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ABSORPTION AND TRANSPORT BY THE TENTACLES OF DROSERA CAPENSIS

V. INFLUENCE ON THE TRANSPORT OF SUBSTANCES INHIBITING ENZYMATIC PROCESSES

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In the preceding publications absorption and transport by the tentacles of *Drosera capensis* has been analysed. The last two communications having been published in Dutch in 1944, the contents will be given a little more extensively in this paper. The researches have been continued after 1945, but owing to the limited plant material and the fact that the experiments require a great deal of time, the results have not yet been closed. In the first part of this publication, some results of the two preceding communications will be discussed and new data will be added, after which data on the influence of inhibitors on the transport process will be dealt with.

I. METHOD

Method of cultivation. All experiments have been made with leaves of *Drosera capensis L.* The plants were cultivated in a hot house in earthenware bowls (diameter 20 cms, height 6 cms) divided into 3 parts by two vertically placed glass plates. In the middle space some six Drosera plants grew on ground peat dust. In the side spaces water was poured.

Variability of the experiments. The experiments were made with six series of six leaves taken from six plants of the same age. Each series was composed of six leaves of different ages, one of each plant. In this way the variability of the series was made as slight as possible. Young plants with not too large leaves give the best results. Combining leaves of various ages into one series, however, presents difficulties for metabolic experiments, because age may influence metabolism, as for instance THIMANN (1949) showed for the growth of oats coleoptiles. A few weeks after six leaves have been taken from the plants, they are again fit for use for fresh experiments.

Arrangement of the experiments. The experiments were arranged as follows (ARISZ and OUDMAN 1937). From agar layers about 7 mms thick, strips were cut of about 7 mms width and 5 cms length. Two of such

strips were placed on a glass plate in such a way that they were a little further apart than the width of the leaf. A leaf was placed with the marginal tentacles on the two agar strips so that the leaf blade was quite clear of the agar strips and likewise of the glass plate. Next a strip of glass was placed lengthwise across the middle of the blade and firmly pressed into two bits of plasticine lying at the ends of the glass plate (Fig. 1). Finally two strips of agar of the same shape as the first were placed on the marginal tentacles, so that they lay between the strips of agar and were capable of absorbing the substance dis-

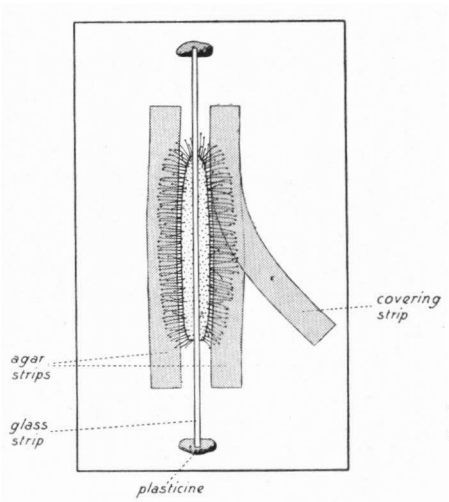

Fig. 1. Leaf of Drosera capensis with the marginal tentacles between strips of agar in which substances have been dissolved.

solved in the agargel. On either side about 80 to 160 tentacles were in touch with the agar. Besides the substance to be transported other substances, such as inhibitors, antibiotica and sugar may be solved in the agar in the concentration required.

Keeping quiet the tentacles. The tentacles, which are stimulated by amino acids, phosphates and similar substances try to curve, in consequence of which they would lose contact with the agar. To prevent them from curving, various methods have been used. Initially caffeine was added, which inhibits curving, when in a sufficiently high concentration. It inhibits, however, absorption as well (Table 1). Keeping the tentacles quiet may also be attained by osmotic dehydration. For this purpose sugar is added to the agar. A 0.30 M sugar concentration had a sufficient osmotic effect to decrease the turgor in the tentacles to such a degree that they remained quietly between the agar strips during the experiment. In later experiments a saltmixture has been occasionally used. We used a mixture of 1.1 gr. KCl + 0.22 gr $CaSO_4$/100 ml. This is isotonic with 0.26 M sucrose. The concentration of the sugar or the saltmixture should be kept as low as possible, because in higher concentrations osmotic actions take place, having an inhibitory effect on the active transport (table 2). To what extent

sugar is taken up by the tentacles was discussed in a previous publication (ARISZ III 1944). The result is uncertain. Reducing sugar increases but at the same time the starch contents of the leaves decrease. It is possible that the influence of the sucrose is indirect and starch is converted into sugar by osmotic influence.

TABLE 1

Simultaneous uptake of phosphate and caffeine. Uptake 48 hours of 1/200 M KH_2PO_4 with addition of sucrose to the agar and of increasing concentrations of caffeine.

	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.32 M sucrose.	0.51
1/200 M „ + 0.32 M „ + 1/80 M caffeine	0.26
1/200 M „ + 0.32 M „ + 1/40 M „	0.24
1/200 M „ + 0.22 M „ + 1/10 M „	0.08

TABLE 2

Influence of addition of sucrose and of a salt mixture on the uptake of 1/200 M KH_2PO_4 during 48 hours.

	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.28 M sucrose.	0.83
1/200 M „ + 0.32 M „	0.90
1/200 M „ + 0.36 M „	0.83
1/200 M „ + 0.40 M „	0.53
1/200 M KH_2PO_4 + 0.10 M KCl + $CaSO_4$	0.50
1/200 M „ + 0.12 M „	0.12
1/200 M „ + 0.14 M „	0.02
1/200 M „ + 0.16 M „	—0.11

Sterility. Sucrose moreover has the drawback of promoting the development of bacteria. Though this is not a serious objection in many experiments, it may be important on examining organic substances like amino acids and their amides, or urea, thiourea, phenylurea, etc. It has been investigated whether addition of an antibioticum to the sugar containing agar could prevent the development of bacteria. It appeared that p. amino benzene sulfonamide in a concentration of 0.05 % did not yet delay the uptake and kept the development of microorganisms within reasonable bounds (table 3) Penicillin and streptomycin inhibited the uptake of some transport substances and were therefore unfit for use. To the action of penicillin we shall revert. A disinfectant as germisan was toxic and therefore likewise unfit for use to obtain sterility. Exposure to ultraviolet light did not give a solution either. Therefore the experiments were made with sugar, to which aminobenzene-sulfonamid was added and the

results were compared with those of experiments in which the salt-mixture had been added to the agar.

TABLE 3

a. Influence of addition of sulfonamid on the uptake of phosphate during 48 hours, *b.* uptake of thiourea, urea and phosphate during 24 hours.

a.	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.32 M sucrose	0.88
1/200 M „ + 0.32 M „ + 0.02 % Sulfonamid	0.82
1/200 M „ + 0.32 M „ + 0.03 % „	0.88
1/200 M „ + 0.32 M „ + 0.05 % „	0.83

b. Addition of	Uptake of 1/20 M thiourea Increase of N	1/80 M urea Increase of N	1/100 M KH_2PO_4 increase of P_2O_5
0.35 M sucrose	0.62	0.37	
0.15 M KCl + $CaSO_4$	0.60	0.35	0.48
0.35 M sucrose + sulfonamid . . .	0.62	0.34	0.52
0.15 M KCl + $CaSO_4$ + sulfonamid	0.60	0.34	

Mucilage secretion. The glands of the tentacles secret mucilage and this mucilage remains sticking as a viscous drop to the tentacles. It is difficult to remove from the tentacle glands in a fresh condition. The quantity of it is large; in the experiment of table 4 it amounts in a fresh leaf to about 57 % of the fresh weight of the leaf without mucilage. After its removal another 19 % mucilage is secreted in the next 24

TABLE 4

Determination of the secretion of 3 series of 6 leaves with tentacles.

Weight fresh weight,	after washing,	secretion	Weight after 24 hrs. on filter paper	Weight after washing	Newly secreted
373 mg	221 mg	152 mg	265 mg	225 mg	40 mg
368 „	236 „	132 „	287 „	242 „	45 „
344 „	234 „	110 „	284 „	236 „	48 „
1085 mg (157 %)	691 mg (100 %)	394 mg (57 %)	836 mg (121 %)	703 mg (102 %)	133 mg (19 %)

hours. Therefore the leaves with the tentacles were washed in tap-water at the end of the experiment in order to remove the mucilage altogether. Next they were dried on the outside with filter paper and the fresh weight was determined. It is evident that while being washed the leaves may take up water, which influences the fresh weight. A number of weighing tests proved that this procedure gives usable results in many cases.

