Acta Botanica Neerlandica **7** (1958) 1—32

INFLUENCE OF INHIBITORS ON THE UPTAKE AND THE TRANSPORT OF CHLORIDE IONS IN LEAVES OF VALLISNERIA SPIRALIS

W. H. ARISZ
(*Botanical Laboratory, Groningen*)

(*received December 24th, 1957*)

INTRODUCTION

From previous researches it has appeared that the uptake of chloride into *Vallisneria* leaves is an active process that accumulates chloride ions in the leaf, owing to energy available in the cell or supplied when exposed (1947, 1952, 1956). The question of the absorption and the transport of the accompanying cations is not raised here. Respecting the influence of light on chloride absorption we refer to a recent publication by R. N. VAN LOOKEREN CAMPAGNE.

ARISZ showed that the uptake is a complex process consisting of various biochemical component processes. He distinguishes a process of ion uptake into the symplasm beside a secretion of ions from the symplasm into the vacuole and a transport from cell to cell in the symplasm outside the vacuoles. Both uptake and secretion are active processes, i.e. processes connected with metabolism. In how far the transport process itself may be called active will be discussed later.

In this publication the term "accumulation" will be used for a process that accumulates salt ions in the cell. For a considerable part this is accumulation in the vacuole owing to secretion of ions from the symplasm. This appears from the increase in osmotic value of the cellsap, demonstrated by A. van Schreven and A. van der Molen (ARISZ 1943, 1956). This does not exclude that in *Vallisneria* in mitochondria or in other parts of the plasm accumulation of ions can occur (cf. Robertson). It is desirable to state here that by ion transport is meant the transfer of ions both over very short distances in the cytoplasm and over longer distances in the symplasm. Transport from the medium into the cell plasm is called uptake into the symplasm. The transport mechanism has still been little investigated. The chloride ions, free or bound to carriers are easily transportable in the plasm and are not released to the medium during the transfer. This might point to the presence of an outer layer of the plasm difficult to permeate for these ions.

Of late years the view has been stressed that in many cells and

tissues there are spaces in the plasm in open communication with capillary spaces in the cell wall and with the external solution so that a reversible penetration of salts from the medium into the plasm can occur. Wall- and plasmatic spaces would then form a "free space", which is calculated as "apparent free space", (Hope and Stevens, Hope and Robertson, Robertson, Butler, Lundegårdh, Burström, Hylmö, Epstein for roots and Cowie and Roberts for *Escherichia coli*). COWIE and ROBERTS (1955) assume "that free metabolites in this space can diffuse out, metabolites involved in the metabolic reactions of the cell are not free, but attached to some larger molecules and thereby prevented from diffusing out". EPSTEIN (1956) suggests that also in *Vallisneria* leaves ions may be bound to larger molecules according to the concept discussed by COWIE and ROBERTS (1955). The problem of a free plasmatic space in *Vallisneria* will be more fully treated elsewhere. Here it may be observed that if the hypothesis is correct that the ions in the cytoplasm are bound to large molecules (Overstreet), nevertheless the complexes formed in *Vallisneria* leaves must be easily transportable in the symplasm without being released to the medium. Essentially the two representations don't differ much. In the one case uptake into the symplasm is binding to carriers present in the symplasm. In the other case it is a membrane passage at the cost of energy provided by the cell, the mechanism of which has not been explained yet, but may likewise be connected with binding to a penetrating carrier. An important difference is, however, that the free ions have a charge, whereas the complexes of ion and carrier have none (cf. p. 25).

On treating this problem other investigators have not paid attention to the transportability of the ions in the symplasm either alone or as a complex together with the carriers to which they are bound. In the discussion we shall revert to the uptake into the symplasm. Here it may be stressed that in a system consisting of various processes an uptake into the symplasm can only be physiologically demonstrated, if this process is limiting for the accumulation of ions into the cell. This sometimes seems to be the case. ARISZ and SOL (1956) for instance found a considerable increase in the uptake of chloride ions into the symplasm on exposure to light of the absorbing part. This indicates that in the dark the uptake process was limiting. The uptake into the symplasm will be dependent on various factors, of which the regulation of the permeability of the membrane for cat- and anions is one and the provision of energy for the transport of ions, for the formation of carriers or for the binding to a carrier a second. Also if one uses an inhibitor which influences one of the processes involved in the uptake into the symplasm, it is to be expected that under the influence of that inhibitor the uptake into the plasm may become limiting for the accumulation of ions.

In a previous investigation (1953) the effect of inhibitors, among which 2,4-dinitrophenol and KCN on the uptake and transport processes was discussed. Only a preliminary communication has appeared hitherto. The plant material used in 1952 had developed

vigorously and was very resistant, which made it capable of enduring fairly high concentrations of dinitrophenol without the protoplasm being injured. This was proved by the fresh look of the leaves at the end of the experiment, by the absence of exosmosis and by the undisturbed continuance of the transport. In this material dinitrophenol appeared to inhibit the vacuole secretion locally, but not the uptake into the symplasm and the transport. We have not yet succeeded in repeating these experiments with dinitrophenol with an entirely satisfactory result. In 1953 and subsequent years a number of other inhibitors were examined among which some giving a similar result as dinitrophenol, whereas others behaved like KCN. Here we mention only hydroxychinoline, sodium azide, KCN, sodium arsenate and uranyl nitrate.

Especially because meanwhile more experience has been obtained about the culture of *Vallisneria* and about the circumstances favourable for uptake and transport these inhibitors have given satisfactory results, which have corroborated and supplemented the results previously obtained. Especially in the spring of 1957 the *Vallisneria* material used was sufficiently resistant to endure inhibitors and consequently at the same time suitable for an operative interference to investigate the paths for transport.

In a discussion at the Conference of the Society for Experimental Biology at Groningen held on 31 March 1955, Dr. Sutcliffe made the suggestion that inhibitors may affect the transport in *Vallisneria* leaves. This might be based on an influence of the inhibitors on the transport of chlorides by the bundles. It is true these bundles are reduced in *Vallisneria* in connection with life under water, but distinct sieve tubes are present. This suggestion has given cause to investigate the parenchymatous transport and the one by bundles separatelv on their sensitiveness to inhibitors in connection with our experiments on "The path of salt transport" (ARISZ and SCHREUDER 1956). In doing so for azide a difference was found that may be interpreted in the sense meant by Dr. Sutcliffe.

METHOD

The method of investigation links up with that of previous publications, especially that by ARISZ and SCHREUDER (1956 a and b).

The first series of experiments was carried out in 1953 on plants grown in tap water in the daylight. In later years material was used, grown in artificial light in water that had been made free of salt by ion exchange resins. The plants were kept in a cellar in concrete tanks (110 by 110 cms surface and 50 cms depth), in soil mostly rich in organic manure. For the transport experiments leaf lengths were always used of 7.5 cms length and 4 mms width; 8 of such leaf lengths form a series of which after the uptake period of 24 hours, 3 series of 8 segments of 2.5 cms length were cut which were analysed for chloride. The difference in chloride per series, present at the beginning and at the end of the experiment gave the increase during the time

of experimenting. The leaf lengths were destructed in nitric acid with silver nitrate. Chloride was determined according to Volhard, van Slyke and Sendroy. With the experiments the numbers are given, because experiments with material of the same age and pre-treatment give analogous results. Seeing an average of two experiments was made per week, from the numbering of the experiments an impression may be obtained on the lapse of time between the different experiments.

During transport the leaves were in closed perspex boxes, divided into compartments. The first 2.5 cms, the absorbing zone, was put into contact with the salt to be taken up, which was administered either in agar (0,01 M KCl + $CaSO_4$) or in a solution (0,001 M KCl+ $CaSO_4$). The adjoining 5 cms, the free length was either entirely kept in distilled water or between agar strips or there was a separate compartment for the middle zone. This lay in a solution or between agar strips, to which, if needed, an inhibitor could be added. It was usually prevented that in two adjoining compartments a liquid was present in both. The Difco agar used was free of chloride. The experiments with operated leaves were made in the same way as mentioned by Arisz and Schreuder. In the greater part of the experiments Mrs. van der Helm–Schreuder lent her assistance. I am much indebted to her for the accurate determinations and the extraordinary skill in the treatment of the sensitive material, for which also in case of the operated leaves reliable and reproducible data were obtained.

Results

Before the influence of inhibitors on the transport is treated, the course of the transport as to time ought to be discussed. Here an experiment is being discussed on transport after 3, 6, 12 and 24 hours (Fig. 1). In the first three hours 90 μg Cl is accumulated in the

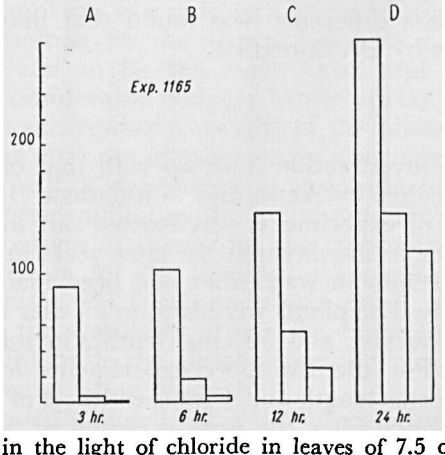


Fig. 1. Transport in the light of chloride in leaves of 7.5 cm length at 25° C. The increase of chloride after 3, 6, 12 and 24 hours for three segments of the leaves is estimated. The first zone is the absorbing zone. On the ordinate the amount present in the segments in μ g Cl.

absorbing zone, while but few chloride ions have been accumulated in the two transport zones. In the absorbing zone accumulation starts at once, the rate decreasing a little in the following hours; in the middle zone accumulation is first weak, increases constantly and does not reach its maximal rate before twelve hours have past. In the third zone hardly any accumulation can be demonstrated in the first three hours; during the next three hours it is greater and from 12 to 24 hours the accumulation in the second and third zones occurs at a fairly equal rate. In the absorbing zone the velocity is but little greater in that period. This course indicates that only after some hours the supply of chloride ions in the plasm is sufficiently great to permit of a maximal accumulation. It seems as if an induction period must elapse before the supply to each zone has reached a sufficiently high level. The length of this induction period depends on the distance over which the transport has to take place. A competition for chloride ions available for accumulation among the various zones is not to be observed in this series of experiments. This is connected with the strong uptake of ions into the symplasm in the light. ARISZ and SOL (1956) did find competition when the absorbing zone was in the dark.

