the protoplasm into the vacuole general rules have been found concerning the connection between the nature of the organic substances and their power of permeating. So COLLANDER assumes that substances permeate better according as they are more lipoid-soluble, while at the same time the molecular volume has its influence. When we trace whether there is any connection between the lipoid-solubility of the substances and the strength of their transport, we should distinguish between the activated transport in aerobic conditions and the transport by diffusion when oxygen is withdrawn. If the latter was purely a diffusionprocess, influence of lipoid-solubility and molecular volume might be expected. For lipoid-soluble substances permeate rapidly through the protoplasm into the vacuole and diffusion in the vacuole takes place more rapidly than in the protoplasm. The data obtained (table I, last column) do not yet enable us to answer this question. Thio-urea having a greater lipoid-solubility than urea, it might be expected that anaerobically more thio-urea than urea would be taken up. This is indeed the case, but only in a slight degree. Caffeine, of which the lipoid-solubility is great and which gives a precipitation in the vacuole, is transported anaerobically more strongly than urea, which is lipoid-soluble to a less degree and has a much smaller molecule. The transport of caffeine is also stronger than that of thio-urea and urotropine, which have a smaller molecule. The very slight anaerobic transport of asparagine and amino-acids is very likely connected with the dissociation of these substances. On the whole dissociated substances permeate badly. In connection with what has been said above on the comparability of the various experimental series, the data mentioned are insufficient to prove a connection between lipoid-solubility and anaerobic transport.

Let us now consider whether there is any connection between the quantity of substances absorbed under normal, aerobic conditions their lipoid-solubility and their molecular

TABLE IV. Absorption of urea, methyl-urea, thio-urea and phenyl-urea in a concentration of 1/20 m and of 1/80 m. All experiments with addition of sucrose to the agar. Column I contains dates on the molecular refraction, column II gives the oil/water distribution coefficients.

	I	II	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
	Mol. refr.	$\frac{\text{Oil}}{\text{Water}}$	24 hours 1/20 m	42 hours 1/80 m
Urea	13.7	0.00015	0.66	0.71
Methyl-urea	18.5	0.00044	0.42	0.18
Thio-urea	20.9	0.00120	0.26	0.14
Phenyl-urea	35.7		0.38	0.23

volume. Table IV and V contain some data which may be mutually compared. Every figure is the average of two or more experiments in which the uptake of the different substances is determined in the same series. In table IV data are given relating to the active absorption of urea, methyl-urea, thio-urea and phenyl-urea. Though in permeation experiments methyl-urea and thio-urea are found to permeate better according to their greater lipoid-solubility, here the active absorption of urea from $^1/_{80}$ m solution is about 4 times as large as that of methyl- and thio-urea.

TABLE V. Absorption of acetamide, lactamide, malonamide, butyramide and succinimid. All experiments with addition of sucrose to the agar. In column I the molecular refraction, in column II the oil/water distribution-coefficients.

	I	II	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
	Mol. refr.	$\frac{\text{Oil}}{\text{Water}}$	24 hours 1/20 m	48 hours 1/80 m
Acetamide	14.9	0.00083	0.25	0.17
Lactamide	21.—	0.00058	0.18	0.12
Malonamide	22.9	0.00008	0.28	0.07
Butyramide	24.1	0.00950	0.27	0.12
Succinimid	27.1	0.00490	0.27	0.11

Also in the series with amides (table V) butyramide and malonamide though greatly differing in lipoidsolubility are taken up in the same amount. Moreover the molecular volume of the different amides seems to have no great influence on the active uptake. Here therefore there is certainly no connection in the sense that substances are absorbed better according as they have a smaller molecular volume or are more lipoidsoluble.

On the contrary it appears from the data obtained that as a substance permeates better through the protoplasm it is less activated in the transport (see also table II). It stands more or less to reason that a substance which easily permeates through the protoplasm and arrives in the vacuole is but slightly transported by the plasm.