Reference value. As a reference value for the uptake of transport substance the fresh weight was used in most experiments, the absolute value of the absorbed substance per series of six leaves being determined at the same time. By expressing the uptake per 1000 parts fresh weight the errors are not objectionably great in the kind of experiments treated in this publication. In another kind of experiments, such as those with permeating substances, difficulties by washing arise which will not be discussed here. The blank series has always been treated perfectly analogically to the experimental series and continued in contact with the agar until the end of the experiment, the only difference being that the transport substance had been omitted from the substances added to the agar. This is necessary, because the whole treatment affects the final fresh weight.

Agar. To the use of agar-agar as a medium for adding the substances to be absorbed there are various objections. Agar contains various substances. To be sure it is possible by digesting and electrodialysing to obtain a purer agar, but experience teaches that it is often less fit for use and that it is especially desirable to use a uniform product, though it may contain some nitrogen and phosphate. In the experiments it is a question of difference in uptake between control- and experimental series. Especially in the later experiments we have used commercial products such as Difco agar and Pectacon. In control experiments it appeared that from this agar no nitrogen or phosphate in analysable quantity was absorbed. It should, however, be taken into account that the agar does contain substances which are taken up by the tentacles and cause aggregation. Experiments with digested agar, in which evidently decomposition products were formed from the proteins, often showed highly aggregated tentacles.

The leaves with the tentacles between the agar strips are put in closed Beyerinck boxes in which the atmosphere is kept humid. The experiments were made in a room of constant temperature at 25°. Absorption always took place in the dark.

Analysis. The presence of nitrogen was shown by the Micro-Kjeldahlmethod according to PREGL. Usually total nitrogen was determined after destruction by the salicilic acid method. Phosphate was determined with ammoniummolybdate after destruction of the leaves with concentrated sulphuric acid. The phospormolybdenic acid formed, is reduced with metol and sulphite of soda to a blue colour, which with the aid of a Cenco photelometer is compared with standard phosphate solutions treated in the same way.

For experiments under anaerobic conditions an anaerobic jar of Mc Intosh and Fildes was used. First purified nitrogen gas is passed through next the oxygen still present is combined with added hydrogen gas with the aid of a palladium catalyst.

To Mrs H. KNOBBE-MEESTER I am greatly indebted for the careful execution and help with the experiments and the analyses in the years 1946 to 1952.

II. Active and activated absorption

Drosera tentacles are organs capable of transporting a number of substances from the insects caught to the leaf blade. Besides a secretory function they bring by their curving the adhering insects in touch with a great number of tentacles, and subsequently they also have a transport function (Darwin). Our reason for these researches has been the assumption that the transport process in parenchymatous tissue can best be studied in organs specialized for this function. The uptake of asparagine by the tentacles and the transport to the leaf was first quantitatively demonstrated in Oudman's thesis (1936). He found for this process a high temperature-coefficient and inhibition through narcosis. Arisz and Oudman (1937) and Arisz (IV 1944) pointed out a possible relation between active transport and cytological changes in the tentacle cells, known as aggregation. These experiments are still being continued.

Independence of the transport of water and that of substances. Arisz (I 1942) showed that the transport process is not influenced by water currents in the tentacles, caused by transpiration of the leaf blade or withdrawal of water from the tentacles by adding to the agar an osmotic substance. It is for instance possible to add sugar to the agar and by doing so to withdraw water from the tentacles osmotically. Water and substance transport are therefore independent processes. Water transport mainly occurs in the spiral vessel of the tentacles, the substance transport in the parenchyma.

Diffusion. In the case of some substances as caffeine, we get the impression that they diffuse from cell to cell in the tentacles; this process can be studied under the microscope (Kok 1933). The caffeine permeates into the vacuoles and causes there granulation, which may be used as an indicator for the rate of the transport. From the microscopic findings it appears that diffusion takes place in rows of cells. From this it may be concluded that not the longitudinal wall, but the vacuole is the transport track, while diffusion must take place from vacuole to vacuole through the transverse wall and the adjoining plasm. Yet the transport of caffeine is not purely a diffusion process, because in an anaerobic medium its progress is slower than in an aerobic one (Arisz II 1942). This indicates an influence of metabolic processes in the plasm.

Activating the transport by oxygen. For a great number of substances it has been traced whether they are taken up by the tentacles and transported and whether these processes are influenced by withdrawal of oxygen. A separation of uptake- and transport processes is impossible. When transport is mentioned, we must consider that the uptake may be repeated in every cell, while uptake always implies transport.

It has appeared that the uptake and the transport of all substances which are carried by the tentacles, are to a certain degree dependent on oxygen, so that some substances are transported anaerobically fairly equally, some less strongly or even not at all. This dependence on oxygen we have called activation and as a measure has been taken

the difference of the total aerobic transport from the transport in anaerobic conditions as a percentage of the total transport.

$$\text{Activation} = \frac{\text{transport dependent on oxygen}}{\text{total aerobic transport}} 100.$$

Table 5 gives a survey of the activation for the transport of a number of substances. The data previously published (ARISZ II tables 1 and 2) mainly correspond with this. Deviations are due to the greater number of observations now available and especially to

TABLE 5

Dependence of the transport on oxygen.

$$\text{activation} = \frac{\text{total aerobic transport-anaerobic transport}}{\text{total aerobic transport}} 100$$

	Activation
amino acids	± 100 %
asparagine and glutamine	
NH_4 (from NH_4Cl)	± 100 %
phosphate (from KH_2PO_4)	± 100 %
urea.	± 76 %
thiourea	± 52 %
caffeine	± 20 %
ammonium carbonate	± 20 %

the use of lower concentrations of the substances transported. The relative inaccuracy of the experiments does not admit of a more accurate determination. It appears that there isn't a close separation between substances actively taken up and substances penetrating through diffusion, but that there exists a gradual transition.

The transport of amino acids, asparagine and glutamine, likewise of phosphate and ammonium is 100 % activated in sufficiently low concentrations. In higher concentrations some transport also takes place in an anaerobic medium. In the series urea, thiourea, caffeine and ammonium carbonate, activation decreases and in the same order these substances permeate better into the vacuole. This is in accordance with the rule previously mentioned (ARISZ 1942) that the transport is more strongly activated according as the substances permeate less well through the plasm.

By permeation we understand the passive diffusion of substances into or through the protoplasm. If absorption takes place through processes connected with metabolism, we do not speak of permeation, but of active absorption or secretion.

Active transport. For those substances for which the activation amounts to about 100 % we speak of active transport. Active transport is only possible for substances which can hardly or not at all permeate into the cell through diffusion. Dissocia ted substances, such as amino acids and salts on the whole penetrate the boundary surfaces of the protoplasm with difficulty, as is known from plasmo lysis experiments. Once taken up by

TABLE 6

Absorption of *glycine* by the tentacles at 25° C, in darkness. 0.35 M sucrose has been added to the agar for keeping quiet the tentacles

Conc. of glycine in mM in the agar	Absorbed nitrogen per 1000 parts fresh weight during 24 hrs	Conc. of glycine in mM in the leaves	Accumulation factor
50	0.99	70.7	1
25	1.11	79.3	3
12.5	1.04	74.3	6
6.25	0.96	68.6	11
3.125	0.84	60.0	19
	during 42 hours		
3.125	1.96	140.0	45
0.781	0.73	52.2	67
0.195	0.37	26.4	135

Absorption of *asparagine* by the tentacles at 25° C in darkness during 24 hours.

Conc. of asparagine in mM in the agar	Absorbed nitrogen per 1000 parts of fresh weight	Conc. of asparagine in mM in the leaves	Accumulation factor
50	1.47	52.5	1
12.5	1.90	67.9	5
3.125	1.50	53.6	17
0.781	0.80	28.6	37
0.195	0.20	7.1	37

Absorption of *phosphate* by the tentacles in 24 hours at 25° C

Conc. of KH_2PO_4 in mM in the agar	Absorbed P_2O_5 per 1000 parts of fresh weight	Conc. of KH_2PO_4 in mM in the leaves	Accumulation factor
50	0.71	10	0.2
12.5	0.71	10	0.8
3.125	0.72	10	3.2
0.781	0.66	9.3	11.9
0.195	0.41	5.8	29.6

Absorption of *caffeine* by the tentacles in 24 hours at 25° C

Conc. of caffeine in mM in the agar	Absorbed N per 1000 parts of fresh weight	Conc. of caffeine in mM in the leaves	Accumulation factor
50	0.81	14.5	0.29
12.5	0.38	6.8	0.54
3.125	0.17	3.0	0.96
0.781	0.—	—	—

the tentacles, they are no more secreted to the medium. Substances' the transport of which is due to a certain degree to passive permeation and diffusion, may be given off again, if the tentacles are put in water.