The experiments made here on the influence of inhibitors on uptake and transport all lasted 24 hours. On the influence of inhibitors in shorter experiments and the reversibility of the inhibition experiments are desirable. Data on the influence of external factors on the uptake process for short periods are being gathered. Such data on the transport are much more difficult to obtain as the rate of transport has to be determined from data on the quantity of ions fixed by accumulation in various zones.

Influence of Sodium azide on the uptake and the transport of chloride ions

Experiments 852, 854 and 857 were made in 1953 on leaves, the absorbing zone of which (2.5 cms) was in the dark and the free leaf part (5 cms) exposed to light. From these experiments it appears that 1.5×10^{-4} M. azide added to the agar with KCl causes a considerable decrease in accumulation of chloride in the absorbing zone (Fig. 2). As is known from a publication by Arisz and Sol, uptake and transport in leaves exposed in this way will be comparatively weak, because in the dark the uptake into the symplasm becomes limiting for the accumulation in the various zones. Also when azide is added to agar in contact with the middle zone, an inhibition of the accumulation is obtained, but this continues more or less limited to the place of administration. As the middle zone does not take up the chloride ions direct from the medium, but via the symplasm from the absorbing part, this means that azide is capable of inhibiting the accumulation in the vacuole locally. The transport through the symplasm to the adjoining length in these experiments is but slightly inhibited by azide, sometimes not at all (exp. 854 B, 857 B). This means that in the zone treated with azide the transfer of ions in the symplasm continues in a normal way, while the active secretion process causing

accumulation in the vacuole is prevented. On administration of azide to the absorbing part the second and third zones forming together the free part can continue accumulating chloride (experiment 854) the uptake from the medium and the transport not being inhibited by azide. Sometimes (experiments 852 and 854) the secretion of

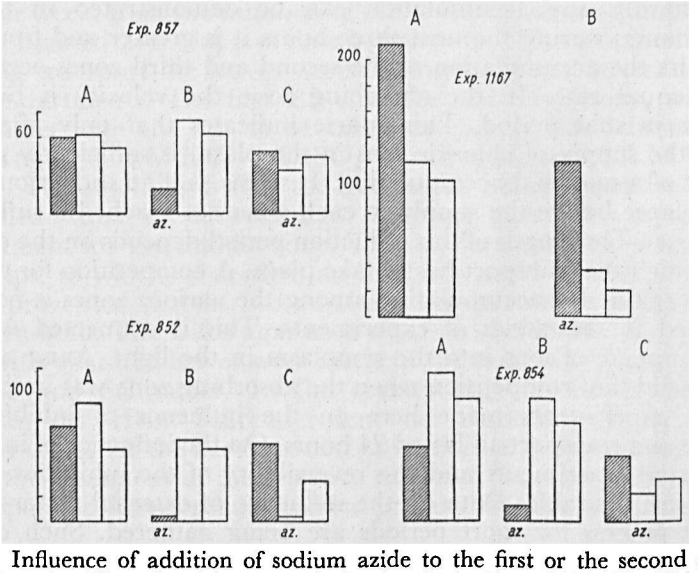
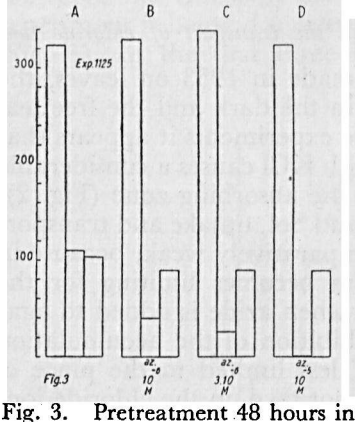


Fig. 2. Influence of addition of sodium azide to the first or the second zone of the leaflengths. The absorbing zone is in the dark. Exp. 857, 852, 854 uptake of 0.005 M KCl + $CaSO_4$ 1.5 × 10^{-4} M azide. Exp. 1167 0.001 M KCl + $CaSO_4$, 10^{-5} M azide. 24 hours 25° C.



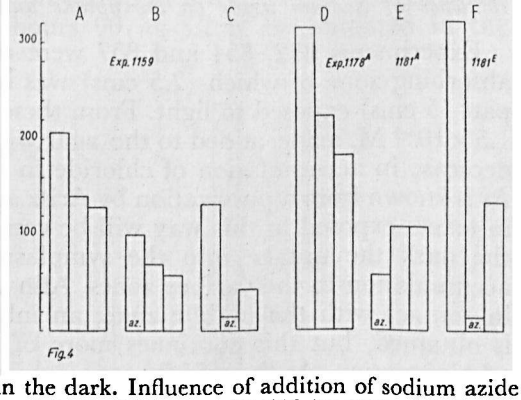


Fig. 3. Pretreatment 48 hours in the dark. Influence of addition of sodium azide in B, C and D to the middle zone of the leaf. Uptake of 1/100 M KCl with $CaSO_4$ by the absorbing zone. Conc. of azide in B 10^{-6} M in C 3.10^{-6} M and in D 10^{-5} M. Exp. 1125, 24 hours 25° C.

Fig. 4. Influence of addition of sodium azide in B and E to the absorbing zone in C and F to the middle zone. Pretreatment 24 hours in the dark. Uptake of 0.001 M KCl + $CaSO_4$ in the light. 10^{-5} M azide is administered to the absorbing zone in B and E, to the middle zone in C and F. Two experiments 1159 and 1178 + 1181.

chloride has slightly decreased in these zones. This indicates that azide can also influence the transport process direct or indirect. These experiments made in 1953 have been repeated in 1956 and 1957. The uptake and the transports were much greater in these experiments, owing to the plants now being grown in water poor in salt while the uptake into the absorbing zone occurred in the light except in experiment 1167 (Fig. 2), where azide was administered to the absorbing zone which was in the dark, and inhibited the accumulation in all zones, especially in the free part.

In experiment 1125 (Fig. 3) in which the entire leaf was exposed to light, administration of azide to the middle zone in increasing concentrations causes a stronger local inhibition of the accumulation, whereas the accumulation in the absorbing zone, the transport to and the accumulation in the third zone continue equally great. Here therefore the effect of azide is local.

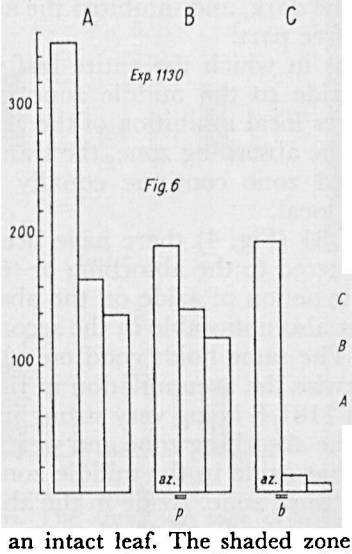
In experiments 1159 and 1181 (Fig. 4) there have been compared the influence of azide administered to the absorbing or to the middle zone. In these experiments the action of azide on the absorbing zone is not limited to this zone, but also noticeable in the second and third zones be it in a less degree. The same holds good on administration to the second zone, when likewise the accumulation in the third zone is inhibited. The inhibition in 1181 F being very strong in the middle zone, not noticeable at all in the absorbing zone and weak in the third zone it is obvious to assume that azide in the middle zone sometimes influences the transport to the third zone. Azide in the absorbing part may inhibit the transport to the second and third zones.

Transport by tissue bridges

As communicated in the introduction, some experiments have been made to trace whether azide affects the transport in the vascular bundles and in the parenchymacells. In a previous publication (Arisz and Schreuder) it was investigated along what paths transport of chlorides can occur in the *Vallisneria* leaf. Just as in previous experiments (Arisz and Oudman) on the transport of asparagine in these experiments the transport in the parenchyma or in the bundles was prevented by locally removing the parenchyma or the bundles over a distance of 4–16 mms (Fig. 5). As a rule for transport by parenchyma bridges (B), some parenchyma paths were left intact and all intervening bundles removed over a certain distance. In the bundle bridges (C) only the central vascular bundle was left intact, while over a distance of 4, 8 or 10 or 16 mms the tissue on both sides of this median bundle was removed over the entire width. Seeing that in doing so the median bundle had to remain intact as much as possible, some parenchyma cells in close contact with it, have not been removed, so that in case of a bundle bridge it cannot be said there was an exclusive transport through sieve tubes, though they will doubtless have provided the most important path. From the experiments by Arisz and Schreuder it is known that after a pretreatment of 24 hours after making the injury, the chloride transport has been

sufficiently recovered as well by a parenchyma bridge as by a bundle bridge. The one vascular bundle left is capable of an intensive transport. The zone of 4–16 mm length over which the operation extended was not included in the analysis of the intact leaves (A) either. Also in longer isolated bundles (8, 10 and 16 mms length) a but slightly weakened transport is to be shown (Fig. 10).

