

Botany. — *Uptake and transport of chlorine by parenchymatic tissue of leaves of Vallisneria spiralis.* III. *Discussion of the transport and the uptake. Vacuole secretion theory.* By W. H. ARISZ.

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From the data obtained in the first part of this publication it appeared that *Vallisneria* leaves accumulate chlorine from a sufficiently balanced solution. This uptake is favoured by light and depends for a large part on the conditions prevailing in the period before the uptake. Since the cutting of the leaf in smaller pieces has to take place before the beginning of the experiments a wound influence has to be considered.

We have now shown that the wound has not only a great effect upon the uptake but also upon the transport. This is in itself already an indication that the transport is to a high degree determined by the protoplasm. While the transport is almost checked by the wounding, absorption proves not to be dependent on it to that degree and appears to continue though with less strength.

The first question we discuss here, is the one concerning the cause and the path of the transport. As a cause we may think of diffusion forces or suction pressures, which may give rise to a passive flow of particles and liquids. In the research made here on transport in leaves the occurrence of suction pressures owing to transpiration in part of the leaf is excluded because all experiments were made in a closed box which was quite saturated with water vapour, while moreover the free part of the leaf was lying on or between moist filterpaper. In their research on the transport of asparagine in leaves of *Vallisneria* OUDMAN and ARISZ have shown that transpiration in the free part does not cause an increase of transport. Some experiments not mentioned here on the influence of transpiration on the transport of chlorine have given an entirely analogous result. It is therefore not possible to suck salt through the leaf by a locally stronger transpiration. These data, on one side the dependence of the transport on plasmatic influences, on the other side the lack of effect from suction pressures in the free leaf length caused by transpiration, show that a transport from cell to cell of chlorine no more than of asparagine through the cell walls takes place to any considerable degree.

The cell wall transport found by some investigators (STRUGGER and ROUSCHAL) was no free diffusion, but a streaming under influence of strong suction pressures and occurred only over a short distance. We therefore conclude that *the transport of chlorine must take place in the plasm of the living cells.* The cell wall must be passed during the uptake in the epidermis cells and in the transition from cell to cell, unless it may be brought about by plasmatic connections from cell to cell. As this transport goes across

the cell wall over a slight distance this is in no way contrary to the experience mentioned above that diffusion through a wall in longitudinal direction is of no consequence.

No doubt the processes of transport and uptake must take place in the protoplasm. The difficulty lies in the fact that the permeability of the surface layers is insufficiently known. There are data in literature which indicate that the tonoplast is less permeable to ions than the outer layer.

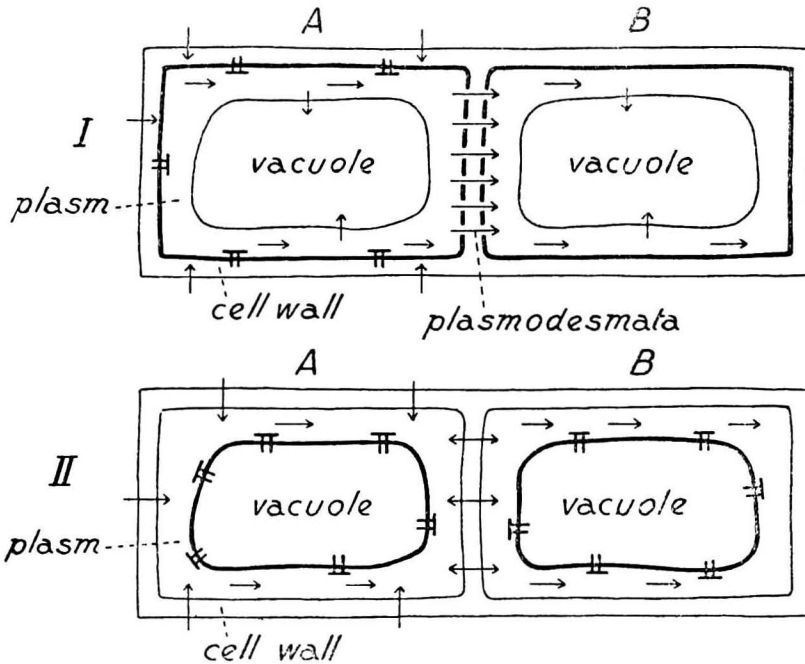


Fig. 3. The scheme represents the two parts of the leaf, the contactzone and the free part by two cells A and B. In scheme I the outer boundary of the protoplasm is impermeable for Cl ions, and the tonoplast permeable. An active mechanism for the introduction of ions must be situated in the outer layer of the plasm. By plasmatic connections between the two cells the substances invade cell B. Scheme II represents the condition that the outer layer of the plasm gives passage to Cl ions. The tonoplast is here impermeable and the accumulating mechanism must be situated in the plasmatic layers bordering the vacuole. π active processes \rightarrow diffusion.