Aggregation. Aggregation of the cells, by which the vacuoles are strongly dehydrated and break up in small pieces of a different shape, water being secreted to the plasm, is especially met with substances with highly activated transport. The cytological picture indicates that the active transport must take place in the plasm. Some substances with weaker activation, as urea and ammonium carbonate likewise cause aggregation. The latter substance, however, only in an extremely diluted concentration.

Besides in the behaviour during exosmosis and in the dependence on oxygen the difference between active and activated transport also appears from the relation between uptake and medium concentration. With actively transported substances already from a low concentration a relatively great quantity of substance is absorbed (table 6). Saturation is attained for glycine and asparagine at about 1/80 M, for phosphate already at 0.003 M. For non-actively transported substances, as caffeine the uptake diminishes much more on decrease of concentration. The uptake in the time takes place at a fairly constant rate (see fig. 1. p. 238. ARISZ III 1944).

Transport to the leaf. The substances are taken up by the glands at the end of the tentacles and transported to the leaf by the about 4 mm long marginal tentacles. The tentacles don't seem to take up anything at their lateral walls, the pedicel being surrounded by badly permeable outer cell walls. On the sides of the pedicel one or more simple glands may occur through which certain substances penetrate (COELINGH). This, for instance, obtains for caffeine, ammonium carbonate, methylene blue and others. Substances absorbed by the tentacles in 24 hours or longer are for the greater part transported to the leaf. In the tentacles little of the transport substance remains. Naturally this depends on the nature of the transport substance. Caffeine and ammonium carbonate give a clearly visible deposit (granulation) in the vacuoles. In quantitative determinations this quantity proves in most cases to be hardly significant (table 7).

It has not been investigated whether the absorbed substances are

TABLE 7

Uptake of 1/20 M $(NH_4)_2 CO_3$ during 24 hours

	N in γ	N increase in γ	Absorbed N per 1000 parts of fresh weight
before the uptake	724		
after the uptake			
in intact leaves	1364	640	2.60
in leaves without tentacles. . . .	1218	494	2.49
in the tentacles	67		

transported as such or after conversion and how they are deposited in the leaf. Formation of protein from the absorbed amino acids or asparagine OUDMAN (1936) could not demonstrate. If one calculates the concentration of the absorbed substance per fresh weight of the leaf, one finds an accumulation factor, which, for instance, for 0,0002 m asparagine amounts to 37 after an uptake of 24 hours (ARISZ I), for 0,0002 m glycine after 42 hours 135 (ARISZ III), for 0,0002 m KH_2PO_4 after 24 hours 30 (ARISZ II). For activated transport lower values are found, for instance for caffeine 0.003 m (ARISZ II) the accumulation factor is 1. This indicates that active transport takes place independent of the fall in concentration medium-leaf, but an investigation to what extent the absorbed substances are converted, is necessary for a clear insight.

Selectivity of the transport mechanism. Active transport is very selective, i.e. only certain substances are taken up by the tentacles and transported to the leaf. They are either polar substances or electrolytes. Only a few saltions are taken up, such as phosphate and ammonium. Whether ammonium is taken up as an ion or as a molecule is still uncertain. For the ions and the amphoions of the amino acids the transport mechanism must have a specific affinity (ARISZ III). It is possible that the active mechanism is not specifically directed to potassium ions, but that they are absorbed simultaneously with the actively absorbed anions.

III. SIMULTANEOUS ABSORPTION

An insight into the specificity of the absorption mechanism may be obtained by having various substances or ions taken up simultaneously and investigating whether these processes progress independently, so that summation occurs or that they use the same transport mechanism and consequently influence one another (ARISZ IV).

The specific difference between asparagine and phosphate transport appears from experiments with simultaneous transport. Both substances are transported actively. For each of them the strength depends on the concentration of the substance in the medium, saturation already setting in at a low concentration. If these substances are presented in concentrations higher than the saturation value the absorption summates, so that each substance is taken up to its saturation level (table 8). This indicates that the uptake of phosphate is based on a different process from that of asparagine. Just like asparagine various amino acids behave on simultaneous absorption with phosphate.

Combining two substances of a same type (table 9) has as a result, that from the two together no more can be taken up than the saturation level for this type amounts to. This is for instance the case on simultaneous uptake of asparagine and glycine or of alanine and glycine (ARISZ 1944 IV, tables 1 and 2). Also on combination of NaH_2PO_4 and KH_2PO_4 these phosphates proved not to be absorbed independently. In taking up the plasm does not make any difference between

sodium and potassium salts which is to be expected when the anion is taken up actively. These data show that the tentacles do not distinguish between substances of a similar type, but they do distinguish between amino acids and phosphates. The active absorption, therefore, must be connected with binding to components of the

TABLE 8

Simultaneous absorption of asparagine and KH_2PO_4 during 24 hours. 0.35 M sucrose is added to the agar. (From ARISZ IV)

Agar	Increase of phosphate as P_2O_5 per 1000 parts of fresh weight	Increase of nitrogen per 1000 parts of fresh weight
1/20 M KH_2PO_4	0.39	
1/20 M asparagine		1.21
1/20 M KH_2PO_4 + 1/20 M asparagine	0.31	1.18

TABLE 9

Simultaneous absorption of glycine and alanine. A 48 hours B 24 hours; only in B 0.35 M sucrose is added to the agar

Agar	Increase of N per 1000 parts of fresh weight	
	A	B
1/20 M glycine	1.16	0.91
1/20 M alanine	1.27	0.74
1/20 M glycine + 1/20 M alanine .	1.10	0.99

plasm. ARISZ (III 1944) thinks that for amino acids and asparagine it is the amphoions, for salts the an- or kations which are bound to the plasm during the uptake. This theory of binding to the plasm explains the specific absorption of different substances.

A special case was noted on the simultaneous absorption of amino acids, asparagine or phosphates with substances as caffeine, ammonium carbonate and antipyrine. (ARISZ IV 1944 tables 6–12). Tables 1

TABLE 10

Simultaneous absorption of KH_2PO_4 and $(NH_4)_2CO_3$ during 46 hours. 0.35 M sucrose is added to the agar

Agar	Increase of phosphate as P_2O_5 per 1000 parts of fresh weight
1/80 M KH_2PO_4	0.85
1/80 M „ + 1/80 M $(NH_4)_2CO_3$	0.68
1/80 M „ + 1/40 M „	0.38
1/80 M „ + 1/10 M „	0.04

and 10 demonstrate this phenomenon. The three last mentioned substances penetrate into the tentacles and cause, as has already been discussed, a granulation in the vacuole. In a sufficiently strong concentration they inhibit the active absorption and the active transport (ARISZ IV tables 7–12). They do not inhibit through an osmotic influence, as sucrose and salts do in a sufficiently high concentration but through an influence on the plasm. In table 1 this has been shown again for caffeine, when through a higher caffeine concentration in spite of the fact that sucrose in lower concentration has been added, absorption is greatly inhibited. It is a remarkable fact that these same substances also inhibit the curving of the tentacles (DARWIN, ÅKERMAN) and inhibit the setting in of aggregation or cause an aggregation already set in to decrease. This too points to a relation between active uptake and aggregation (ARISZ IV 1944).