In the series of the aminoacids (table VI) the strength of the uptake from $^1/_{20}$ m solutions is about the same for glycocoll and alanine being less for leucine and phenyl-alanine. From $^1/_{320}$ m solutions the absorption is much smaller for phenylalanine than for the other aminoacids. As the aminoacids are highly dissociated they cannot easily permeate in the living cell. (SCHÖNFELDER 1930) It is interesting that SCHMENGLER (1933) in experiments with a collodion-membrane obtained a much better permeation of phenyl-alanine than of aliphatic aminoacids. According to SCHMENGLER the better permeation of this aromatic aminoacid depends on the formation of amphions in a smaller amount by

aromatic than by aliphatic aminoacids (BJERRUM 1923). In the here mentioned experiments with active uptake we see the opposite phenomenon. Phenyl-alanine, that permeates better, is here less strongly absorbed than the aliphatic aminoacids. It is probable that the amphions of the aminoacids are actively taken up. In experiments on the active absorption of asparagine by the leaves of Vallisneria spiralis (ARISZ and VAN DIJK 1939, p. 830) we arrived at the same supposition. When phenyl-alanine forms amphions in a smaller degree its smaller absorption can easily be understood. So we find here the same rule that *substances are more actively absorbed and transported in proportion as they permeate less well through the plasm.*

TABLE VI. Absorption of amino-acids. All experiments with addition of sucrose to the agar.

	Mol. refr.	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
		24 hours 1/20 m	24 hours 1/320 m
Glycocoll	16.4	0.80	0.32
Alanine	21.01	0.70	0.32
Leucine	34.87	0.47	0.30
d Phenyl-alanine	45.2	0.29	0.13

§ 3. *Influence of oxygen on the absorption and the transport of inorganic substances, especially of phosphates.*

SCHMID (1912) and RUSCHMANN (1914) have shown by micro-chemical methods that after feeding with phosphates the presence of phosphoric acid could be shown in the leaf of DROSERA. In addition they could prove the absorption of potassium.

The phosphates appeared to be very suitable for an investigation on absorption, though a few difficulties were encountered. In the first place the determination of the absorbed phosphates was not so accurate as that of nitrogen with the Micro-Kjeldahl method, while the phosphates are less strongly absorbed than the amino-acids. Owing to the mono-potassium phosphate the tentacles curve, so that most of the experiments mentioned in this paper have been made with addition of sucrose to the agar. Di-potassium phosphate does not cause curving of the tentacles. The absorbtion is very slight in this case.

In the quantitative determination of phosphate we encounter the difficulty that the secretion of the tentacles influences the pH of the agar, which factor also affects the dissociation of the phosphates. We shall first discuss the uptake and transport of the KH_2PO_4. Table VII column I gives the concentration of the KH_2PO_4 in the agar; in the second column we find the quantity of phosphate absorbed, calculated as P_2O_5 and in the third column the concentration KH_2PO_4 in the leaf, assuming that the substance

TABLE VII. Accumulation of KH_2PO_4 in leaves of Drosera capensis. In all experiments 0.35 m sucrose is added to the agar. Duration of absorption 24 hours, 25° C.

m conc. of KH_2PO_4 in agar	Increase of P_2O_5 in $^0/_{00}$ of fresh weight of leaves	m conc. of KH_2PO_4 in leaves	Accumulation factor
0.05	0.71	0.01	0.2
0.0125	0.71	0.01	0.8
0.003125	0.72	0.01	3.2
0.000781	0 66	0.0093	11.9
0.000195	0.41	0.0058	29.6

remains unchanged in the leaf and is equally divided over all leaf cells. In the last column the accumulation-factor has been given.

The result of the experimental series is clear. Up to a concentration of about 1/320 m the absorption increases, according as the concentration increases. With a further rise in the concentration of KH_2PO_4 in the agar the absorption does not increase any more and the maximum transport-capacity has been attained. Then the concentration of the phosphate is no more a limiting factor for the strength of the transport, so that the maximum transport strength has been attained for this temperature. The accumulation factor for a concentration of 1/5120 m is about 30. Therefore the uptake of phosphate is just like that of amino-acids and asparagine an accumulation process. In this case we find for the uptake of phosphates almost the same connection between concentration and strength of uptake as in the preceding publication for asparagine. Also for glycocoll (cf. this series No. III) a similar connection has been found.