This problem was discussed at length in a publication: Contribution to a theory on the absorption of salts by the plant and their transport in parenchymatic tissue, (ARISZ 1945). It was pointed out there that in roots a salt transport through the plasm of epidermis and cortex cells to the xylem may occur without passing the vacuoles and that various data show (HÖFLER, WIERSUM) that some ions can follow plasmatic tracts very well on their way from medium to xylem. The recent results of various American and English investigators (i.a. HOAGLAND and BENNETT CLARK), which were not at my disposal at the time, also point in the direction that the external layer of the protoplasm does not check the ions. In the above

publication I suggested to use by the side of *intrability*, i.e. the permeating of a substance through the outer boundary layer or plasmalemma, the term of *transmeability* to indicate the passing of both plasmalemma and tonoplast as occurs in deplasmolysis experiments. Its consequence is that the above tissue permeability of the root only indicates intrability of the plasm, whereas deplasmolysis experiments show the transmeating of a substance. It is then the permeability of the tonoplast that renders a passive penetration of substances from the plasm into the vacuole possible. This conception forms the basis of our further discussions.

If the *Vallisneria* leaf piece used in the transport experiments, for convenience' sake is represented by two cells: cell A representing the contactzone, and cell B representing the free part of the leaf, it has been shown that when A absorbs chlorine ions, they penetrate into B as well, especially when B is exposed to light (cf. fig. 3).

There is no loss of chlorine ions which have been absorbed in A, on a change of external solution, e.g. on transmission to distilled water, even if the tissue is brought into an anaerobic medium. Seeing that during the uptake the osmotic value of the cell sap increases, we are justified in assuming that the absorbed Cl ions are for the greater part present in the cell sap in a free condition. Their concentration can considerably surpass that of the liquid from which they have been taken. We conclude from this that the accumulation of chlorine in the vacuole is an irreversible process. A transport in only one direction must take place through the membranes and the plasm. The active accumulation mechanism is capable of taking the ions through the enclosing layer. It also appears from these data that the condition of impermeability of one or more of the boundary layers is stable and does not depend on a process that is kept up at the expense of energy. In that case as soon as this active process should be checked by withdrawal of oxygen it would pass into a loss of the substance first absorbed. The impermeability is also preserved for some time in an anaerobic condition. Only through injury of the plasm the state of impermeability is discontinued and exosmosis takes place.

It is incontestable that this accumulation mechanism lies in the protoplasm, but concerning the permeability of the boundary surfaces no conclusion can be drawn from this as yet.

Three cases may be distinguished:

System I. The outer boundary surface or plasmalemma is impermeable to chlorine ions, but the tonoplast allows them to pass.

System II. The outer boundary surface is permeable to chlorine ions, but the tonoplast does not allow them to pass.

System III. Both boundary surfaces are impermeable to chlorine ions. These must be transferred by special mechanisms from the medium to the plasm and thence to the vacuole.

The first conception of the permeability of the boundary surfaces is usually accepted. It must then be assumed that the accumulation mechanism transfers the ions from the outer solution across the outer boundary surface to the plasm and that thence they pass unimpeded into the vacuole. At the expense of energy the chlorine ions are as it were, pumped into the cytoplasm and the vacuole.

We have now to consider, how in the scheme of fig. 3 I when cell A has absorbed chlorine ions in this way these ions can be given up to cell B in spite of the impermeability of the plasmalemma. Cell B might do this either actively by means of a mechanism that at the boundary surface of the two cells withdraws the ions from cell A, or the chlorine ions already accumulated in the plasm of cell A might be transferred by diffusion along plasmatic strands in the partition wall into the protoplasm of cell B. The first possibility seems very unlikely, as it cannot be seen how through the two impermeable plasmalemmas of the adjoining cells, the ions could be pumped from cell A into cell B. Moreover in the partition wall no Cl ions will be present, as exosmosis of Cl ions does not take place. We therefore arrive at the hypothesis that with impermeability of the outer boundary surfaces cell B can only absorb chlorine, if the plasm of cell A is connected with that of B, so that ions can pass from A to B without their being obliged to pass the outer surface layers. MÜNCH's hypothesis, viz. that the plasm of adjoining cells is connected by plasmodesmata satisfies this condition. The experiments made in 1944, could for the greater part be accounted for in this way. There was only a difficulty concerning the explanation of the result mentioned in II § 3 sub 5, that the transport from the contactzone, which was not exposed itself, was greater to the free part if this was exposed than if it was in the dark. For if exposure of the free part affects the uptake by this leaf length, it proves that not the uptake of the contact zone determines how much chlorine gets into the free part, but the active processes in the free part itself. This was the reason why the whole process was again investigated extensively in a second series of experiments, the result of which has been communicated above.