IV. INFLUENCE OF TEMPERATURE

The influence of the temperature on the uptake and transport processes has been investigated extensively. Table 11 gives a survey of the results obtained. The absorption has been given in millimol. The substances have been arranged in accordance with the magnitude of the Q_{20}. For caffeine and phenylurea this is 2,6, for a low concentration of $(NH_4)_2CO_3$ 3: for a high concentration of NH_4Cl 5, for asparagine likewise 5, for urea 9, and for phosphate 18. It is evident that these determinations cannot lay claim to great accuracy, though

TABLE 11

Influence of the temperature on the absorption of different substances. The uptake is given in millimol per 24 hours

	25° C	Q 15°–25°	15° C	Q 5°–15°	5° C	Q 5°–25°
1/20 M caffeine	20	1.6	12.6	1.6	7.6	2.6
1/20 M phenylurea . . .	22.8	1.4	16.–	1.8	8.6	2.6
1/80 M $(NH_4)_2CO_3$ . . .	77.–	1.3	57.5	2.2	25.4	3.–
1/20 M NH_4Cl	56.4	1.5	36.4	3.4	10.7	5.–
1/100 M asparagine . . .	22.7	1.6	14.6	3.4	4.3	5.–
1/20 M urea	28.5	1.5	19.6	6.5	3.–	9.–
1/200 M KH_2PO_4	3.6	1.5	2.3	11.–	0.2	18.–

they are averages from a great number of observations. The uptake at 5° C lies for most substances hardly beyond the limits of error. It is a striking fact that the Q 10 for the temperature interval 15–25° C is fairly equal for all substances and rather low, 1.5; whereas greater differences occur for the interval 5–15° C. Here the temperature coefficient is distinctly higher for a more activated transport.

This gives rise to the supposition that the temperature coefficient for active transport is higher than for the weakly activated transport but that between 15° and 25° it does not show, because another factor becomes limiting.

V. Influence of inhibitors

As it had appeared from the dependence of transport on oxygen concentration in the medium, that there must be a relation between transport and metabolism, it seemed expedient to investigate the influence of inhibitors of respiration enzymes in the transport process. These are added to the agar containing the transport substance, in consequence of which they can exercise an influence on the uptake by the gland of the tentacle. Apparently the influence of these inhibitors is *local*. Whether they can be absorbed themselves and transported to other places in the tentacles is uncertain. Sometimes in stronger concentrations they do have an influence on the phenomenon of aggregation, as dinitrophenol and jodoacetate. This is in favour of the supposition that such an inhibitor penetrates. In experiments with oxygen withdrawal the whole tentacle together with the leaf have to be put in surroundings free from oxygen. So here the effect is not localised and it is uncertain whether the processes inhibited by oxygen withdrawal are to be found in the tentacles or in the leaf or in both. This localisation of the active processes can be traced by inhibitors. Besides it is possible to investigate the influence of inhibitors on the transport in tentacles without glands and to decide in this way whether a transport in tentacles without glands is still sensitive to inhibitors. We will first discuss the transport in intact tentacles.

Tentacles with glands. The following inhibitors were examined: KCN, Na-azide, jodoacetate, NaF, Na-arsenate, Na-arsenite and 2–4, dinitrophenol. In addition penicillin, phloridzin, are involved in this research. In concentrations lying between 10^{-2}–10^{-7} mol the inhibitors were added to the agar containing the transport substance. The pH was raised to 6 by means of a Beckman pH meter with glass electrode. Taking into consideration the great variability of the determinations already discussed, which is a result of difference in age of the leaves and the plants and of the season, it is impossible to give graphs in order to compare the inhibitory action of different concentrations of the inhibitors. We restrict ourselves, therefore, to communicating the results of a number of experiments. In one experiment 5 concentrations could be compared. This gave a clear result. But experiments made in different seasons of the year gave results which, though quantitatively noticeably different, did correspond qualitatively. We, therefore, restrict ourselves to giving the limits within which in various experiments 50 % inhibition of the uptake was found. Moreover a choice has been made from the great number of experiments made. In the tables some characteristic inhibitions have been inserted. As already mentioned on discussing the method, collecting in *one* series 6 leaves of various ages is inconvenient for metabolic researches, because the concentrations working inhibitively, are certainly also connected with the ages of the plants and leaves. We restrict ourselves in this discussion to experiments made with two transport substances, asparagine and phosphate (KH_2PO_4). These have been chosen, because it had appeared from the preceding

investigations that both substances are transported actively, but that the mechanism of the uptake of these substances must be different. Asparagine was examined mostly in a concentration of 1/100 M, potassium phosphate in one of 1/50 or 1/80 M. The uptake usually lasted 24 hours, for phosphate sometimes 48 hours. Table 12 gives the concentration of the inhibitors giving 50 % inhibition. In table 13 the results of some experiments are collected. KCN, NaN_3, Na-arsenite, jodoacetate are all substances known as inhibitors of processes forming part of respiration and glycolysis. From the figures

TABLE 12

Average conc. of inhibitor which gives a 50 % inhibition of the absorption of 1/80 M KH_2PO_4 and of 1/100 M asparagine during 24 hours

KCN	10^{-4} M
Na-azid	10^{-5} M
Na F	10^{-2} M
Na-arsenite	10^{-5} M
jodoacetate	10^{-5} — 10^{-6} M

TABLE 13

Inhibition of uptake of asparagine and phosphate by KCN, NaN_3, Na-arsenite and jodoacetate

M conc. of KCN	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	24 hours' uptake of 1/20 M asparagine, N increase in ‰ of fresh weight	Uptake of 1/100 M KH_2PO_4, 48 hours increase of P_2O_5 in ‰ of fresh weight
—	1.09	0.80	0.55
10^{-4}	1.06	0.62	0.53
3. 10^{-4}	0.78	0.34	0.50
10^{-3}	0.20	0.28	0.43
3. 10^{-3}	0.02	0.18	0.10
M conc. of NaN_3			
—	0.78	0.92	0.74
10^{-6}	0.57	0.77	0.82
10^{-5}	0.32	0.33	0.79
10^{-4}	0.05	0.21	0.51
M conc. of Na-arsenite			1/80 M KH_2PO_4 24 hours
—	0.80	0.94	0.77
10^{-6}	0.57	0.74	0.55
10^{-5}	0.14	0.29	0.37
10^{-4}	0.09	— 0.02	0.27
10^{-3}	0.02	0.02	0.17
M conc. of jodoacetate			1/100 M KH_2PO_4 24 hours
—	0.92	1.69	0.58
10^{-6}	0.82	0.48	0.35
10^{-5}	0.35	0.08	0.25
10^{-4}	— 0.16	0.05	0.16
10^{-3}	— 0.96	— 0.02	0.10

the conclusion may be drawn that inhibition of respiration also causes inhibition of uptake of asparagine and phosphate. None of these substances behaved differently with regard to transport of asparagine or phosphate. Neither is this to be expected, when the two processes are connected with respiration in a similar way. An application of these inhibitors to the gland of the tentacles, therefore, is sufficient to bring about an inhibition of the transport.

Dinitrophenol is regarded by various investigators as a substance which likewise interferes in the respiration system, does not influence oxydation processes, but represses transphosphorylation (GREEN, LOOMIS and LIPMANN). Under influence of the dinitrophenol the phosphate is freed from the cyclophorase-gel as an inorganic phosphate (TEPLY). This so called gel-phosphate has proved in certain cases indispensable for the transfer of phosphate to other substances. HUNTER (1951) finds the action of dinitrophenol limited to phosphorylations coupled with electron transfer from $DPNH_2$ to oxygen.

It appeared that in Drosera tentacles dinitrophenol inhibits the uptake of both substances (table 14). From this it may be inferred that the uptake of both asparagine and phosphate depends on transphosphorylation processes, in which energy-rich phosphates are

TABLE 14

Inhibition of asparagine and phosphate uptake by 2–4 dinitrophenol

M conc. of dinitrophenol	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	24 hours' uptake of 1/80 M KH_2PO_4 increase of P_2O_5 in ‰ of fresh weight
—	1.06	0.50
10^{-7}	0.74	0.40
10^{-6}	0.19	0.07
10^{-5}	— 0.01	— 0.07

formed. A similar behaviour shows dinitrophenol in other processes of the plant which require energy, such as growth (THIMANN and others, BONNER and BANDURSKI) saltabsorption by the root (ROBERTSON and others) and active water absorption (HACKET and THIMANN).

According to GALE (1949) 8-hydroxyquinoline inhibits the uptake of glutamic acid in bacteria. It was also active in Drosera (table 15). It is a substance that combines with heavy metals.