In experiment 1130 (Table 1 and Fig. 6) a normal transport (A)



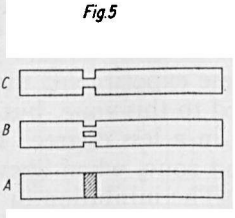


Fig. 5. A is an intact leaf. The shaded zone is not analysed. B two bridges of parenchyma cells. C one bridge of a bundle.

Fig. 6. Influence of azide administered to the absorbing zone in B and C on the transport. In B a bridge of parenchyma p, in C a bundle bridge b. Exp. 1130, 24 hours, 25° C.

TABLE I

Influence of sodium azide on the uptake and transport of Cl in intact leaves, in leaves with a parenchyma bridge and in leaves with a bundle bridge. Length of the bridges 4 mm. I is the absorbing zone, II middle zone, III end zone of a leaf length. Each zone is 2,5 cm long and 0,4 cm wide. Amount of Cl in μg Cl/8 leaflengths 2.5 . 0,4 cm; 25° C. 24 hours. Exp. 1130.

	Cl content in μg Cl present in zone			Cl increase in μg during uptake		
	I	II	III	I	II	III
After pretreatment in water	263 absorbing zone	270 middle zone	— end zone	—	—	—
after uptake of:				—	—	—
0,001 M KCl + $CaSO_4$ . . .	618	433	405	351	166	138
idem + 10^{-5} azide	454	398	383	187	131	116
idem + 10^{-5} azide . . . . (parenchyma bridge)	462	419	390	195	152	123
idem + 10^{-5} azide	447	277	263	180	10	— 4
idem + 10^{-5} azide . . . . (bundle bridge)	476	284	284	209	17	17

has been compared with the effect of azide on a transport through parenchyma bridges, in which the bundles have been removed over a 4 mm distance (B) and on a transport by one median isolated bundle, the tissue on either side of this bundle being removed over a 4 mm distance, both bundles and parenchyma (C). The two series have been carried out in duplicate. It is a striking fact that in C the transport via the bundle bridge is greatly decreased by azide, whereas that via the parenchyma in B is hardly reduced. This follows from the slight accumulation of chloride in the cells of the free length in C. The parenchymatous transport is apparently not being inhibited by azide, but the transport via the sievetubes is. This phenomenon will be referred to as the "Sutcliffe effect" (cf. p. 3).

In experiment 1134 with parenchyma bridges and 1135 with bundle bridges this phenomenon has been further investigated. In experiment 1134 Fig. 7 A is an intact leaf, B a leaf where at 25 mms distance from the one end the bundles have been removed over a distance of 4 mms

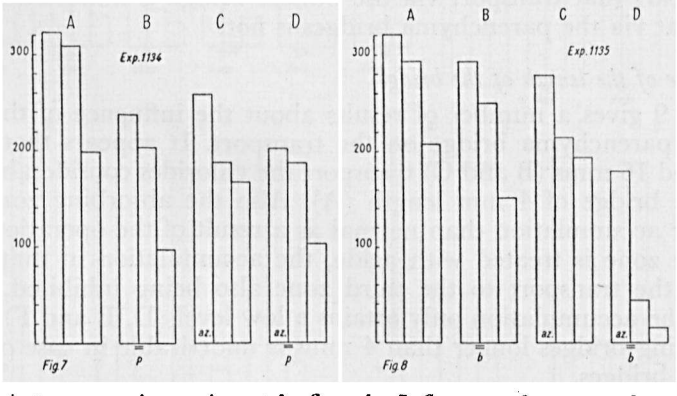


Fig. 7. A transport in an intact leaflength. Influence of a parenchyma bridge on the Cl transport. (B and D). In C and D 10^{-5} M azide is administered to the absorbing zone. Length of the bridges 4 mm. 24 hours, 25° C. uptake of 0.001 M KCl + $CaSO_4$ in the light. Exp. 1134.

Fig. 8. The difference with fig. 5a is that the bridges of B and D are bundle bridges. Exp. 1135, 24 hours, 25° C.

so that transport in the bridges only occurs through parenchyma cells. C is an intact leaf treated with 10^{-5} M sodium azide to the absorbing part. The absorbing part in D has likewise got azide, the bundles being removed over a distance of 4 mms. The operation zone of 25 to 29 mms has not been included in the analysis neither in A, nor in B, C and D.

After 24 hours an increase in chloride is found, indicated in Fig. 7. In agreement with previous results (Arisz and Schreuder) it appears that parenchyma bridges of 4 mm length permit of a proper transport of chloride ions. Owing to the operation the accumulation in B in all three zones is weaker than in the intact leaf. In C azide gives a slight local inhibition in the absorbing zone of the intact leaf, the transport to the free leaf part being only slightly affected. If the

transport to the free part occurs via parenchyma bridges (B and D) administration of azide to the absorbing part has only a slight influence.

The result of experiment 1135 (Fig. 8), where the transport via the zone of 25 to 29 mms occurs through one isolated bundle only, is different. The transport through the isolated bundle in series B is almost equally strong as the one in the intact leaf. If one considers that here mainly the sieve tubes are active, these elements must permit of a comparatively stronger transport than the parenchyma cells.

Azide added to the absorbing part gives a slight inhibition in all zones in series C in comparison with series A. It is a striking fact that in series D where beside the inhibition by azide in the absorbing zone transport must moreover take place through one bundle bridge, just like in Fig. 6 C, the transport to the free part is greatly inhibited, as appears from the slight accumulation. From these experiments it appears that transport via the bundle bridge is inhibited by azide but that via the parenchyma bridges is not.

Influence of the length of the bridge

Fig. 9 gives a number of results about the influence of the length of the parenchyma bridge on the transport. It appears that bridges of 8 and 16 mms (B and C) transport the chlorides considerably worse than a bridge of 4 mm length (A). Also the absorbing zone has a weaker accumulation than normal as a result of the operation. If the middle zone is treated with azide, the accumulation in that zone is weak, the transport to the third zone also being inhibited, so that there the accumulation only attains a low level (D, E and F). Therefore using bridges longer than 4 mms is undesirable in case of parenchyma bridges.

Contrasted with this is the effect of a longer bundle bridge (Fig. 10). Still with an 8 mm bridge there is a fairly normal transport to the free part. The accumulation in the absorbing part continues quite normal.

From experiment 1146 (Fig. 11) it may be seen that the middle zone to which azide has been administered, has a slight accumulation while both at 4, 8 and 16 mm bundle bridges the transport to the third zone and the accumulation there is equally great or even a little greater than in the intact leaf. Here therefore in spite of addition of azide to the middle zone, transport from the second to the third zone occurs with an unaltered strength. It cannot be indicated here whether this transport occurs in the symplasm or in the bundle.

Experiment 1151 (Fig. 12) gives the influence of azide in leaflengths with a bundle bridge of 10 mm length. The greater length of the bridge enables us to locally administer azide, besides to the absorbing part (B) and to the middle zone (D), also to the 10 mm bridge (C).

In series C azide, locally administered to the isolated bundle causes only a slight decrease in accumulation in the adjoining middle zone, the third zone having an almost normal chloride accumulation.

If, however, in series B azide is administered to the absorbing zone, the transport to the second and third zones is considerably inhibited ("Sutcliffe effect"). Azide added to the middle zone (D) inhibits the accumulation in this zone, the accumulation in the third zone being lower than without azide (A) in this case.

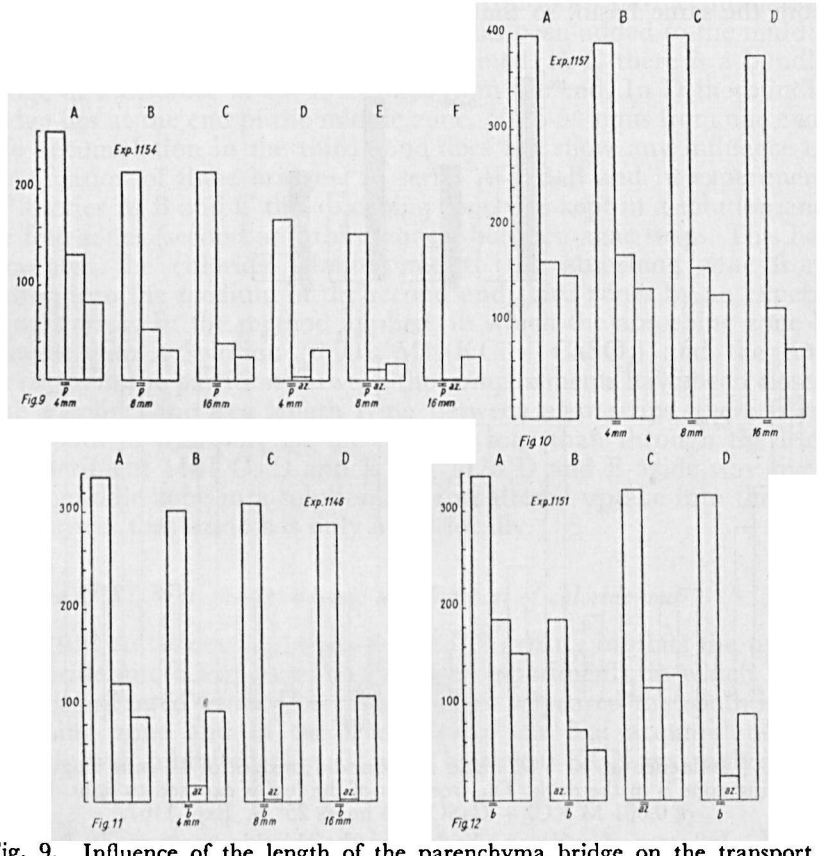


Fig. 9. Influence of the length of the parenchyma bridge on the transport of chloride. In A and D 4 mm, in B and E 8 mm, in C and F 16 mm length. In D, E and F 10^{-5} M azide is administered to the middle zone. Uptake of 0.01 M KCl + $CaSO_4$ in the light; 24 hours, 25° C. Exp. 1154.

