ACTIVE UPTAKE, VACUOLE-SECRETION AND PLASMATIC TRANSPORT OF CHLORIDE-IONS IN LEAVES OF VALLISNERIA SPIRALIS

BY

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Leaves of *Vallisneria* accumulate substances e.g. aminoacids and salts in their vacuoles. This appears from the increase of the osmotic value of the vacuole sap. The accumulation of chloride and phosphate ions depends on the intensity of the illumination to which the leaves are exposed. We have termed this process photoaccumulation and found that it does not depend on the presence of carbon dioxide. The leaves absorb the salts as well from a solution as from a 2 % agar gel. We confine ourselves here to the process of uptake of chloride-ions and use the method of uptake from agar strips because it provides the opportunity to localize the uptake in a definite part of the leaf. The rest of the leaf gets the chloride-ions by means of transport from the absorbing part. So we have an absorbing and a transport zone. The length of the leaves used was mostly 7.5 cms, the absorbing zone being 2.5 cms and the free part of the leaf 5 cms. 2.5 cm leaf lengths were analysed before and after the uptake. The methods used have been described in a preceding paper (ARISZ 1947). A survey of the results of the translocation experiments formerly obtained will be given here with the aid of figs. 1–4. Fig. 1 gives the results

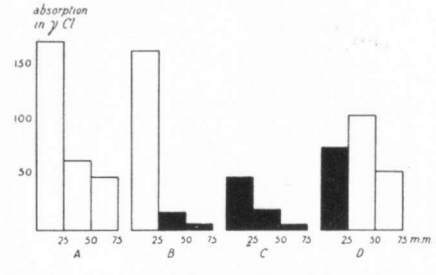


Fig. 1. Influence of exposure to light on uptake and transport of chloride in leaves of *Vallisneria*. In A the whole leaf is in the light, in B only the first zone. In C the whole leaf is in the dark, in D the first zone in the dark and the free part in the light. The first zone of 25 mms is 24 hours in contact with a solution of 0.01 mol KCl + $CaSO_4$ in 2 % agar. The second and third zone are free in moist air on wet filterpaper. On the ordinate the increase in Cl-content; temp. 25° C. Pretreatment during 24 hours in aerated distilled water in the light. (From ARISZ 1947). In this and the following figures on the ordinate the amount of the uptake in γ Cl.

of 4 different ways of exposure to light. In A the whole leaf is exposed to the light, in B only the absorbing zone, in C the whole leaf is in the dark, in D the absorbing zone is in the dark and the free part in the light. The first 2.5 cm zone, the absorbing zone, is in contact with a solution of 0.01 M KCl in 2 % agar with an addition of $CaSO_4$. The second and third zones forming the free part are in moist air on wet filter paper. A series of 8 leaves is put in a closed glass box the bottom of which was covered with water. In fig. 1A (whole leaf in the light) there is a strong accumulation in the absorption zone and a slight translocation to the free part. In 1B (free part of the leaf in the dark) this translocation is still slighter. In 1C (whole leaf in the dark) both uptake and transport are slight. Only in 1D there is a remarkable translocation of chloride from the absorbing zone, which is in the dark, to the free part of the leaf, which is in the light (Confer fig. 2).

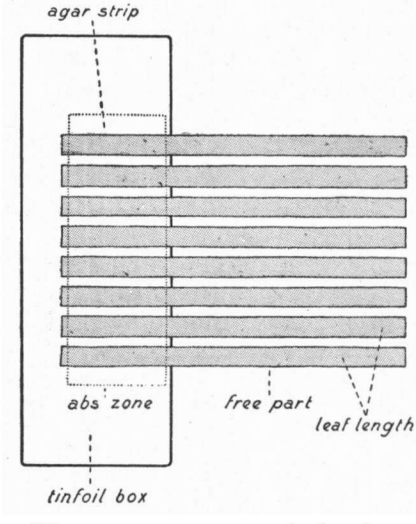


Fig. 2. 8 leaf lengths are with their absorptionzone between agar plates in a tinfoil box, which is closed during the experiment. The free parts of the leaf lengths protrude through perforations in the side wall of the box.