TABLE 15

Inhibition by 8-hydroxy-quinoline

M conc. of hydroxy-quinoline	24 hours uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	48 hours uptake of 1/100 M KH_2PO_4, increase of P_2O_5 in ‰ of fresh weight
—	0.78	0.43
10^{-4}	0.82	0.46
$3.\,10^{-4}$	0.81	0.45
10^{-3}	0.66	0.27
$3.\,10^{-3}$	0.18	0.14

A special discussion requires the behaviour of the remaining substances examined, phloridzin, penicillin and Na-arsenate. These substances proved to work more specifically than the ones already mentioned, because they inhibited the transport of one definite substance either of asparagine or of phosphate. Phloridzin inhibits the transport of phosphate, whereas penicillin and Na-arsenate, only inhibit the aparagine transport. Of the many experiments made with these substances, we have collected some in the table subjoined (table 16). It is known that some preparations of phloridzin have no good effect, unless they have first been purified (STREET and LOWE). The

TABLE 16
Inhibition by phloridzin

M conc. of phloridzin	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	24 hours 'uptake of 1/80 M KH_2PO_4 increase of, P_2O_5 in ‰ of fresh weight
—	0.76	0.60
2.10^{-6}	0.80	0.45
2.10^{-5}	0.79	0.32
2.10^{-4}	0.75	0.27
2.10^{-3}	0.71	0.19

preparation we used first (MERCK) also gave a proper inhibition without purification. The preparation of the British Drug H. gave inhibition after purification. It has not been investigated to what extent the addition of sucrose or KCl to the agar to keep the tentacles quiet, influences inhibitive action. Phloridzin was the only substance with which a specific inhibition of the phosphate absorption was obtained. It is known that in a high concentration phloridzin inhibits the phosphorylation of glucose. According to Shapiro it already inhibits in a low concentration the formation of energy-rich phosphates, which is coupled with pyruvate and citrate oxidation.

Penicillin had been used by us as an antibioticum to promote the sterility of our experiments. It then appeared, however, that it had an inhibitive effect on the transport of asparagine; in the phosphate transport no inhibition occurs (table 17). By GALE and TAYLOR, GALE and RODWELL the inhibition by penicillin of the uptake of amino acids especially of glutamic acid has been examined for "growing" cells of *Staphylococcus aureus*. GALE (1949) ascribes the action of penicillin to a disorganisation of the metabolism of ribonucleic acid. GROS and MACHEBOEF (1949) were of opinion that the consumption or the synthesis of mononucleotides are factors in the action of penicillin, other investigators (HOTCHKISS) think that penicillin affects the synthesis of proteins from amino acids. It is evident that this problem has not yet been solved (BROWNLEE 1951). At any rate it is noteworthy that in Gram positive bacteria and in tentacles of Drosera the uptake of amino acids, asparagine and glutamine is inhibited by penicillin. The fact that penicillin does not inhibit the uptake of phosphate and does inhibit the uptake of asparagine even if phosphate

has been added, is in favour of a specific inhibition of the binding of asparagine to a plasm component. An analogous inhibition of penicillin we found for *Vallisneria* leaves in which the uptake of chlorine and phosphate is not inhibited, but the uptake of asparagine is.

TABLE 17
Inhibition by penicillin

conc. of penicillin	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	48 hours' uptake of 1/200 M KH_2PO_4, increase of P_2O_5 in ‰ of fresh weight
—	1.35	0.75
1 O.U./ml	1.16	0.75
2 „	0.74	0.77
4 „	0.58	0.76
conc. of penicillin	24 hours' uptake of 1/100 M glutamine, N increase in ‰ of fresh weight	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight
—	1.00	0.77
2 O.U./ml	1.05	0.83
3 „	0.92	0.70
4 „	0.55	0.19
8 „	0.28	0.14

Na-arsenate replaces inorganic phosphate in oxidative systems, in which the uptake of phosphate is coupled to oxydation of the substratum (WARBURG and CHRISTIAN). Addition of phosphate inhibits the action of the arsenate in proportion to the quantities in which these substances are present. BONNER found that arsenate inhibits growth while respiration remains unchanged.

In Drosera tentacles arsenate inhibits the uptake of asparagine, but not the uptake of phosphate (table 18). This need not indicate a specific behaviour of arsenate with regard to the uptake of asparagine, because as we saw above addition of phosphate removes the inhibition by arsenate.

TABLE 18
Inhibition by Na-arsenate

M conc. of arsenate	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	1/100 M asp. + 1/80 M KH_2PO_4, N increase in ‰ of fresh weight	24 hours' uptake of 1/50 M KH_2PO_4, increase of P_2O_5 in ‰ of fresh weight
—	0.97	0.48	0.44
10^{-7}	0.74	0.49	0.47
$3.\ 10^{-7}$	0.59	0.49	0.48
10^{-6}	0.40	0.34	0.46

Tentacles without glands. Before discussing the influence of inhibitors on the uptake and the transport in tentacles without glands, we must first find out to what extent transport by tentacles without glands takes place. It has been known for a long time that tentacles without

glands and also cut tentacles absorb substances as caffeine and that this process is not polar (KOK 1933). Of more importance is here the behaviour of phosphate and asparagine, substances which are polarly and actively transported by tentacles with glands. The transport in tentacles without glands of diluted solutions of phosphate is almost

TABLE 19

Influence of NaN_3 on the absorption during 48 hours of 1/50 M KH_2PO_4 by tentacles with and without glands. 3 experiments A, B, C

M conc. of NaN_3	With glands			Without glands		
	A	B	C	A	B	C
—	0.72	0.92	0.54	0.71	0.96	0.51
10^{-4}	0.62			0.60		
$3.\ 10^{-4}$			0.23			0.22
10^{-3}		0.11			0.21	

as strong as in tentacles with glands (table 19). This process is likewise dependent on oxygen. This is clear from table 20.

The transport of asparagine in tentacles without glands was already examined by OUDMAN. He found that the uptake from a 1/20 M solution amounts to 73 % of the uptake by intact tentacles. On these experi-

TABLE 20

Uptake of phosphate during 48 hours, in air with and without oxygen by tentacles without glands

	aerobic	anaerobic
48 hours 1/50 M KH_2PO_4 + (KCl + $CaSO_4$) . .	0.95	0.07

ments being repeated (ARISZ I table 3), this result was corroborated (table 21). Besides it appeared that the transport in tentacles without glands to a certain extent also depends on oxygen, seeing that in an anaerobic medium half or less than half is taken up. This also indicates that transport is probably not 100 % activated in this case. This need

TABLE 21

Uptake of asparagine during 24 hours by tentacles with and without glands. Two experiments A and B. Uptake as increase of N per 1000 parts of fresh weight

	with glands		without glands	
	A	B	A	B
24 hours 1/20 M asparagine + (KCl + $CaSO_4$)	0.97	1.03	0.55	0.77
1/100 M asparagine + (KCl + $CaSO_4$)	0.70	0.52	0.13	0.12

not surprise us, because also in intact tentacles transport is not 100 % activated in these high asparagine concentrations, in contrast to lower concentrations such as 1/100 M. Moreover on our cutting off the gland the pedicel has been cut and as a result the spiral vessel is opened. Already on this ground it may be expected that in such

tentacles diffusion from high concentrations through the spiral vessel may occur. Therefore it would be preferable to make the experiments with inhibitors at low asparagine concentrations. We found, however, that tentacles without glands take up hardly any asparagine from low concentrations such as 1/100 M (table 21). At any rate this uptake is so slight that a quantitative investigation into the influence of inhibitors is next to useless. (table 22b). So there is an essential difference between the transport of phosphate and asparagine in tentacles without glands. Phosphate is transported as well in an active

TABLE 21*a*

Uptake of asparagine during 24 hours in air with and without oxygen by tentacles without glands

	aerobic	anaerobic
24 hours 1/100 M asparagine + ($KCl + CaSO_4$)	0.14	— 0.10

process as in intact tentacles, but the transport of low concentrations of asparagine is considerably weaker. This gives the impression that the gland is necessary to render an active transport through the pedicel possible. This might be connected with a phenomenon found by COELINGH, i.c. that phosphate causes aggregation also in tentacles without glands, whereas it is not caused by asparagine.