Fig. 10. Influence of the length of the bundle bridge on the transport of chloride. In B 4 mm, in C 8 mm and in D 16 mm. Uptake of 0.01 M KCl + $CaSO_4$ in the light 24 hours, 25° C. Exp. 1157.

Fig. 11. The difference with fig. 10 is the administration of azide 10^{-5} M to the middle zone in B, C and D. Exp. 1146.

Fig. 12. Influence of bundle bridges of 10 mm length and azide on the transport of chloride. In B azide is administered to the absorbing zone, in C to the isolated bundle bridge and in D to the middle zone. Uptake of 0.01 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1151.

An experiment in which the absorbing zone was in the dark, gave the same results. In experiment 1167 (Fig. 13) the absorbing zone is in the dark and the rest of the leaf in the light. Azide administered to

the absorbing zone (B) again gives inhibition of the accumulation in all leaf zones. In the presence of a bundle bridge the effect is fairly equal.

Experiments 1178 and 1181 (Fig. 14, 15) are supplementary. They have been made in successive weeks on particularly good material from the same basin, so that the absorbed quantities of chloride are

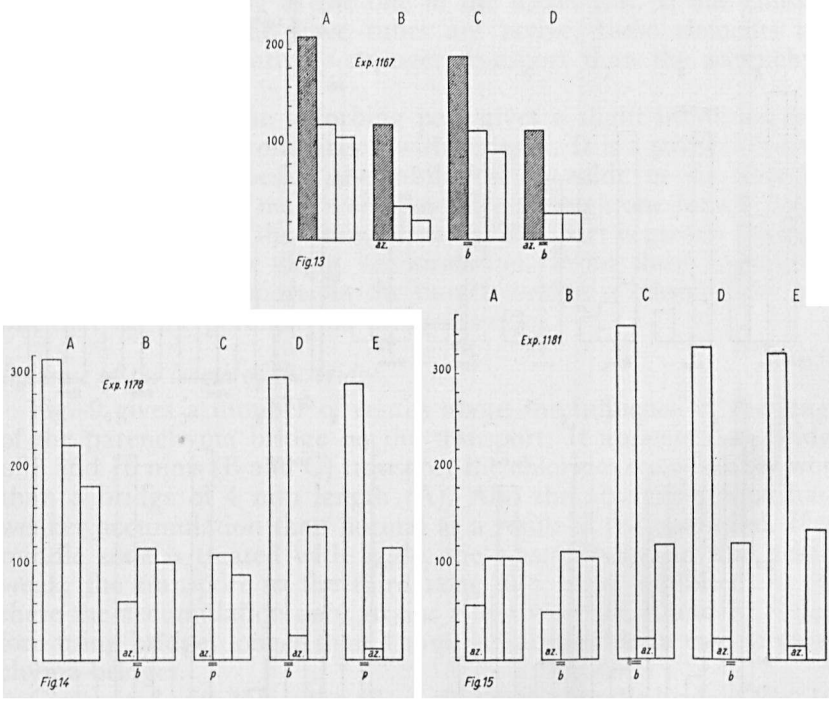


Fig. 13. Influence of 10^{-5} M azide and bundle bridges of 10 mm length. The absorbing zone is in the dark. The free part of the leaf is exposed to light. Uptake of 0.001 M KCl + $CaSO_4$, 24 hours 25° C. Exp. 1167.

Fig. 14. Influence of azide and bridges on the chloride transport. In B and D bundle bridges of 4 mm length, in C and E parenchyma bridges of 4 mm length. Azide is administered to the absorbing zone in B and C, to the middle zone in D and E. In this experiment young *Vallisneria* leaves were used. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1178.

Fig. 15. Influence of azide on the transport through bundles. In A and B azide is administered to the absorbing zone, in C, D and E to the middle zone. A 4 mm bundle bridge is situated in B and C between the absorbing and the middle zone, in D between the middle and the third zone. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1181.

comparable and the experiments supplementary. In experiment 1178 the azide inhibition in bundle- and parenchyma bridges of 4 mm length has been compared. In B and C azide has been given to the absorbing length, in D and E to the middle zone. In this experiment the nature of the bridge has no influence. Azide in the absorbing zone also inhibits the accumulation in the other zones. When azide is

administered to the middle zone the inhibition of the accumulation in the adjoining zones is slight (also confer experiment 1181 C and E).

In experiment 1181 azide administered to the absorbing zone (A and B) causes inhibition of the accumulation in all zones. A is a transport inhibited by azide without operation, in B a bundle bridge is present. The latter does not influence transport and accumulation in the free length. In C, D and E azide has been added to the middle zone, in E no operation has been performed, in C there is a bundle bridge at a distance of 25 to 29 mms from the end. In D the bundle bridge lies at the end of the middle zone, 50 to 54 mms from one end. The accumulation in the third zone does not show any influence of the situation of these bridges. In series A and B and in experiment 1178 series A, B and C the absorbing zone was kept in a solution and the free zones (second and third zones) between agar strips. This has prevented the chloride administered to the absorbing zone from getting into the medium of the second and third zones by an experimental error. In the method applied, in which the absorbing zone is brought into a solution (0,001 M) ($KCl + CaSO_4$) and the tiny apertures in the partition between the compartments have been closed with vaseline, the free length lying between agar strips, there is no other path of transport for the chloride ions than through the leaf. In experiment 1181 C, D and E and 1178 D and E azide was given to the middle zone in a solution. The unaltered uptake into the first zone proves that azide has only acted locally.

Influence of KCN on the uptake and the transport of chloride ions

In 1953 Arisz demonstrated that KCN greatly inhibits the uptake of chloride into a leaf zone. In transport experiments, in which KCN was administered to the absorbing zone, it appeared that both in the absorbing zone and in the free part of the leaf accumulation of chloride was inhibited (Fig. 16). If, however, KCN was only given

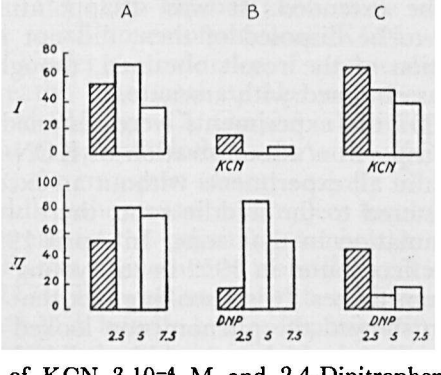


Fig. 16. Influence of KCN 3.10^{-4} M and 2.4-Dinitrophenol 10^{-4} M on the uptake and the transport of chloride. In all experiments absorption of 0.01 M $KCl + CaSO_4$. The absorbing zone is in the dark and the free part in the light. (From Arisz 1956).

to the middle zone, neither the uptake nor the accumulation of chloride was inhibited in this zone. From these data important conclusions could be drawn. The lack of influence of KCN administered to the middle zone shows that the accumulation of chloride ions is not inhibited by KCN, the transport experiencing no influence either.

The strong inhibition of uptake and transport, when KCN is administered to the absorbing zone, can therefore not be based on a local inhibition of the accumulation and inhibition of the transport from cell to cell. The accumulation of chloride ions being inhibited both in the absorbing zone and the free zone, this must be due to an inhibition of the uptake of ions into the symplasm.

If a transport of chloride takes place outside the plasm via the cell walls of the absorbing zone to those of the free zones, it would be expected that also on administration of KCN to the absorbing zone this wall transport would continue. KCN being only administered to the absorbing zone, the free leaf part would take up this chloride supplied by the cell walls into the symplasm and accumulate it in their vacuoles. It appears, however, that this does not occur. This speaks against chloride transport via cell walls outside the plasm.

This also appears from the continuation of the transport to and the accumulation in the middle zone on local administration of KCN to this zone. For, KCN administration to the middle zone would certainly have inhibited the uptake of chloride supplied via the cell walls into the plasm of this zone, just as it does in the absorbing zone. Also the accumulation in the free part not inhibited by KCN indicates that no transport of chloride ions takes place from the medium solution through the cell walls to the free zones. This is the physiological proof that transport from cell to cell must take place through a symplasm. The symplasmatic transport and the accumulation in the vacuole is not influenced by KCN in this experiment, whereas the uptake into the symplasm is.

It was desirable that the small number of experiments carried out in 1952 under particularly favourable conditions with perfect *Vallisneria* material should be extended. It was disappointing that the less resistant material to be disposed of then, did not permit of a conclusive corroboration of the result obtained, though in a few cases a similar effect was obtained with arsenate.