The pretreatment of the leaves after cutting has an important influence on the power of the plant for uptake and transport. This is elucidated by fig. 3. In A the leaves are directly used after cutting;

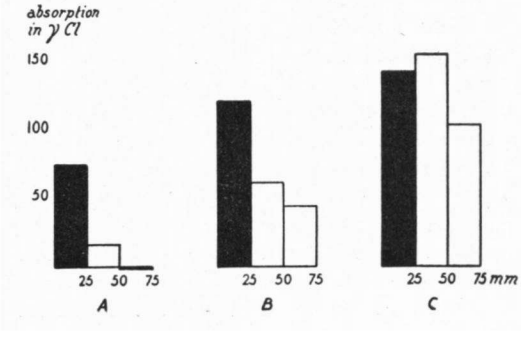


Fig. 3. Influence of the pretreatment on the transport of chloride. Exposure as in Fig. 1D:A, no pretreatment, B, 16 hours and C, 24 hours pretreatment in distilled water. In C a strong transport to the free part of the leaves. (From Arisz 1947).

in B there is a pretreatment of 16 hours in distilled water in the light and in C the pretreatment lasts 24 hours. Only after 24 hours pretreatment the translocation effect of fig. 1D is obtained. This is indicative of the strong influence of cutting on the condition of the leaves and points to the activity of the protoplasm in translocation.

As a result of these experiments the conditions are known which favour translocation of chloride ions. The absorbing zone has to remain in darkness which brings about a small accumulation in this zone and the free part has to be exposed to light in order to promote the photoaccumulation of transported chloride ions in these parts.

To explain these phenomena two schemes were considered (ARISZ III 1948) which we have reproduced in fig. 4. The schemes represent

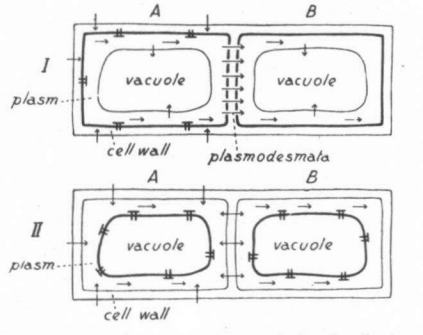


Fig. 4. The scheme represents the two parts of the leaf, the contactzone and the free part by two cells A and B. In scheme I the outer boundary of the protoplasm is supposed to be impermeable for Cl ions, and the tonoplast permeable. An active mechanism for the introduction of ions is situated in the 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 is situated in the plasmatic layers bordering the vacuole. (From ARISZ 1948)

the two parts of the leaf, the absorbing zone and the free part, by two cells A and B. In Scheme I the accumulating mechanism is situated near the outer boundary of the cytoplasm and the tonoplast is assumed to be permeable to the chloride ions. In Scheme II the accumulating mechanism is situated near the inner boundary of the cytoplasm, the tonoplast and the ions of the medium having free entrance into the cytoplasm. According to scheme I the chloride ions are accumulated in the plasm and the vacuole. Translocation in the plasm of absorbed chloride ions from cell to cell is only possible if there are plasmodesms which connect the cytoplasm of adjacent cells. Evidently there is also the possibility that the chloride ions are translocated outside the plasm through the cell walls and that they are subsequently absorbed by the active accumulating mechanism of the cells in the free part. The transport of chloride ions from cell to cell through plasmodesms does not agree with the result obtained in Fig. 1D that the free part of the leaf can obtain more Cl ions than the absorbing zone itself. This is not in accordance with a diffusion

process from cell to cell which requires that concentrations gradually decrease. This means that it is not the active process of uptake in the absorbing zone that determines how much chloride ions will be accumulated in the different parts of the leaves, but that this depends on the strength of the accumulation in the different parts of the leaf.