We have now arrived at the discussion of the effect of the experiments with different inhibitors on the phosphate transport in tentacles without glands. For a few inhibitors this yielded indistinct results. The results were variable, which was not the case with our experiments with intact tentacles. It is obvious that these variable results are due to the injury inflicted on the tentacles through cutting off the glands. As a rule KCN, Na-azide, Na-arsenite had a strongly inhibitive influence, NaF weaker, but jodoacetate and dinitrophenol did not inhibit in concentrations which had inhibited in intact tentacles (table 22). First (ARISZ 1952) we were inclined to conclude that the latter two inhibitors should inhibit the transport in the gland, but not in the pedicel. For this conclusion, however, there did not seem to exist proper grounds now that we have a greater quantity of material at our disposal. For the present it is safer to consider only the positive inhibiting effect of KCN, Na-azide and Na-arsenite on the transport in the pedicels of the tentacles without glands as proved. Possibly the other inhibitors do not show their effect in a normal concentration as a result of the injury. This is for instance indicated by the fact that dinitrophenol in a higher concentration 10^{-3} does sometimes act inhibitively. An influence of the pH we could not show. Therefore we must leave the question whether certain substances specially inhibit a reaction in the gland, undecided. The positive result obtained that the active phosphate transport in the pedicel is inhibited independently of the presence of the gland is of essential importance,

TABLE 22*a*

Influence of inhibitors on the uptake of 1/50 M KH_2PO_4 during 48 hours by tentacles with and without glands. Uptake as P_2O_5 increase per 1000 parts of fresh weight

	A		B		Normal	3.10^{-4} M KCN	Normal	10^{-5} M jodoacetate
	Normal	2.10^{-5} M dinitrophenol	Normal	2.10^{-5} M dinitrophenol				
with glands	0.75	0.10	0.50	0.06	0.77	0.46	0.66	0.06
without glands	0.77	0.73	0.47	0.17	0.86	0.48	0.70	0.71

	A		B	
	Normal	2.10^{-3} M phloridzin	Normal	2.10^{-3} M phloridzin
with glands	0.93	0.39	1.01	0.49
without glands	0.95	0.94	—	0.28

because it indicates that not only in the uptake in the gland, but as well in the tentacle pedicel processes take place which these inhibitors influence.

We have omitted to investigate the influence of carbon monoxide on the transport, because CO brought into the atmosphere will act as well on the tentacles as on the leaf, so that it cannot be proved whether it influences the processes localized in the tentacle.

As discussed above, the active transport of asparagine in tentacles without glands is slight. This slight transport was markedly inhibited by KCN, Na-azide and Na-arsenite (table 22b). Dinitrophenol and jodo acetate do not inhibit here either. Regarding the specific inhibitors phlo-

TABLE 22*b*

Influence of NaN_3 on the uptake of 1/100 M asparagine during 24 hours by tentacles with and without glands

	normal	10^{-4} M azide
with glands	0.80	0.11
without glands	0.18	0.05

ridzin for phosphate transport and penicillin for asparagine transport, it may be observed that with the exception of one experiment, the inhibition by phloridzin did not occur in tentacles without glands. This one exceptional case makes us doubtful about the significance of the absence of the inhibitory effect found by us. Penicillin, however, was always active also in tentacles without glands and inhibited the weak asparagine absorption. Also the stronger asparagine absorption in tentacles without glands at an asparagine concentration of 1/20 mol was distinctly inhibited (table 23). Summarizing we

TABLE 23

Influence of penicillin on the uptake of asparagine by tentacles with and without glands

	normal	10 O.U./ml penicillin
24 hours 1/20 M asparagine with glands	0.92	0.48
24 hours 1/20 M asparagine without glands	0.68	0.39

think we may state that the investigation into inhibitors in tentacles without glands shows that an active transport takes place in the pedicels.

VI. Influence of some dye-stuffs on the transport

Schumacher used eosin to make the sieve tubes in *Pelargonium* unfit for use. He showed that if eosin is put on a leaf in conc. 1 : 25000–100000 this penetrates into the vascular bundle through the parenchyma and makes the sieve tubes unfit for transport. The sieve tubes

die off and after two or three days callus-formation is found near the sieve plates. After dying the sieve tubes are compressed. All other cells continue living. After that the leaves were no more capable of translocating nitrogen compounds. The same phenomenon was found by SCHUMACHER for a great number of other plants. OUDMAN used this method to inhibit the translocation of N-compounds from the leaves of *Drosera*. It seemed interesting to discover whether eosin also influences the transport in the tentacles. Besides the action of fluorescein-potassium was examined. According to SCHUMACHER (1933) fluorescein gives no callusformation in the sieve tubes. It is easy to recognize by the yellowish green fluorescence under a fluorescence microscope. When put on a *Pelargonium* leaf the sieve tubes and companion cells fluorescence after some hours. Therefore it has been used

TABLE 24

Influence of eosin on the uptake of 1/100 M asparagine and of 1/50 M phosphate during 24 hours

Conc. of eosin	Uptake of asparagine, N increase ‰ fresh weight	Uptake of phosphate, P_2O_5 increase ‰ fresh weight
—	1.22	0.55
0.00003 %	1.00	0.12
0.0003 %	0.60	— 0.02
0.003 %	0.—	0.02

by SCHUMACHER as an indicator of the transport. Also the uptake and the transport of fluorescein in parenchymatous tissue has been extensively examined by SCHUMACHER. It is, therefore, very important for us to know whether this dye influences the transport in parenchymatous tissue.

Eosin appeared to be an extremely toxic substance in *Drosera* tentacles too (table 24). The experiments were made in the dark, so that a photodynamic influence of the eosin was avoided as much as possible. In a concentration of 3.10^{-7} M, it causes a strong inhibition of the

TABLE 25

Influence of K-fluorescein on the uptake of 1/100 M asparagine and of 1/80 M phosphate

Conc. of fluorescein	Uptake of asparagine, N increase ‰ fresh weight	Uptake of phosphate, P_2O_5 increase ‰ fresh weight
—	1.03	0.64
0.001 %	0.90	0.51
0.003 %	0.66	0.51
0.01 %	0.45	0.38
0.03 %	0.26	0.11

transport both of the phosphate and of the asparagine uptake. This result indicates that this substance not only in the sieve cells, but also

in the parenchyma cells, has an inhibitory influence on transport processes in the protoplasm.

Fluorescein-potassium, which according to SCHUMACHER is not toxic, influences the plasmatic transport in *Drosera* tentacles as well (table 25). Lethal action is out of the question, as the tentacles keep living. There is, however, a pronounced inhibition of the transport of asparagine and of phosphate. A 50 % inhibition appears, when 2.7 10^{-4} M fluorescein-potassium is added to the agar in which the transport substance is present. It is unknown what the inhibition by fluorescein is based on. It is known, however, that triphenyl methan dyes to which both eosin and fluorescein belong, inhibit various metabolic processes. As these dyes act on the uptake of phosphate and of asparagine in the same way, it must be assumed that they influence a general process that is essential for the transport. The tentacles absorb the dye strongly in the protoplasm, but in a phase which does not act a part in the protoplasmic streaming. Already before we could corroborate SCHUMACHER's observation that the extension of fluorescein in the plasm is entirely independent of the plasmic streaming. This makes us surmise that the inhibitive action of the normal transport might be connected with a displacement of the transport substance from a protoplasmatic surface. At any rate it appears from the action of fluorescein that it will have to be used with great caution as an indicator for a normal plasmatic transport. Further investigation is necessary.

VII. INFLUENCE OF INHIBITORS DURING THE TRANSPORT OF OTHER SUBSTANCES

As discussed the transport of caffeine is different from that of phosphate and amino acids: it is more like a diffusion process, but it is, be it in a slight degree, dependent on oxygen. The nature of the process finds expression in the following features:

1. In the dependence on the concentration of the medium: in a low concentration little caffeine is absorbed, whereas with actively absorbed substances relatively more is taken up from low concentrations.
2. In the dependence on the temperature; for caffeine the temperature coefficient for lower temperatures is lower.
3. In the dependence on oxygen. Without oxygen the transport is but slightly inhibited. The activation of the transport is slight.
4. In the non-polarity of the transport. The gland of the tentacle has no specific influence, while uptake takes place as much by the base as by the tip of a cut off tentacle.
5. In the non-occurrence of aggregation, as with actively transported substances. Caffeine gives rise to granulation (coacervation) in the vacuole.
6. In the transport track. In caffeine transport the vacuole acts an important part. Passing the transverse wall and the adjoining plasm offers a 160 times greater resistance than passing through the vacuole (KOK).

7. In the leaching of the absorbed caffeine on putting the leaves in water. This is in contrast with the retention of the absorbed substances after uptake of asparagine and phosphate.

Taking into account the slight influence of oxygen on the transport of caffeine, it is not to be expected that inhibitors will have a considerable influence on the transport. The differences found are often too slight to conclude to an inhibitory action with certainty. Let it suffice, therefore, to state that KCN, Na-azide and dinitrophenol gave a slight inhibition at higher concentration.