In 1956 and 1957 the experiments were resumed. The inhibition of uptake and transport on administration of KCN to the absorbing part occurred then in all experiments without an exception, but when KCN was administered to the middle zone, there was a pronounced decrease in accumulation in that zone. In April 1957 we succeeded in recovering the effect found in 1952 in still young plants cultivated in favourable circumstances. It is a striking fact that only plants from one concrete basin showed the phenomenon looked for, while plants from an adjoining basin which were older, did show inhibition of accumulation by KCN in the middle zone. It has repeatedly appeared in the experiments made with *Vallisneria* during these years that the previous history and the age of the plants is determining for the way

in which the biochemical processes of uptake and secretion of chlorides progress. With various results obtained with different material it cannot be said that one result is correct and the other wrong, but both may be right, but only hold good for material that underwent a special pre-treatment. The material of the south east basin reacted in 1957 not only on KCN in the way discussed (Fig. 19), but completely analogously on uranyl nitrate (Fig. 26, 27), and arsenate (Fig. 24). On the contrary azide, as already discussed (Fig. 15) did give with this same material an inhibition of the accumulation on administration to the middle zone, but no "Sutcliffe effect". So azide and KCN react on the same material with a different effect. Experiments simultaneously made with material from the south west basin which was older, did give an inhibition of the accumulation in the middle zone with KCN.

Though both results with KCN are correct, the behaviour rediscovered in 1957 is of a particular theoretical importance, because in this case KCN only inhibits the uptake of ions into the system and not the accumulation in the vacuole, which proves that the transport of ions does not occur in the walls, but in the symplasm.

We have distinguished wall and plasm as separate paths of transport. Of course it is conceivable that especially in younger walls such a distinction is not permitted, as in these there is also cytoplasm in the wall. So a safe conclusion is that a wall transport not controlled by the cytoplasm, is not present in *Vallisneria*.

We will now discuss the experiments with KCN, arsenate and uranyl nitrate separately.

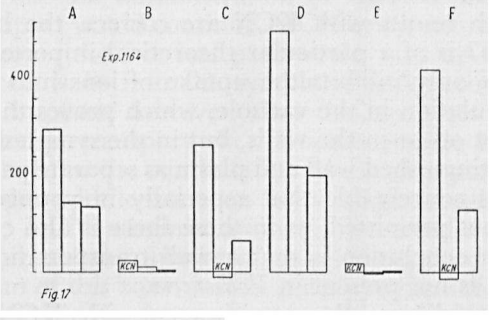
Experiments with KCN

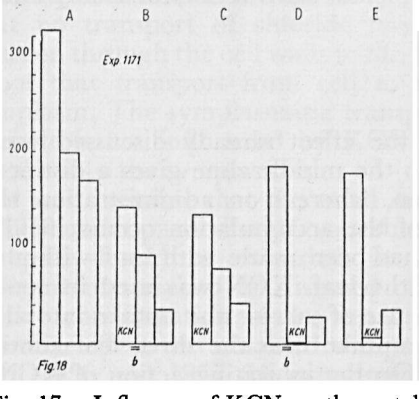
In experiments 1164 and 1171 the effect already discussed was obtained that KCN administered to the middle zone gives a distinct local inhibition of the accumulation, whereas on administration to the absorbing zone an inhibition of the accumulation occurs in all zones. Experiment 1164 (Fig. 17) has been made with and without addition of sucrose to all zones of the leaf. KCN was used in concentration 10^{-5} M. Under the influence of sucrose the uptake into all zones increased greatly. The total uptake into the three leaf zones increases from 575 to 915 μgr. Cl. On the inhibiting action of KCN sucrose addition has no specific influence. At this KCN conc. (pH 7) the uptake is slight, the transport almost entirely inhibited. Addition of KCN to the middle zone causes a complete local inhibition of the accumulation, whereas in the adjoining zones the accumulation is slightly inhibited. To this phenomenon we will revert in the discussion.

In experiment 1171, likewise made with addition of sucrose and a KCN conc. 3.10^{-6} M a bundle bridge is present in B and D. From Fig. 18 it appears that these bridges have hardly any perceptible influence on the inhibition by KCN and the accumulation in the free part. Here too (D and E) administration of KCN to the middle zone causes a strong local inhibition and at the same time a decrease in

accumulation in the two adjoining zones. Any effect due to the isolated bundle bridge is not present.

Experiment 1176 (Fig. 19) gives the result of administration of KCN 5.10^{-6} (pH 7) to leaflengths with isolated bundle or with parenchyma bridges. As a rule the accumulation is a little weaker in the case of parenchyma bridges. The bridges always have a length of 4 mms. In these experiments the perspex boxes were divided into three compartments by partitions so that KCN could only act on one certain zone. It is astonishing that in spite of the presence of large intercellular spaces in the leaf, the action of KCN is so strongly localised to the spot where it has been administered. In this experiment a series of leaves is omitted in which KCN has been administered,





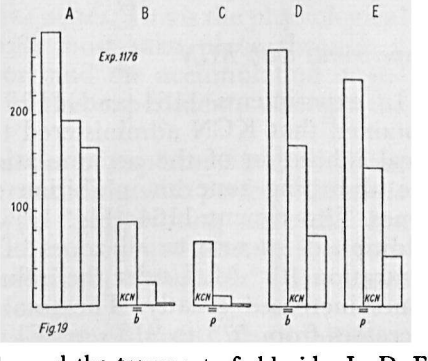


Fig. 17. Influence of KCN on the uptake and the transport of chloride. In D, E and F sucrose 0.05 M is administered to all zones of the leaves. In B and E KCN 10^{-5} M administered to the absorbing zone, in C and F to the middle zone. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1164.

Fig. 18. Influence of KCN on the uptake and the transport of chloride. In B and C KCN is administered to the absorbing zone, in D and E to the middle zone. In B and D bundle bridges of 10 mm length. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1171.

Fig. 19. Influence of KCN on the uptake and the transport of chloride. KCN 5.10^{-6} M, pH 7 is administered to the absorbing zone in B and C, to the middle zone in D and E. In B and D a 4 mm bundle bridge, in C and E a 4 mm parenchyma bridge. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1176.

but no operation has been performed. As a result it cannot be said in this case whether the difference in accumulation of the first and third zones in D and E with respect to A is due to the operation or to a KCN influence.

It clearly appears from D and E that KCN administered to the middle zone gives hardly any inhibition of the accumulation in that zone. This is in contrast with the two preceding experiments and corresponds with the result obtained in 1952. To be sure this experiment was more successful than the one in 1952, because uptake and transport are much greater here. In this experiment with plants normally treated the total uptake of chloride was four times greater than in 1952. This is due both to the way of exposing to light and to the cultivation of the material.

Experiments with arsenate

A number of experiments have been carried out with arsenate; some with uptake in the dark and transport zones exposed to light will be discussed here first. Experiment 845 (Fig. 20) gives the same picture for arsenate as figs. 16 and 19 for KCN, i.e. arsenate does not inhibit when administered to the middle zone. Experiments 858 and 1063, however, give the deviating behaviour that arsenate also acts inhibitingly in the middle zone on the accumulation in that zone.

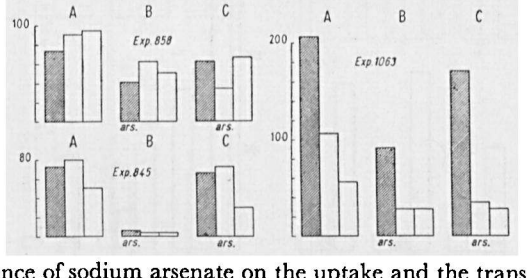


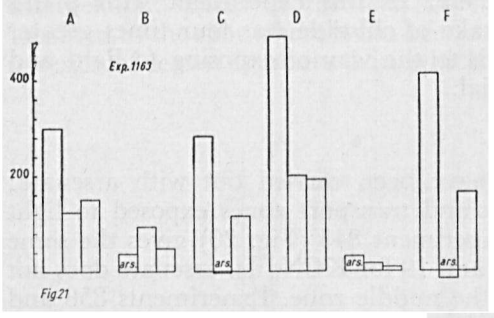
Fig. 20. Influence of sodium arsenate on the uptake and the transport of chloride. Uptake of 0.005 M KCl + $CaSO_4$ in exp. 858 and 845 and of 0.01 M KCl + $CaSO_4$ in exp. 1063, 25° C. Conc. of arsenate in all experiments 10^{-5} M. The absorbing zone is in the dark, the free part in the light.

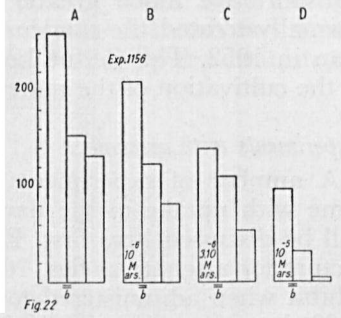
Experiment 1163 (Fig. 21) on the inhibition with arsenate has been made simultaneously with experiment 1164 (Fig. 17), in which KCN was administered. The right of the figure (D, E and F) contains a repetition of the experiment with the only difference that sucrose was added to all zones. The results of Fig. 21 and Fig. 17 are strikingly identical. In both administration of sucrose increases uptake and transport, the inhibitions continuing to be equal.