From the experiments mentioned in table VIII on the influence of removal of oxygen

TABLE VIII. Absorption of phosphates in normal and anaerobic conditions. The concentration of all substances is 1/20 m. 25° C.

Substance	Duration of absorption in hours	Increase of P_2O_5 in ‰ of fresh weight		Anaerobic as % of normal
		Normal	Anaerobic	
KH_2PO_4	24	0.46	0.18	39.1 %
KH_2PO_4	48	1.09	0.15	13.8 %
KH_2PO_4	48	0.57	0.08	14.— %
KH_2PO_4	44	0.21	0.01	

on the uptake of KH_2PO_4 it appears that this process also depends on the oxygen-pressure of the air and therefore in this respect also corresponds with amino-acids. How strong the activation of the transport in this case is, cannot be ascertained with great accuracy. From the figures obtained it appears that the average activation is 78 %. It seems, however, very well possible, that the activation is considerably greater, but the accuracy of the determinations does not allow of our pronouncing an opinion on this. At any rate it appears from this figure that the protoplasm also in the transport of phosphates acts a very important part. All these data indicate that phosphates are transported in essentially the same way as amino-acids and asparagine. In a following publication we shall revert to this.

In table VIII figures have also been given about the uptake of KH_2PO_4 in normal and in anaerobic conditions. The uptake of this substance is slight, but it is clear that here too aerobic respiration is necessary for the absorption. In experiments with this alkaline-reacting substance we encounter the difficulty that owing to the secretion of acid by the tentacles the pH is altered during the experiment and there will be found di-potassium phosphate by the side of mono-potassium phosphate.

The absorption of phosphate by the DROSERA tentacles reminds us of the absorption of phosphates by sugar-cane, about which a research was made by VAN DEN HONERT. He found that in greatly diluted concentrations the uptake by the roots depends on the phosphate concentration of the liquid nutrient, but that with stronger concentrations than 1 mg per liter the absorption does not increase. Suffice it to point out here the correspondence between the uptake of phosphate by the root and the transport by the tentacle of DROSERA. There are more points of correspondence, which however require a special discussion.

§ 4. *Summary and Discussion.*

In the preceding publication it had been found that the absorption and the transport

of asparagine by the tentacles are processes which only occur in the presence of oxygen. Here it is shown that amino-acids, glycocoll, alanine and leucine behave in the same way and are actively transported like asparagine.

For these substances the not active transport by diffusion is probably but extremely slight, because for these substances the protoplasm is not permeable.

Other organic substances as urea, methyl-urea, thio-urea, phenyl-urea, urotropine, caffeine are in the presence of oxygen transported more or less actively as well. The degree of activation depends on the nature of the substance and is probably greater for substances which cannot diffuse so well through the protoplasm, either on the ground of their dissociation or because of their great molecular volume or owing to a low solubility in lipoids. The activation decreases in the series urea, methyl-urea, thio-urea, phenyl-urea, urotropine, caffeine.

The tentacles also absorb inorganic substances. The uptake of KH_2PO_4 is entirely analogous to that of dissociated organic substances, as amino-acids and asparagine. It is also an accumulation process, which is dependent on oxygen.

The data on the transport mentioned here can be explained, if we assume that the transport consists of two processes. In the first place there is a diffusion process as far as the substances permeate through the protoplasm. These substances are then spread by diffusion in the protoplasm and the vacuole. As the vacuole offers the least resistance and takes up the greater part of the cell volume, the diffusion through the vacuole of well-permeating substances will be the most important process. In addition to diffusion an active transport takes place. The active transport will be more pronounced according as the substances permeate less well through the protoplasm. In the slightly permeating salts, amino-acids and asparagine there is hardly anything but active transport. In those cases in which diffusion and active plasmatic transport take place side by side, the term activated transport has been used. This activated transport was found for urea, methyl-urea, thio-urea, phenyl-urea and caffeine.

For a theoretic discussion of these processes we must refer to a following publication.