In the first place it was found in the second series of experiments that wounding affects the transport, a result that is not compatible with a passive transport through the cell-walls. In the second place it appeared that, in experiments in which the influence of the woundstimulus after a 24 hours' stay in distilled water had largely disappeared, in the free part an active uptake can take place, which may be greater than the one in the contact zone (II Fig. 1 D). In the simplified scheme this means that cell B itself absorbs actively. If A is in the dark and B exposed, the uptake in B is greater than in A which absorbs direct from the medium. As we have already discussed, the ions absorbed by B must have passed through cell A. Such an active uptake by cell B from cell A can hardly be imagined, if the plasmalemma does not allow chlorine ions to pass. It might be supposed that a transfer along the protoplasm of cell A is not necessary

at all and the chlorine ions can get into B through the cell wall. The objections against this conception we have already discussed. Neither can it be realized how such a transport in cell walls could be checked by the wound stimulus.

There is a third phenomenon that is hard to be accounted for in this way. This is the phenomenon discussed in § 5, that the absorption by cell B, which must take place through the plasm of cell A need not occur at the expense of what is accumulated in A. If the accumulation took place at the outer boundary surface of cell A this would be inexplicable.

Finally we may consider what happens, if a leaf after having absorbed chlorine, e.g. with an exposure as represented in fig. 1 B (contactzone in the light, free part in the dark), is put in distilled water. The uptake from the medium is then finished and only a redistribution of the chlorine between the different parts of the leaf can occur. If there are plasmatic communications between the plasm of the cells of the contactzone and those of the free part, we can expect according to scheme I a redistribution by diffusion of chlorine from cells with higher to those with lower chlorine concentration, as the chlorine which is accumulated in the vacuoles can pass through the permeable tonoplast to the protoplasm. This problem has been investigated, but a considerable redistribution could not be shown. A small decrease of the amount of chlorine in the contactzone may be present after 12 hours, but if it is significant, it can be caused as well by a slight exosmosis. *This proves that a free transfer of chlorine accumulated in the vacuoles of the contactzone to the adjacent cells is impossible.*

All these data show that both the system I and III (see p. 27) in which an impermeability of the plasmalemma and a permeability of the tonoplast to ions is assumed, are unacceptable. The plasmalemma must allow the ions to pass and the tonoplast must be impermeable. This leads us to accepting system II (fig. 3 II). In this the plasmalemma is permeable, the transport of chlorine takes place in the cytoplasm and the accumulation is brought about by a mechanism in the plasm adjoining the vacuole. Now the tonoplast is impermeable to chlorine ions. In this system we have to deal with a secretion of chlorine from the protoplasm into the vacuole. This system can account for all the results obtained, and we shall call this conception the vacuole secretion theory. Seeing that now the outer layer of the plasm passes chlorine ions they can get into the plasm. The accumulation mechanism which must lie in the cytoplasm, surrounding the tonoplast, takes the ions from the plasm through the tonoplast into the vacuole. A loss by the vacuole cannot be shown as long as the plasm is in a normal condition. It may be imagined that a loss takes place, but it must occur so slowly that it makes the impression that this layer does not pass the ions from the vacuole to the remaining cytoplasm. In abnormal conditions at too high or too low a pH, there is a marked loss of chlorine ions, which ooze from the cells to the medium. If the supply of chlorine ions in the plasm is great, the secretion into the vacuole in the cells of the contactzone will

occur without the transfer of ions in the plasm towards the free zone being influenced by it. In that case the plasm of a leaf zone not in direct touch with the medium can be supplied with chlorine, which is thereupon secreted into the vacuoles of the cells of this zone.