TABLE 26

Influence of inhibitors on the uptake during 24 hours A of caffeine, B of NH_4Cl and C of $(NH_4)_2CO_3$

A

M conc. of KCN	Uptake of 1/20 M caffeine, N increase in ‰ fresh weight
—	1.34
10^{-4}	1.35
3.10^{-4}	1.32
10^{-3}	0.87
3.10^{-3}	0.51

B

M conc. of dinitrophenol	Uptake of 1/80 M NH_4Cl, N increase in ‰ fresh weight
—	0.76
10^{-7}	0.60
10^{-6}	0.27
10^{-5}	0.07
10^{-4}	0.—
M conc. of jodoacetate	
—	1.19
10^{-6}	0.68
10^{-5}	0.68
10^{-4}	0.54
10^{-3}	0.22

C

M conc. of NaN_3	Uptake of 1/80 M $(NH_4)_2CO_3$, N increase in ‰ of fresh weight
—	3.34
10^{-6}	3.37
10^{-5}	3.28
10^{-4}	2.77
10^{-3}	2.66

For the transport of other substances, urea, ammonium chloride and ammonium carbonate the influence of some inhibitors has also been examined (table 26). Ammonium from ammoniumchloride behaves like phosphate. For the others the influence may be compared with that of caffeine. For urea the inhibition is stronger, which was to be expected, the transport being more strongly activated here. Ammonium carbonate was absorbed very strongly (table 11). It is distinctly inhibited by withdrawal of oxygen (table 5), also at low temperature at 5° C. Anaerobically there arises a green precipitate in the cells of the pedicel, which turns into black when exposed to the air. Na-azide 10^{-4} gives a distinct but slight inhibition (table 26).

VIII. Discussion

From the above it has appeared that to making experiments on uptake and transport in leaves of *Drosera capensis* various difficulties are attached. We summarize them below:

1. Growing regularly developed plants in an adequate quantity. For each experiment 36 leaves, divided into 6 series, are used. Each plant has to possess six successive suitable leaves.
2. The long duration of the experiments, in which the uptake depending on the nature of the substance absorbed must last 24 or 48 hours to obtain a sufficiently strong transport. As a rule two or three experiments a week could be made in the most favourable season.
3. The presence of mucilage on the tentacle glands necessitates washing off the leaves before the fresh weight can be determined. In order to avoid injury this washing can only be done after finishing the experiment. Washing results in an alteration of the fresh weight and with some substances there is leaching due to exosmosis.
4. As a reference quantity the fresh weight of the leaves at the end of the experiments after washing has been used. The uptake can also be determined per series of six leaves. This matters for series of which the leaves are sufficiently equal in length and development and for which owing to the nature of the experiments the final fresh weight is unsuitable as a reference value.
5. The lack of sterility of plants and medium through which organic substances in the medium can be decomposed. The substances have been administered in agar by which we should take into account the impurities which the agar may contain and which may have an influence on the tentacles.
6. Keeping the tentacles quiet, so that they cannot curve from the agar and absorb the substance from the agar unequally. Adding caffeine, sucrose or salts to the agar keeps the tentacles quiet, but at the same time influences the uptake.
7. The composition of a series from 6 leaves of different ages means that leaves that react differently in their metabolism, have been put together in one series.
8. The influence of the season and of external factors during the growth on the reactive power of the plants.

9. The variability in the observations which is due to the many sources of error.

The difficulties mentioned above have been met as well as possible, but some of them are inherent to the material used.

It is a striking fact that with the uptake of all substances an influence of oxygen has been found, which indicates that also with substances that penetrate through diffusion, the influence of the plasm that has to be passed, is expressed in a dependence on respiration. This gives the impression that there is *no strict separation between passively and actively absorbed substances.* In taking up certain substances "physical" and "physiological" permeability, i.e. passive and active uptake can cooperate or in Overton's terminology permeation and adenoid absorption can go together. On the simultaneous action of the two processes we have not yet any data for other objects. Neither has it been taken into account that this possibility exists, so that in researches on permeability active processes are frequently made impossible by the way of experimenting. Injury due to cutting, infiltration to make the preparations more transparent and lack of oxygen will have to be avoided. It is quite possible that if these items are taken into consideration, cases will be met which will correspond with our results with *Drosera.*

Before discussing the results obtained, we must remind the reader of the fact that it is essentially impossible to separate processes of uptake and transport.

In the two preceding communications (ARISZ III and IV 1944) we have arrived at a theory to interpret the phenomena found. We will discuss this theory first and next consider to what extent the results newly obtained correspond with it.

Of the active absorption of substances as asparagine, amino acids and phosphates the following presentation was given in 1944(ARISZ III. p. 246).

"The first phase of active absorption is based on a combining of ions from the medium with components of the protoplasm in the boundary surface. In the case of amino acids and asparagine it is the amphoions, in the case of the salts the anions which combine with the plasm. According as the concentration in the medium is higher, more ions are combined, but in low concentrations, as is the rule in adsorption processes relatively more ions are combined than in strong ones. On further increase of the concentration the number of particles which can be combined at the same time reaches a marginal value. This marginal value fixes a limit to the loading of the transport system. In this connection we leave the question open of what nature the linkage is between the parts of the substance transported and the protoplasmic carriers. The quantitative relation between rate of absorption and concentration in the medium indicates an adsorption process in which at the boundary surface a quick adjustment of equilibrium takes place, yet we can also think of chemical combinations.

On the first phase of the absorption or transport process follows the introduction of the particles of the transport substance into the cyto-

plasm, in consequence of which fresh particles can be combined at the boundary surface. The view that in order to be absorbed the substances are combined with protoplasmic carriers and do not permeate into the cell through free diffusion we call *the theory of plasmatic binding.*

From the subsequent publication (ARISZ 1944 IV) we quote as follows: If when absorbing the cell does not make any difference between one amino acid and another or between amino acid and asparagine, this means that the protoplasmic patches which can combine with asparagine, can also combine with amino acids. The number of patches, therefore, available for combining with an amino acid or an asparagine amphoion, is limited and these substances compete for a place on the boundary surface. Phosphates are absorbed independently of amino acids and asparagine. This means that the patches capable of combining phosphates, cannot combine with the amphoions of amino acids at the same time. There are, therefore, patches of a different type in the protoplasm, some of which are adapted for combining with amino acids and asparagine and others for combining with phosphate. Of what nature the combining with phosphates is, has not yet been cleared up. It does follow from the experiments that it does not make any difference whether potassium or sodium phosphate is present" (l.c. p. 257).

We now arrive at the question what supplement and alteration is necessary of the standpoint taken up by us in 1944. For this purpose we summarize the data newly obtained on the influence of inhibitors on the active transport. On the whole it may be said that the chemical nature of combining the substances actively absorbed in the protoplasm has become more prominent.

1. The result obtained before that the absorption of asparagine and of phosphate is dependent on oxygen only indicates the connection of the transport with metabolism, but does not show where the active processes are localized. For the analysis of these processes, therefore, inhibitors are of great importance, as they can be administered locally and render conclusions on the localization of the active processes possible.

2. Inhibitors of the respiration such as KCN, Na-azide and Na-arsenite inhibit the transport through the intact tentacles and likewise through the pedicels after the glands of the tentacles have been removed. This indicates that the dependence of active transport on respiration is not limited to a process that takes place in the boundary surface between gland and medium. No more can the active transport be exclusively due to processes that are restricted to the leaf and for instance keep the concentration of the transport substance there at a low level, so that a continuous diffusion from the medium with high concentration through the tentacles to the leaf would become possible. *The cellular transport in the whole tentacle must depend on the respiration.*

3. The deviating behaviour of iodo acetate and dinitrophenol with regard to tentacles without glands could be interpreted in two ways. It may be that as a result of the wounding the inhibitors are inactivated and less active. This is indicated by the fact that in a

higher concentration of dinitrophenol sometimes a strong inhibition in the tentacles without glands is obtained. A second possibility is that these inhibitors are only active in the gland. This is possible, but it does not seem probable, seeing the varying results, in which in a few cases an inhibition is occasionally found.

4. Na-arsenate inhibits the uptake of asparagine, as a result of its inhibiting phosphorylation, so that no phosphates rich in energy are made available. It has, therefore, a similar effect as dinitrophenol. Na-arsenate, however, does not influence the uptake of phosphate. This different effect may be explained satisfactorily by assuming that this inhibitor is displaced from the substratum by inorganic phosphate which is administered to the medium. Inhibition of asparagine absorption by Na-arsenate therefore, is not a specific process attuned to the uptake of one special substance. This is an interesting indication that one should be cautious in ascribing specificity to a particular inhibitor. This was a reason to consider for the other inhibitors working specifically on asparagine whether addition of inorganic phosphate to the medium influences inhibition.