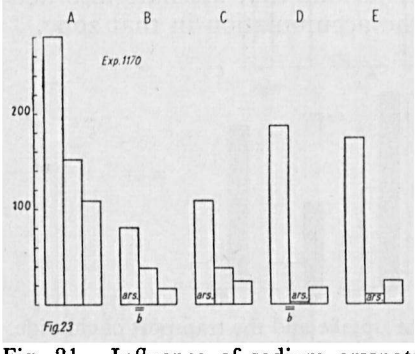
Experiment 1156 (Fig. 22) gives the results of various concentrations of arsenate in operated leaves. In all leaf lengths there is a bundle bridge of 10 mm length. Arsenate has always been administered to the absorbing zone. It inhibits the accumulation in all zones proportional to the concentration.

In experiment 1170 (Fig. 23) the influence of a 10 mm bundle

bridge has been investigated, arsenate in a 3.10^{-6} M conc. being given to the absorbing zone in series B and C or to the middle zone in series D and E. There is no specific influence of the bundle bridge, the inhibition by arsenate being in entire correspondence with those in the previous experiments. It is a striking fact that arsenate in D and E has not only got a local effect on the accumulation in the middle zone, but also clearly inhibits the accumulation in the first and third zones. Inhibition of the accumulation in the first zone does not occur in all experiments. In experiment 1177 (Fig. 24) with arsenate as an







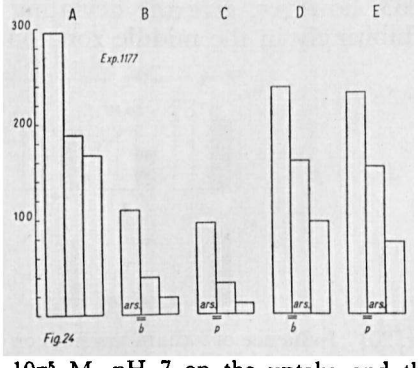


Fig. 21. Influence of sodium arsenate 10^{-5} M, pH 7 on the uptake and the transport of chloride. In D, E and F sucrose is administered to all leaf zones. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1163.

Fig. 22. Influence of different conc. of sodium arsenate. In all series a bundle bridge of 10 mm length. Conc. arsenate in B 10^{-6} M, in C 3.10^{-6} M, in D 10^{-5} M. Exp. 1156.

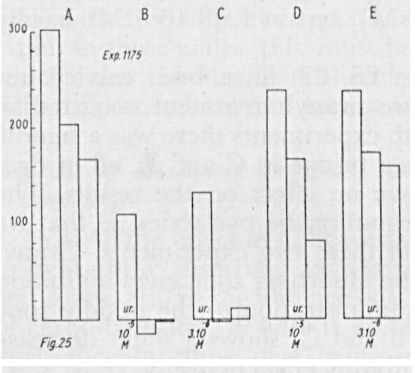
Fig. 23. Influence of sodium arsenate 3.10^{-6} M, pH 7. In B and D bundle bridges of 10 mm length. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1170.

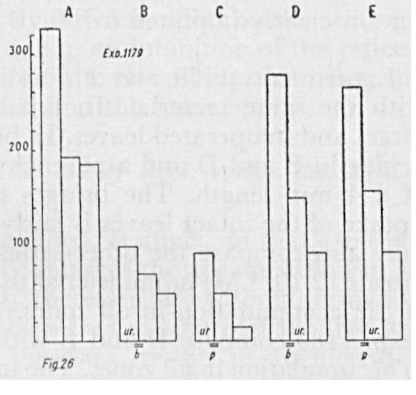
Fig. 24. Influence of sodium arsenate 5.10^{-6} M on uptake and transport of Chloride. In B and D a bundle bridge of 4 mm length, in C and E a parenchyma bridge of 4 mm length. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1178.

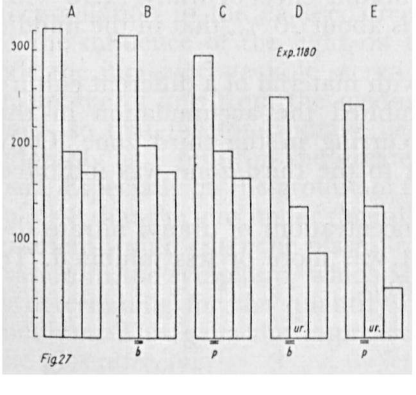
inhibitor which was carried out simultaneously with experiment 1176 (Fig. 19) with KCN as an inhibitor on material from the same basin (south east basin), a perfectly identical effect was found for arsenate as has already been discussed for KCN. Here too arsenate administered to the absorbing zone gives inhibition of uptake and transport, whereas it gives hardly any inhibition, if administered to the middle

zone. These effects are equal for bundle and parenchyma bridges. The slighter accumulation in the third zone will presumably be connected with the operation.

It should be mentioned here that in some experiments the effect of arsenate was very weak. This was likewise the case in some uptake experiments with arsenate on 2.5 cm leaf lengths. Such a lack of effect of arsenate is to be expected, when the material is rich in phosphate. Addition of phosphate to the medium also prevents the inhibition by arsenate. In agreement with this is the fact that the uptake of phosphate in *Vallisneria* is not inhibited at all by low arsenate concentrations.







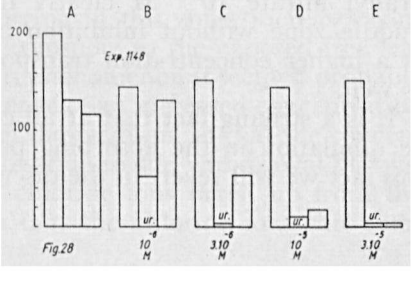


Fig. 25. Influence of uranyl nitrate on uptake and transport of chloride. In B and D a conc. of 10^{-5} M uranyl nitrate, in C and E 3.10^{-6} M. The inhibitor is administered in B and C to the absorbing zone, in D and E to the middle zone. Uptake of 0.001 M KCl + Ca sulfate, 25° C. Exp. 1175.

Fig. 26. Influence of uranyl nitrate 2.10^{-6} M on uptake and transport of chloride. In B and D a bundle bridge of 4 mm length, in C and E a parenchyma bridge of 4 mm length. Uptake of 0.001 M KCl + Ca sulfate 25° C. Exp. 1179.

Fig. 27. Uranyl nitrate administered to the middle zone in D and E. B and D with bundle bridges (4 mm length). C and E with parenchyma bridges (4 mm length) Exp. 1180 with same material as 1179.

Fig. 28. Influence of uranyl nitrate administered in the middle zone on different concentrations. Uptake of 0.01 M KCl + Casulfate, 25° C. Exp. 1148.

Experiments with uranyl nitrate

Some experiments have been made on the inhibition by uranyl nitrate. A few were carried out on the same material as experiment 1177 with arsenate and 1176 with KCN. They gave identical results.

Experiment 1175 is an experiment with intact leaf-lengths and two different concentrations of uranyl nitrate 10^{-5} M and 3.10^{-6} M (Fig. 25).

Uranyl nitrate administered to the absorbing leaf zone inhibits the accumulation in this zone and prevents the transport to the free leaf part. Uranyl nitrate administered to the middle zone does not influence the accumulation in that zone. In D and E the accumulation in the absorbing zone shows a very slight inhibition; that in the third zone is clearly inhibited in D (10^{-5} M), but in E (3.10^{-6} M) hardly at all.

Experiments 1179 and 1180 (Fig. 26, 27) have been carried out with the same material. In this case uranyl treatment occurred in intact and in operated leaves. In both experiments there was a bundle bridge in B and D and a parenchyma bridge in C and E, all bridges of a 4 mm length. The bridges have no effect on the results. The uptake of the intact leaves is fairly equal in the two series, so that we may also compare the other series of these two experiments. Uranyl nitrate (2.10^{-6} M) administered to the absorbing zone gives inhibition of the accumulation in all zones. Administration to the middle zone (exp. 1180 compare D and E with B and C) shows a slight decrease in accumulation in all zones. The inhibiting effect of uranyl (2.10^{-6} M) administered to the absorbing zone is about 70 %, that in the middle zone about 20 %.

In experiment 1148 (Fig. 28, B) with material of a different culture uranyl nitrate 10^{-6} M clearly inhibited the accumulation in the middle zone without inhibition occurring in the third zone. Only at a higher concentration transport to the third zone was inhibited as well.

It is a striking fact that at all concentrations of uranyl nitrate the accumulation in the absorbing part was more or less inhibited. To this fact we will revert in the discussion.

Discussion

From the researches on *Vallisneria* leaves it has appeared that the uptake of salts consists of a number of processes in which the ions first enter the plasm, are transported there over a longer or shorter distance and are finally accumulated in parts of the plasm or in the vacuoles.

When we compare uptake processes in cells with and without vacuole, we should realise that on uptake into a cell without vacuole we only think of uptake into and binding to the plasm, whereas in a cell with vacuole uptake often implies accumulation in the vacuole. The two processes might progress more or less separately.

Separation of the uptake into the symplasm from the secretion into the vacuole

The principle of the analysis of the uptake processes carried out on *Vallisneria* is based on transport experiments, in which the influence of an exterior factor on the accumulation in a zone which takes the chloride ions direct from the medium, is compared with the one in an adjoining part which has to obtain the ions from the absorbing part. In both zones accumulation in the vacuoles takes place, but only in the absorbing zone the ions from the medium are taken up into the plasm. By exposing both or one of the zones to the light or by allowing inhibitors to act on these zones, it is possible to obtain valuable data on the processes concerned in uptake and secretion. If for instance an inhibitor on administration to the absorbing zone and to the free zone has a qualitatively different effect on the accumulation in those zones, this must be due to an inhibition of the process which takes the ions from the medium into the plasm, this being the only difference between the absorbing and the free zone.