Because in the light secretion is stronger than in the dark, the case may arise, as we saw in fig. 1 D that the exposed free part of the leaf absorbs more chlorine than the darkened contactzone. The secretion of ions into the vacuoles of those parts of the leaf which are not in direct touch with the external solution, is added to that of the contactzone, so that it is comprehensible that a greater leaflength can absorb more Cl than a shorter one, though the contact zone is equally long in both cases. As soon, however, as the supply through the plasm is slighter, the uptake of the free part will take place more or less at the expense of what is absorbed by the contactzone. In this case there is a rivalry for the ions supplied by the plasm.

From the experiments it appeared that both uptake and transport are retarded by wounding. The experiments of table 8 do make the impression that after a 16 hours' preliminary treatment in water the accumulative capacity has become fairly normal, notwithstanding the fact that the second and third zones absorb less than after a 24 hours' preliminary treatment. This must be based on the fact that the transport in the cytoplasm has not yet quite recovered by that time. Also the varying results concerning the strength of the transport in leaf lengths which have been entirely exposed in which case the absorption in the contactzone may be very great, but the transport very slight, make the impression that the accumulation, i.e. the secretion into the vacuole and the transport in the cytoplasm are separate processes in which the accumulation may be strong without a proportionally strong transport attending it. This gives a foundation to the hypothesis that transport is a protoplasmatic process, which is particularly sensitive to disturbing influences.

Finally the question remains to be discussed how exposure affects accumulation and transport. Seeing the influence of exposure is also very marked in experiments in which the transport is slight, it may be considered an established fact that exposure affects the accumulation process. The influence of exposure during the preliminary treatment on the subsequent absorption is theoretically of great importance, but difficult to analyze, as long as the mechanism of accumulation or secretion is unknown. This phenomenon, however, which is independent of the presence of carbonic acid and therefore of carbonic acid assimilation, shows, that accumulation is connected with the presence of substances in the plasm which are formed in the light (cf. I p. 12 and 13). May be a further analysis will furnish data on the nature of the accumulation process. Interesting are in this respect the researches of BENNETT CLARK.

About the way in which the ions are transported in the plasm we must refer to previous publications in which we have developed the theory of

transport by binding to plasmatic particles to explain the transport in the tentacles of *Drosera* and to the discussion in the publication of 1945 quoted above (cf. WIERSUM 1947). It was then pointed out that two possibilities will have to be considered, viz., either the chlorine ions move bound to plasmatic-particles or independent of their movements. A clear connection with the visible protoplasmic streaming could not be demonstrated. The visible strong protoplasmic streaming occurring as a result of wounding appears not to go together with an intensive transport. Only after a prolonged stay in distilled water in which the plasm has seemingly settled down, the transport capacity is recovered. In leaf lengths which absorbed chlorine from a salt solution Miss M. HEIKENS did not observe plasmic streaming. Also on this subject a further research will have to throw light.

After all it is surprising that through this research on the influence of light on the process of uptake and transport in *Vallisneria* we have arrived at a corroboration of the conception we had formed on the permeability of the protoplasm and its boundary surfaces in 1945 on the ground of data in literature.

The first series of experiments of 1944 gave a different, seemingly simpler interpretation (ARISZ 1946). A continuation of these experiments, however, has rendered it possible to prove conclusively for the leaf cells of *Vallisneria* that we have to deal with a secretion of substances from the plasm into the vacuole and not with an accumulation through the outer layers of the protoplasm from the medium into plasm and vacuole.

As in a previous publication the literature was already extensively discussed, we shall not go into this again.

Summary of the theoretical part.

The chief theoretical result of this research is that we have succeeded in analyzing the osmotic system present in the cells of *Vallisneria*. The ions permeate through the outer layer of the plasm into the cytoplasm. Here there exists an accumulation mechanism that takes the ions from the plasm to the vacuole. So the principle of the active uptake of chlorine by *Vallisneria* is the secretion of the chlorine by the protoplasmic layers into the vacuole. This conception may be called the vacuole secretion theory.

In the vacuole the ions are present in a free condition and cause a considerable rise in osmotic value. Ions present in the vacuole can only diffuse outwardly in a demonstrable quantity, when the plasm is injured. The movement of ions in the plasm is inhibited by the influence of wounding. This indicates an active assistance of the cytoplasm in the transport. A connection with the visible protoplasmic streaming could not be demonstrated.

A summary of the experimental data may be found in Proc. L, Part I on p. 1032 and Part II on pp. 1240 and 1245.