5. Inhibition of phloridzin, and penicillin seems to be specific. Phloridzin inhibits the uptake of phosphate only, penicillin, only the uptake of asparagine (also that of glutamine, while amino acids have not yet been examined). This result corresponds with what is known about the inhibition of these substances in literature (GALE, NANCE). The biochemical insight, however, into the reactions which these substances affect, is still insufficient.

The result of these experiments is, therefore, a corroboration of the connection between transport and respiration. Inhibition of the respiration renders transport impossible. It is, however, not clear yet what parts of the respiration process are influenced by special inhibitors. In biochemical litterature it is stated that the uncoupling of the formation of energy-rich phosphates from respiration is caused by a great many substances. But they can effect this in quite different ways. Among these substances are 2,4-dinitrophenol, p. nitrophenol, methylene blue, brilliant cresyl blue, arsenite, arsenate, Ca^{++} and malonate (LEHNINGER 1951). SPIEGELMAN, KAMEN and SUSSMAN 1948 and LOOMIS and LIPMANN 1949 found that azide likewise inhibits the formation of energy-rich phosphates (SPIEGELMAN 1952).

About the way in which these inhibitors act in our experiments with *Drosera* there prevails an uncertainty. Suffice it to state the general connection with respiration and particularly the dependence of the transport processes on the production of energy rich phosphates. In accordance with the previously shown specificity of the uptake of asparagine and phosphate it was found that there are inhibitors which inhibit the uptake of a special transport substance specifically. Penicillin acts on the combining of asparagine and phloridzin on the combining of phosphate.

The theory of transport through plasmatic binding therefore, has been vigorously supported by the experiments with inhibitors. The experience that many inhibitors are active as well in tentacles with-

out glands as with glands indicates that the active part of the absorption and transport process is not limited to the gland, but that in every tentacle cell the same reactions take place and the transport through the tentacle must, therefore, be considered a succession of reactions in the parenchyma cells of gland and pedicel. What part the leaf acts in this remains unexplained, but there is no reason to expect that the transport processes will be different there.

This resolves the problem of uptake and transport by the tentacles into the more general problem of the polar transport of substance in parenchymatous tissue, as is for instance known for growth substances. This problem will not be further discussed here.

About the question whether substances taken up in the plasm of a cell through a metabolic process, are set free there in their original or in changed form or continue combined with plasm carriers, we have not yet got any data. Of great importance for the cellular transport are the newer data on the significance of microsomes and mitochondria as enzyme systems (MILLERD e.a. on *Phaseolus*). We may refer to recent summaries (ARISZ 1952, ROBERTSON 1951, SPIEGELMAN and SUSSMAN 1952).

The connection of the aggregation processes often discussed in this and in preceding publications with the transport processes can only be mentioned here. It seems as if here a possibility exists to enter deeper into the nature of the cellular transport process, but various points still require a further investigation before we can give a summarizing opinion about this. Also the remarkable inhibiting influence of fluorescein on the transport of actively absorbed substances requires a further investigation. It does not seem impossible that the action of this substance is closely connected with the mechanism of the transport in the protoplasm. The action of eosin and fluorescein is not specific. Both asparagine and phosphate transport are inhibited, as much in tentacles with as without glands. In the case of fluorescein it may be easily noted that it permeates into and accumulates in the plasm. In these cells we also frequently meet contraction of vacuoles. The actions of these dyes on the transport suggest a displacement of the transport substances from active enzyme surfaces.

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Summary

The tentacles of the leaves of *Drosera* have the function of secreting to the leaf substances which are liberated by the disintegration of the insects. An extensive survey has been given of the method of research. The difficulties met have been summed up in VIII pp. 98 and 99 and in the methodical part it has been stated to what extent it has been possible to find a working method giving reliable results.

The principal results of two previous publications, which had been published in Dutch in war time, have been summarized and supplemented with new data.

The tentacles of *Drosera capensis* are genuine transport organs, which themselves retain the transport substance in hardly analysable quantities, but pass it on to the leaf. Important is the dependence on oxygen in the medium. Some substances are not transported in an anaerobic medium others only inhibited. The first set of substances is absorbed from a low concentration and accumulated in the leaf in a changed or unchanged form. We have called this active transport. In the latter case we have spoken of activated transport since a diffusion process acts an important part; pure diffusion, however, was not found for any substance, as withdrawal of oxygen always effects the transport somewhat. This points to influence of processes in the plasm, connected with metabolism. Amino acids, asparagine, glutamine, phosphates and ammonium are transported actively. For urea, thiourea, ammonium carbonate, caffeine, and others activation of the transport occurs in a different degree. The transport of amino acids, asparagine and glutamine on the one side and that of phosphate on the other side have in common that these substances are transported actively, but the nature of the transport differs for these two groups of substances. This specificity of the transport appears from summation experiments. For each substance the uptake depends on the concentration in the medium; already in fairly low concentrations a maximal rate of uptake is reached. Combination of different amino acids or of asparagine with an amino acid gives as long as the concentration is limiting summation until the saturation level for amino acids and asparagine has been reached. This indicates that they are all absorbed by the same system. Combination of an amino acid or asparagine with phosphate, however, gives of each of these substances absorption till saturation is attained. These systems must work independently. This led us in 1944 to draw up the hypothesis that during the transport the substances are combined with the plasm. Chemical reactions between the substances and the plasmatic particles must take place rendering absorption and transport possible. The summation experiments indicate that the nature of the binding of amino acids and asparagine is different from that of phosphates. The theory of the transport through combining the substances with the protoplasm based on these data (ARISZ 1944) has been discussed in VIII pp 100 and 101.

Some substances such as caffeine, antipyrine, and ammoniumcarbonate inhibit especially in a higher concentration the active transport of phosphates and amino acids. These substances inhibit aggregation as well.

With the aid of substances inhibiting enzymatic processes, the relation of transport to metabolism has been investigated. Such investigations have, for instance, been made by THIMANN and collaborators for the growth, the protoplasmic streaming and the active absorption of water and for instance by LUNDEGÅRDH, MACHLIS and

ROBERTSON for the uptake of salts. The results of our experiments was that the wellknown inhibitors of glycolysis and respiration, KCN, Na-azide, jodoacetate and Na-arsenate all inhibit the transport of phosphate and asparagine. Dinitrophenol likewise inhibits the transport of these two substances, which points to the influence of energy-rich phosphates on the transport process. Na-arsenate inhibits the asparagine transport .This likewise points to the significance of energy-rich phosphates for the transport processes. As phosphate competes with arsenate, it is to be understood that arsenate has no influence on the phosphate transport. More specific is the behaviour of phloridzin and penicillin. The first substance only inhibits the uptake of phosphate, the last only the uptake of asparagine. These specific inhibitions prove that the theory previously formulated that the combination of phosphate with the plasm is of a different nature from that of asparagine and amino acids is correct.

The glands of the tentacles can be cut off with a pair of scissors. Marginal tentacles treated in such a way behave essentially in the same way as tentacles with glands. They transport phosphate as well as tentacles without glands; this transport is likewise dependent on oxygen. The glands of the tentacles are, therefore, not essential to the active transport of phosphates. The transport of asparagine by the tentacles, however, is dependent on the presence of glands. For a low asparagine concentration 1/100 M, the transport is very slight. For a higher concentration, for instance 1/20 M, it may be greater, but the height of the transport in intact tentacles is not reached. The gland, therefore, has a specific influence on the uptake of asparagine. This reminds us of the influence of the gland on aggregation (COELINGH).

Inhibitors of the respiration have partly the same influence on the transport of phosphate in tentacles without glands as with intact tentacles. KCN, Na-azide and Na-arsenite inhibit this transport. This proves that the transport in the tentacle pedicel is of the same nature as the transport in the intact tentacles.

Various inhibitors gave no inhibition or a weaker one in tentacles without glands than in intact tentacles. This phenomenon has not yet been explained. It may be connected with the wounding or point to the fact that certain reactions are localized in the gland.

Eosin is a dye which is used to render transport in the sieve tubes impossible. Eosin gives a complete inhibition of the transport in Drosera tentacles already in a very low concentration.

Fluorescein has been used as an indicator of the transport in parenchyma cells and in sieve tubes. It causes a distinct inhibition of the transport in Drosera tentacles in a low concentration.

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