Analysis of the uptake processes with the aid of light, carbon dioxide and wounding

In 1948 the effect of light was examined. It appeared that exposure to light of the absorbing zone greatly enlarges the accumulation in all leaf zones, also in the absence of CO_2, therefore also without formation of carbo-hydrates. Besides it was shown that exposure of the free leaf part, the absorbing part being in the dark, results in an increased accumulation in the exposed free part. On the one side this points so the influence of the light on the vacuole secretion, on the other tide the increased vacuole secretion at the same time requires a rise in uptake of ions from the medium into the plasm of the absorbing zone, so that the total uptake occurring in this zone, increases considerably as a result of the greater transport to the exposed free leaf part. Especially on the ground of this phenomenon it seemed probable in 1948 that the ions under the influence of an increased concentration gradient would enter the plasm from the medium, so that the concentration in the symplasm, which is low due to the withdrawal of ions, is determining for the quantity of chloride ions taken up from the medium. This gave the impression that the chloride ions can enter the plasm freely.

Besides it was pointed out in 1948 that ion transport takes place in the plasm. After the cutting and wounding of the leaf material to be used for the experiments, the capacity of taking up ions and accumulating them is decreased for some time, but it has already recovered after a few hours. The transport, however, to the free leaf part continues to be considerably weakened for many hours, so that not until 24 hours have passed a normal transport is found. This great sensitiveness indicates that the transport is connected with the plasm. The accumulation in the vacuoles seems to be an irreversible process, the tonoplast almost entirely preventing a leakage of salts from the vacuole under normal circumstances. From redistribution experiments

(Arisz 1954) it has appeared that a slight decrease of accumulated salts may occur, but that under normal conditions these ions do not go to the medium but are transported in the symplasm and thence may be accumulated into the vacuoles or in the cytoplasm.

It is necessary to point out that in less resistant leaves it repeatedly occurs that already on change of the outer solution ions are excreted. It is, however, of essential importance that in resistant material also after a long period of uptake there is no loss of the absorbed chloride ions. Comparatively slight changes in structure of the plasm seem to create the possibility for exosmosis. So in 1957 during some weeks in probably less resistant material under the influence of a treatment in water purified with ion exchangers a great loss of ions was obtained.

On the loss of asparagine various data were already given before (ARISZ 1943). As this publication appeared in Dutch in the years of the second world war, we copy a few results rendered in some two figures. Figure 29 gives the result of transfer after uptake for 24 hours

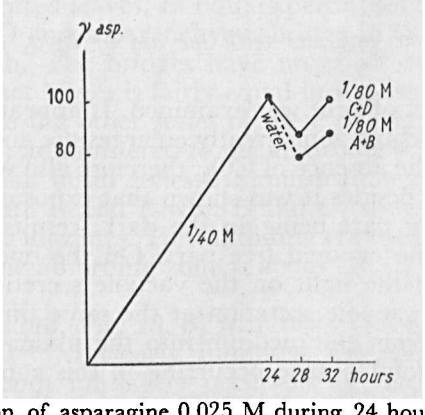


Fig. 29. Absorption of asparagine 0.025 M during 24 hours; afterwards 4 hours in water (A and B) or in 0.0125 M asparagine (C and D). The following 4 hours all series in 0.0125 M asparagine solution.

in a 1/40 M asparagine solution into a different asparagine solution (8 hours in 1/80 M asp.: C and D; 4 hours in water and next 4 hours in 1/80 M asp. solution: A and B).

In fig. 30 the leaves have been taken from a 1/40 M asp. solution to asparagine solutions of various strength after a 24 hours' uptake. Owing to the transfer to a different solution the permeability increases, which is shown as exosmosis or endosmosis dependent upon the concentration gradient between medium and cell sap. After some time the active uptake is resumed. These data indicate that the structure of the plasmatic membranes is not stable and it is influenced by the composition of the medium solution. After some hours an autonomous recovery takes place after which the active uptake is resumed. It may be somewhat upexpected that this lability also holds good for the tonoplast. This, however, must be the case, because Arisz and van Dijk (1939) showed that the asparagine was really

taken up into the vacuole and in the exosmosis returns to the medium for the greater part unaltered. This behaviour indicates that the loss of ions is determined by the change in structure of the cytoplasm and that only so long this structure is intact, the normal uptake into and accumulation in the vacuole can take place.

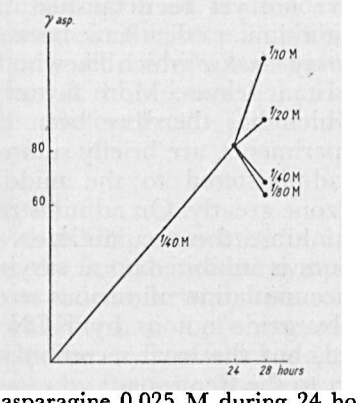


Fig. 30. Absorption of asparagine 0.025 M during 24 hours, afterwards 4 hours in asparagine solutions of different strength.

Analysis of the uptake processes with the aid of inhibitors

In 1953 the influence of inhibitors was investigated. It was ascertained whether an inhibitor administered at the absorbing zone works differently from that in the free part of the leaf. It was found that KCN administered to the absorbing zone can fully inhibit the uptake into the plasm and the accumulation in the vacuoles of the whole leaf. Administered in equal concentration to a zone of the free leaf part, KCN caused no inhibition of accumulation or transport. From this it was concluded that KCN inhibits the uptake into the symplasm and consequently both accumulation and transport to the free part become impossible. The experiments have already been discussed on page 13. Further conclusions inferred from these experiments in 1953 were the following: an ion transport outside the symplasm through the cell walls to the free zones does not occur in a demonstrable degree, because administration of a sufficiently strong concentration KCN to the absorbing zone fully inhibits the transport to the free leaf part and because administration of the same concentration KCN to the free leaf part does not lessen the accumulation. This should be the case if this transport took place through the cell walls outside the symplasm, so that the accumulation in a free zone should be preceded by an uptake from the cell walls.

The behaviour of KCN is the physiological proof of the symplasm theory of the transport, (Arisz 1956).

In 1953 it had likewise appeared from some preliminary experiments that 2,4-dinitrophenol inhibits the accumulation in *Vallisneria* leaves while the transport to other zones and the accumulation in the free leaf part are but slightly decreased by administration to the absorbing

leaf part. On continuing these experiments it was regularly found that 2,4-dinitrophenol inhibits the accumulation in the vacuole. Seeing, however, that this inhibitor at a higher concentration can injure the protoplasm, it cannot be said with certainty whether the uptake into the symplasm and the transport in the symplasm continue unaltered. The experiments have not yet been finished, and in the meantime other inhibitors giving a similar effect have been studied. (Cf. fig. 16B).

One of these is *hydroxychinoline* which likewise is slightly injurious to the plasm of less resistant leaves. More favourable were the results with *sodium azide*, which has therefore been extensively examined. The results of the experiments, are briefly summarised here.

Azide on being administered to the middle zone inhibits the accumulation in this zone greatly. On administration to the absorbing leaf part, it likewise inhibits the accumulation whereas the accumulation in the free zones is inhibited to a varying degree, sometimes hardly at all. This accumulation of various strength in the free leaf part indicates that by azide not as by KCN the uptake into the symplasm is inhibited, but the local secretion into the vacuoles and the chloride transport to the free zones.

Azide administered to the absorbing zone usually inhibits the transport through an isolated bundle bridge greatly ("Sutcliffe effect"). Transport by a parenchyma bridge is not inhibited. In the case of a bundle bridge the inhibition of the transport by azide administered to the absorbing part, is probably not based on a direct influence of azide on the transport in the sieve tubes of the bundle, as azide administered to an isolated bundle causes only a slight inhibition of the transport. It might therefore be that azide inhibits the process that takes the salts to the sieve tubes and not the transport itself. This might explain that in experiments with young material this vascular bundle inhibition has not occurred, because then the differentiation of the sieve tubes has not reached the same stage as in older material, so that they still behave as parenchyma cells.

The result of the new experiments with *KCN* was a corroboration of the results obtained in 1953, though the result that KCN does not inhibit the vacuole accumulation in the free leaf part was not always obtained. This effect is apparently influenced by the age of the leaves. In young leaves KCN does not give inhibition of the accumulation, which is proved, when it is administered to a zone of the free leaf part. In older leaves it does give a distinct inhibition of the accumulation. The young material which with KCN did not show inhibition of the vacuole secretion in the free leaf part, did not show it either with arsenate and uranylnitrate. Administered to the absorbing zone, these inhibitors always cause a strong inhibition of the accumulation in all zones of the leaf. This must be due to an inhibition of the uptake into the symplasm. Neither for KCN nor for arsenate and uranylnitrate a difference has been found between bundle bridges and bridges of parenchymatous tissue.

The corroboration of the former observations with KCN on the different reaction of the absorbing and the free zone with two other

inhibitors is of great value, because the significance of this fundamental result is considerably increased.

No more can there be any doubt of the former observation with 2,4-dinitrophenol that inhibition of the vacuole secretion may take place without the transport being inhibited, on the ground of the observations with administration of azide to the middle zone, in which the vacuole secretion in this zone is inhibited dependent upon the azide concentration used, while transport to the third zone continues unaltered (figs. 3, 11, 14 and 15). This is independent of the presence of the "Sutcliffe effect". (confer Fig. 21 with arsenate and Fig. 28 with uranyl.)