Botanical Laboratory University of Groningen.

LITERATURE.

- ARISZ, W. H., Absorption and transport by the tentacles of *Drosera capensis*. I. Active transport of asparagine in the parenchyma cells of the tentacles. Proc. Ned. Akad. v. Wetensch., Amsterdam, **45** (1942).
- , II. The activation of the transport of different substances by oxygen. Proc. Ned. Akad. v. Wetensch., Amsterdam, **45** (1942).
- , III. De absorptie van aminozuren en zouten door binding aan het plasma. Verslagen Ned. Akad. v. Wetensch., Amsterdam, Afd. Natuurkunde **53** (1944).
- , IV. Gelijktijdige absorptie van verschillende stoffen. Verslagen Ned. Akad. v. Wetensch., Amsterdam, Afd. Natuurkunde **53** (1944).
- , Het actief en passief opnemen van stoffen door *Vallisneria*. Verslagen Ned. Akad. v. Wetensch., Amsterdam, Afd. Natuurkunde **52** (1943).
- , Contribution to a theory on the absorption of salts by the plant and their transport in parenchymatous tissue. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **48** (1945).
- , and P. J. VAN DIJK, The value of plasmolytic methods for the demonstration of the active asparagine intake by *Vallisneria* leaves. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **42** (1939).
- , and J. OUDMAN, On the transport of introduced nitrogenous substances in the leaves of *Vallisneria spiralis*. Proc. Kon. Akad. v. Wetensch., Amsterdam, **40** (1937).
- , and J. OUDMAN, Effect of decreased oxygen pressure on absorption and transport of asparagine and caffeine in *Vallisneria* leaves. *Chronica Bot.*, **4** (1938).
- , and J. OUDMAN, Absorption and transport of asparagine in leaves of *Vallisneria*. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **41** (1938).
- BENNET-CLARK, T. A. and D. BEXON, Water relations of plant cells. III The respiration of plasmolysed tissues. *The New Phytologist* **42** (1943).
- GESSNER, Fr., Die Nährstoffaufnahme der Submersen. *Ber. D. Bot. Ges.* **51** (1933).
- HOAGLAND, D. R., Lectures on the inorganic nutrition of plants. Prather lectures at Harvard University. *Chronica Botanica Co.* (1944).
- , and A. A. DAVIS, Further experiments on the absorption of ions by plants, including observations on the effect of light. *The Journ. of Gen. Phys.* **6** (1924).
- , ———, The intake and accumulation of electrolytes by plant cells. *Protoplasma* **6** (1929).
- , P. L. HIBBARD and A. R. DAVIS, The influence of light, temperature and other conditions on the ability of *Nitella* cells to concentrate halogens in the cell sap. *The Journ. of Gen. Phys.*, **10** (1927).
- HÖFLER, K., Kappenplasmolyse und Ionen antagonismus. *Protoplasma* **33** (1939).
- , Salzquellung des Protoplasmas und Ionenantagonismus. *Ber. D. Bot. Ges.* **58** (1940).
- INGOLD, C. T., The effect of light on the absorption of salts by *Elodea Canadensis*. *New Phytologist*, **35** (1936).
- JÄRVENKYLÄ, Y. T., Über den Einfluss des Lichtes auf die Permeabilität pflanzlicher Protoplasten. *Annales Bot. Soc. Zoologicae-Botanicae Fennicae. Vanamo.* T.9 no. 3 (1937).
- PHILLIS, E. and T. G. MASON, On the effects of light and of oxygen on the uptake of sugar by the foliage leaf. *Ann. of Bot. N.S.* **I** (1937).
- ROUSCHAL, E. und S. STRUGGER, Der fluoreszensoptisch-histochemische Nachweis der Kutikulären Sekretion und des Salzweges im Mesophyll. *Ber. D. Bot. Ges.* **58** (1940).

- STEWART, F. C., Mineral nutrition of plants. *Ann. Rev. Biochem.* **4** (1935).
- , Salt accumulation by plants. The role of growth and metabolism. *Trans. Faraday Soc.* 196. **33** (1937).
- WIERSUM, L. K., Een methode van doorstroming der houtvaten in de wortel in verband met het stoftransport in radiale richting. *Versl. Ned. Akad. v. Wetensch., Amsterdam, Afd. Natuurkunde*, **53** (1944).
- , Transfer of solutes across the young root. *Dissertation Groningen* (1947). *Rec. trav. bot. Néerl.* in press.