Though the assumption that the transport takes place in the cell-walls can give an interpretation of the different strengths of the accumulation in different parts of the leaf, it is not in accordance with our finding that particularly the transport is very sensitive to a wound stimulus, while the photoaccumulation process is less sensitive. This makes a mere translocation of the ions in the walls outside the protoplasm very unlikely.

Scheme II seems to give a reasonable interpretation of the results. Here the accumulation mechanism is situated near the border of the vacuole and takes the chloride ions from the cytoplasm into the vacuole. This process can be compared to a secretionprocess. The ions pass from one cell to an other either by plasmodesms or they diffuse through the transverse walls. When arrived in the cytoplasm of the cells of the free part they can be secreted into the vacuoles by the active accumulating process. It is in close agreement with this scheme that substances once secreted into the vacuoles remain there and do not diffuse to adjacent cells.

In the years after 1947 we have tried to extend and to corroborate the above mentioned experiments but the results were disappointing. The leaves used were less resistant and could not recover in 24 hours from the effect of cutting and wounding. In stead of an uptake by the darkened absorbing zone there was often a loss of salt owing to leakage. Besides it seemed desirable to take special precautions against a spreading of the transportsubstance over the surface of the leaves. This was obtained by coating the leaf between the region of absorption and the free part with a thin covering of vaseline. Experiments made in this way showed that such a spreading outside the cells was out of the question. Not until May 1952 could experiments be made with complete success. During May and June of this year the material remained in that specially good condition which is required for transport experiments, but after that it declined again.

Fig. 5 gives a translocation over a region of 12.5 cms in 24 hours.

In the meantime we managed to show that even leaves in a less satisfactory condition could be used for transport experiments if sugar or fructose was administered to the leaves. A conclusive proof of plasmatic transport can be given by the following experiment with a leaf having a darkened absorbing zone and the free part exposed to the light (fig. 6 and 7). The absorbing zone was put between two agar strips containing KCl and $CaSO_4$ in a tinfoil box. The free part of the leaf was exposed to light. The free parts of the series B and C were placed between two agarplates which contained 2 % fructose. In series B the agar strips with the absorbingzones also contained 2 % fructose. Between the absorbing zone and the free part of the leaf the surface of the leaf was covered with vaseline.

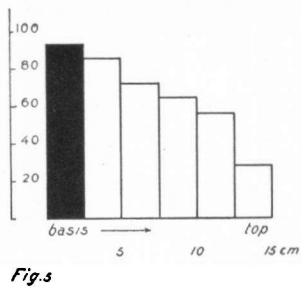
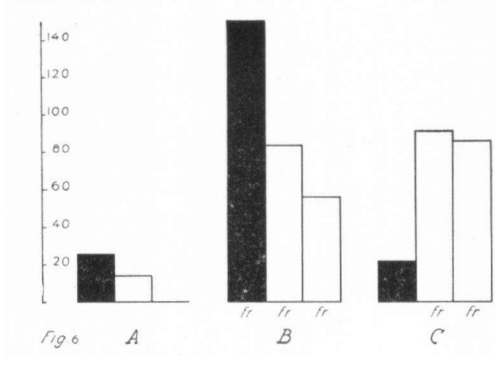
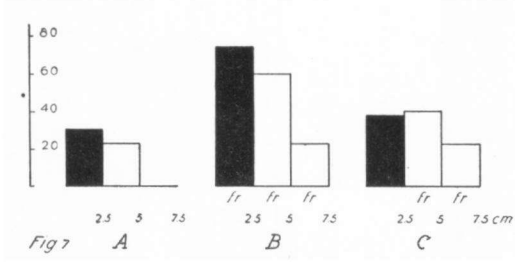


Fig. 5

Fig. 5. Transport of chloride ions in leaf-lengths of 15 cm. The first zone of 2.5 cm, the absorbing part is in the dark. The rest of the leaf-length is exposed to light. 24 hours uptake from a solution of 0.01 M KCl + $CaSO_4$ in agar.





Figs 6 and 7. Influence of fructose on the transport of chloride ions in leaf-lengths of 7.5 cm. A, normal conditions; B, with addition of fructose to all the zones; C with addition of fructose only to the free part.

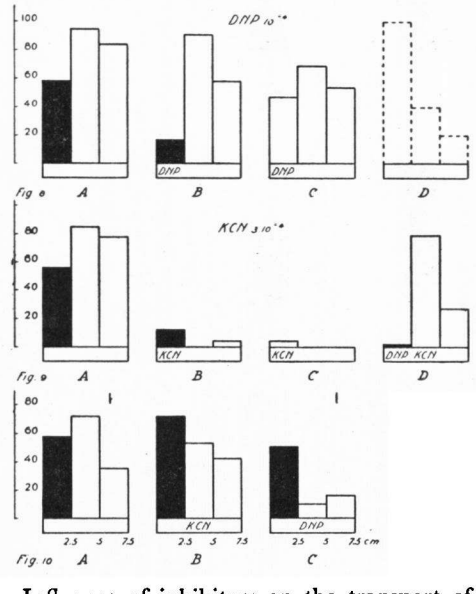
Without fructose the uptake and the translocation in all parts were slight. With fructose administered to all zones (series B) there is a strong accumulation of chloride in the absorbing zone and the translocation to the free part is considerable too. In C with fructose only administered to the free part the accumulation in the absorption zone is slight, but the transport to the free part is of the same strength. If one considers that these leaf lengths were lying between agar strips it will be clear that there can be no question of diffusion or spreading in the cell wall. The transport must be a vital process dependent on the presence of sugar and closely connected with metabolism. Such a transport can only take place in the cytoplasm.

The connection of translocation with metabolism can also be investigated in other ways. We analysed the transport in *Drosera* tentacles by means of substances which inhibit enzymatic processes. It is obvious that we can do the same with the processes in Vallis-

neria leaves. This research has only commenced but seeing the difficulty of getting sufficient material of the quality required for these experiments on transport it seems justified to communicate some preliminary results. They relate to the influence of 2–4 dinitrophenol and KCN on the uptake and transport of chloride ions.

All these experiments have been taken with the arrangement which had formerly given the most favourable conditions for transport i.e. after 24 hours' pretreatment in the light the absorbing zone is placed in the dark and the free part is exposed to the light. The duration of the experiments is always 24 hours at a temperature of 25° C.

In the first experiments the influence of 2–4, dinitrophenol (D.N.P.) on the uptake has been investigated (fig. 8B). It was added to the agar covering the absorbing zone. It gives a strong inhibition of the accumulation. But in the free part of the leaf there is a strong accumulation which proves that the transport is not checked by the inhibitor. As these substances must have passed the absorbing zone, it proves that D.N.P. even in the absorbing zone does not inhibit the uptake of substances into the cytoplasm. Accumulation in the absorb-



Figs 8, 9 and 10. Influence of inhibitors on the transport of chloride ions.

Fig. 8. In A and B the absorbingzone is in the dark and the free part is in the light. In C and D the whole leaf length is in the light. In B and C 2–4, Dinitrophenol 10^{-4} is administered to the absorbing zone.

Fig. 9. In A and B the absorbingzone is in the dark and the free part is in the light. In C the whole leaf length is in the light. In B and C KCN 3.10^{-4} is administered to the absorbing zone. In D DNP is administered to the first zone and KCN to the second zone.

Fig. 10. In A, B and C the absorbing zone is in the dark and the free part in the light. In B KCN 3.10^{-4} and in C D.N.P. 10^{-4} is administered to the second zone.

ing zone being at the same time inhibited, it is obvious that the inhibited accumulating mechanism must be situated in the inner cytoplasm and that the accumulation is a secretion into the vacuole.

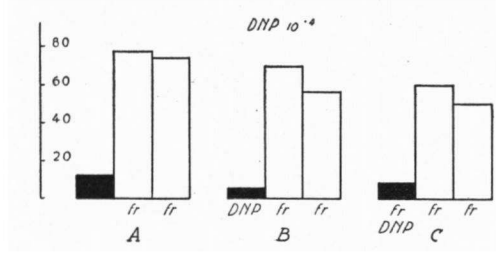
We have further traced the influence of D.N.P. when the whole leaf length was in the light. Fig.8D gives the result without D.N.P. Here the absorbing zone gets a high Cl content, that of the free part being much smaller. If D.N.P. is added to the agar covering the absorbing zone (fig. 8C) the accumulation in this zone is strongly decreased, but the translocation of chloride ions to the free part of the leaf is much larger as the competition of the mechanism which secretes ions into the vacuole of the cells of the absorbing zone is now ruled out. So, the result is approximately the same whether the absorbing zone to which D.N.P. is added is exposed to the light or stays in the dark.

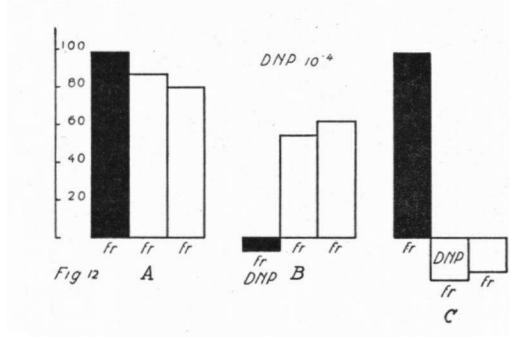
If KCN 3.10^{-4} is added to the absorbing zone a remarkable effect is produced (fig. 9). Now the accumulation in this zone as well as the translocation to the free part are strongly inhibited. Neither does it make any difference in this case whether the absorbing zone is in the light or in the dark (fig. 9 B and C, fig. 9C has to be compared with 8D).

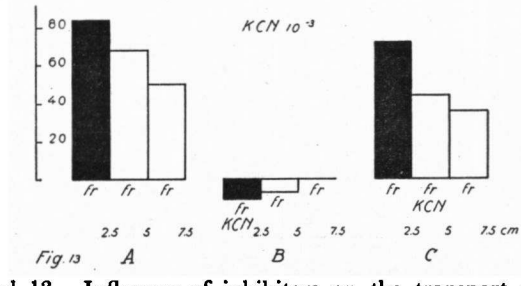
Three suppositions have to be considered to interpret this result: 1. KCN inhibits the translocation in the cytoplasm, 2. KCN inhibits the process of uptake at the outer boundary of the cytoplasm, 3. KCN has moreover an inhibiting influence on the process of vacuole secretion. Also a combination of these actions can be assumed.

By administering KCN to the free part of the leaf a possible influence on the transportprocess and on the secretion into the vacuole can be investigated. We have put the second zone of 2.5 cms between agar strips to which KCN 3.10^{-4} was added. The result is given in fig. 10B. It appears that KCN does not inhibit the secretion into the vacuole of the free part, neither has it an appreciable influence on the plasmatic transport. Fig. 9D corroborates this conclusion. Here dinitrophenol has been added to the first zone and KCN to the second one. D.N.P. inhibits only the secretion of Cl into the vacuoles of the first zone but does not interfere with the translocation to the free part. KCN has no effect if applied outside the absorbing zone. It seems therefore that KCN acts only on the uptake of chloride from the medium by the absorbing zone.

Once the substance has penetrated into the cytoplasm KCN neither inhibits its transport nor its accumulation in the vacuole. The difference in behaviour between D.N.P. and KCN with respect to the transport is also apparent from fig. 10B and C where the accumulation in the free zone is positively inhibited by D.N.P. KCN being without influence. The slighter translocation into the third zone can be ascribed to the less favourable condition of the material in this experiment. In later experiments D.N.P. was so harmful that the cytoplasm could scarcely endure its presence and often gave exosmosis of Cl.







Figs 11, 12 and 13. Influence of inhibitors on the transport of chloride ions. With addition of fructose.

Fig. 11. Influence of D.N.P. administered to the absorbing zone. Addition of fructose to the free part in A and B and to the entire leaf in C. The absorbing zone is in the dark and the free part in the light. In B and C Dinitrophenol is administered to the absorbing zone.

Fig. 12. Influence of D.N.P. administered in the first or the second zone. Addition of fructose to all parts of the leaf lengths. The absorbing zone is in the dark and the free part in the light. In B Dinitrophenol has been administered to the absorbing zone and in C to the second zone.

Fig. 13. Influence of KCN administered in the first or in the second zone on the transport of chloride ions. To all parts of the leaf lengths fructose has been supplied.

From figs 11, 12 and 13 it appears that even in the less favourable circumstances in October 1952 it was possible by addition of fructose to the different parts of the leaf to show the different effect of D.N.P. and KCN when administered either to the absorbing zone or to the free part of the leaf. So this is a conclusive proof that D.N.P. is an inhibitor specifically acting on the secretion of chloride ions into the

vacuole. It does not inhibit the translocation in the cytoplasm nor the uptake through the outer cytoplasmic layer. KCN on the contrary acts particularly on the uptake in the cytoplasm and does not influence, or only in a slight degree, the accumulation in the vacuole and the translocation from cell to cell.

DISCUSSION

The experiments with inhibitors confirm and complete our former results. The view that substances are translocated in the living cytoplasm and are secreted from this plasm into the vacuoles agrees with our findings on the influence of Dinitrophenol. The inhibition of the translocation to the free part by administering KCN to the absorbing zone proves that this translocation is a process which takes place in the cytoplasm and not outside it in the cell walls. Spreading and diffusion outside the protoplasm cannot play a part in this transport process.

A new point is the influence of KCN on the uptake in the absorbing zone. We have formerly assumed that chloride ions easily penetrate into the cytoplasm. The inhibition of this uptake by KCN points to the presence of a metabolic system near or in the outer boundary of the cytoplasm which regulates the introduction of these ions into the cells. KCN has no influence or only a trifling one when it is administered to the transportzones. This indicates that the process which is sensitive to KCN is only connected with the uptake through the outer layer of the protoplasm. By inhibiting this process in the absorbing zone both uptake and transport are inhibited. As there is no chloride present in the cytoplasm the secretion in the vacuole also comes to a stand still.

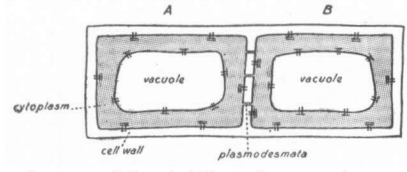


Fig. 14. Confer the schemes of fig. 4. There is an active accumulating mechanism situated near the outer boundary of the cytoplasm, which is inhibited by KCN and there is a second accumulating mechanism situated near the tonoplast, which secretes chloride ions into the vacuole. This process is inhibited by dinitrophenol. The transport takes place in the cytoplasm.

Fig. 14 gives the true scheme for the interpretation of these phenomena. There are two regulating mechanisms in the cytoplasm one at the outer boundary influenced by KCN and a second one at the inner boundary influenced by dinitrophenol. Transport is a vital process in the symplasm.

SUMMARY

The transport of chloride ions in the leaves of Vallisneria is a plasmatic process dependent on cell metabolism. It is checked by a wound stimulus and promoted by administering fructose.

Uptake from the medium is regulated by a process in the outer layer of the cytoplasm. KCN is an inhibitor of this process. There is an active secretion-process by which chloride ions from the cytoplasm are accumulated in the vacuoles. This process is inhibited by 2–4, dinitrophenol.

To Mrs H KNOBBE-MEESTER I am greatly indebted for the careful execution and help with the experiments and the analyses in the years 1947 to 1952.

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