Nature of the uptake processes

The experiments with KCN, uranylnitrate and arsenate indicate that a process active in the uptake into the symplasm uses an energy-rich phosphate such as adenosinetriphosphate. Uranylnitrate and arsenate both influence the formation of energy rich phosphates, while KCN inhibits the cytochrome oxydase which is active in the production of energy rich phosphates by the mitochondria.

The experiments with inhibitors give the impression that KCN, uranylnitrate and arsenate do not inhibit the accumulation in the vacuole, if the material is still young, but they do, if it is older, whereas dinitrophenol and azide in our experiments always inhibit the vacuole accumulation. Considering our slight biochemical knowledge of the uptake processes, it is difficult to indicate what biochemical reaction this might be based upon. We might think of a surplus of energy rich phosphates present in the plasm in the so-called young, vigorously growing plants, so that inhibition of new formation of these substances cannot influence accumulation. Both for 2,4-dinitrophenol and for azide it is plausible that they do not act on the uptake into the cytoplasm. They always inhibit the accumulation in the vacuole. Even in the young material, in which KCN, uranyl nitrate and arsenate did not inhibit the vacuole secretion, azide did inhibit. In this young material, however, azide also behaved differently, in so far it did not give the specific inhibition of the bundle transport ("Sutcliffe effect"). All these experiments indicate the specific action of these inhibitors on certain biochemical processes.

We shall now revert to the interpretation of the uptake processes. The supposition made in 1948, viz. that chloride ions permeate freely in the plasm so that the vacuole secretion is the only active process in the uptake, had to be abandoned, when it appeared in 1953 that KCN inhibits the uptake into the symplasm. This means that both the uptake into the symplasm and the secretion into the vacuole are connected with metabolism and are processes requiring energy. In 1954 ARISZ considered the question whether the process of ions uptake may be active in that sense that anions are passed with difficulty through the plasm with its chiefly negative charge. The ion transfer might be made possible by a reaction that takes away the charge of the ion and may bind it to a carrier, after which the

complex can be moved by diffusion or the ions are transferred by an electron ladder system as supposed by Lundegårdh. In a similar system the anions will be present free or bound in the central plasm. If they are free they should be able to interchange with anions in the medium, if there is no outer membrane or if the outer membrane is permeable for anions.

Experiments carried out by the author with Winter (unpublished results) on uptake of labelled chloride ions gave as a result that after uptake there are no labelled chloride ions present in the plasm, which can be exchanged for unlabelled chloride ions in the medium. This means that if the outer membrane should be permeable for anions, no free or exchangeable chloride ions can be present in the plasm. Chloride ions once taken up into the plasm are no more released to the medium and are no more interchangeable with ions in the outer solution either. They are either separated from the medium by a semipermeable membrane impermeable for chloride ions or in case the membrane is permeable for anions they are irreversibly bound to carriers. The lability of the plasmatic structures which we have already mentioned before, particularly in respect of the asparagine uptake cannot elucidate whether the boundary surfaces of the plasm or the carriers themselves, which may be protoplasma molecules (Overstreet) cause the lability.

In 1948 ARISZ found a stronger uptake of ions from the medium by a non-exposed absorbing zone when the free part was exposed to light. By the stronger secretion into the vacuoles of the exposed free leaf part ions are withdrawn from the symplasm and this increased the uptake. In 1956 the effect of exposure of the absorbing zone was extensively examined by ARISZ and SOL. They found that light increases the uptake into the symplasm, because not only in the absorbing zone but also in the free zones the vacuole secretion greatly increases. So light acts stimulating both on the vacuole secretion and on the uptake into the symplasm. Such a stimulation of the uptake into the symplasm might be explained in various ways. We might consider an increase in permeability of the outer membrane, but this cannot fully explain the light effect, because the uptake is an active process. We may assume that owing to exposure to light more free energy becomes available for the uptake, which can be used for the ion transport direct or indirect. If in the ion transport the ions are bound to carriers the energy must be used either for this binding or for the formation of carriers. Arisz and Sol take it that the stronger uptake after exposure of the free leaf part is due to the formation of substances in the light which are transported to the absorbing zone and are capable of increasing the uptake there. Besides they found that on pre-exposure a transportable substance is formed which in a subsequent exposure is capable of rendering an increased uptake possible for several hours. The nature of these substances is still unknown. They will be called carriers here.

This leads to the question whether in the experiments of this investigation with the effect of inhibitors something has been observed

as to inhibition of the formation of these carriers. It might for instance be expected on administration of inhibitors to a middle zone that the accumulation in the absorbing zone would decrease, because now it is not strengthened by the supply of carriers from the middle zone. This is surely not the case for azide. Azide acts quite locally and the accumulation in the absorbing zone has rather increased than decreased in Fig. 3. Neither is there in Fig. 12, 14 and 15 a significant decrease in the accumulation in the absorbing zone.

With uranylnitrate (Figs. 25, 26, 27 and 28) there is always a distinct decrease in accumulation in the absorbing zone, when this inhibitor is administered to the middle zone. The differences, however, are not sufficiently significant to prove that the formation of these carrierlike substances is not inhibited by azide, whereas it is inhibited by uranylnitrate.

The transport of chloride ions

From an experiment on transport in the time (Fig. 1 and Table 2) it appeared that after a rapid accumulation in the absorbing part for the first 3 hours, the accumulation rate in the second period of 3 hours decreases greatly, while from 6–24 hours it takes place at a

TABLE 2

Rate of accumulation of chloride in μg/8 leaflengths 2,5 . 0,4 cm/3 hours. The first zone absorbs from a 0,001 M KCl + $CaSO_4$ solution, 25° C.

	1–3 hours	3–6 hours	6–12 hours	12–24 hours
First zone	90	14	21	34
Middle zone	5	14	18	23
End zone	0	5	11	23

rate which increases from 14 to 34 μgr. Cl per 20 cms leaflength, 4 mms width per 3 hours. The accumulation in the second and the third zone begins later; it is slow during the first few hours and attains a rate of 23 μgr. Cl in the second 12 hours. From this it appears that it takes a long time before a more distant zone has obtained the full rate of accumulation. The increase in rate indicates an induction process, but it does not yet give an insight in the nature of this process. The formation of carriers mentioned above might bring about such an induction. Apparently a state has been reached after 12 hours, owing to which the accumulation rate in the vacuoles limits the accumulation process. Anyway it is not determined by what has already been taken up into the vacuole.

In connection with the results communicated before (Arisz and Schreuder) the experiments with bridges have yielded some fresh data. The results are clear and corroborate that a narrow bundle bridge transports almost equally well as the intact tissue. It is a striking fact that the chloride ions are also transported well by parenchyma bridges. This indicates the possibilities of transport by ordinary parenchyma cells. Parenchyma bridges of over 4 mms length appear to transport appreciably less. On the other hand 16 mm bundle bridges still give

a proper transport. That is a comprehensible result that indicates a greater suitability for transport of bundle bridges with sieve tubes. In 1913 SOLEREDER mentioned the presence of sieve tubes in these bundles and the lack of xylem vessels.

Particularly surprising is the result of the inhibition of the bundle transport by azide. This removes any doubt one might have that this transport should take place in the plasm. It is also a striking fact that the transported ions even in the parenchyma bridges, which do imply rather drastic injuries, are not released directly to the medium. The results of these experiments are very regular and indicate a diffusion or process of spreading for the transfer of ions in the plasm. About the question whether the ions are transported as such or as complexes with carriers nothing can be said as yet. A connection with metabolism in the transport in the symplasm has not yet been found hitherto. It is, however, possible that we have to deal here with an accelerated diffusion, but experimental data on the rate of transport are still lacking. Vital processes are likely to be involved in the bundle bridges and the transport through the sieve tubes. As discussed on page 24, we see the cause of the inhibition of the bundle transport by azide rather in a process that takes the salts into the sieve tubes than in an inhibition of the transport itself. As already indicated before (ARISZ 1952) the bundles of young leaves will possess sieve tubes which are not yet differentiated. They transport in the same way as ordinary parenchyma cells. There was an indication that in very young leaves the "Sutcliffe effect" with azide does not arise. In this stage apparently the special structure needed for this has not yet been formed.

Distinction of two processes, the uptake into the symplasm and the secretion into the vacuole.

When the uptake processes in *Vallisneria* are compared with other objects the emphasis lies on the possibility *Vallisneria* offers to demonstrate that uptake into the symplasm and accumulation into the vacuole are biochemically different processes.

It is not yet possible to extend this result to other objects like roots. Theoretically it is imaginable that we could prevent the accumulation process in the vacuole by an inhibitor, while the uptake into the symplasm and the secretion into the xylem are continued.

If scientists had not started from the simple diffusion- and permeability phenomena and had not come to the active uptake processes connected with metabolism so gradually, they would probably have distinguished a process of uptake from the medium into the plasm and one of secretion from the plasm into the vacuole from the start. Now that for *Vallisneria* the proof has been yielded for these processes being distinguished, it should be considered whether this distinction is of general validity. It seems of essential importance that it appears from the experiments that the possibility exists of the uptake into the plasm being a process based on a different biochemical reaction from the secretion into the vacuole.

Fig. 31 gives a general scheme of uptake and transport applicable to the root. Uptake from the medium into the central plasm and secretion from the pool of the central plasm into the vacuole are shown as separate processes giving an impression of the possibilities opened up by this constellation. The scheme is based on data obtained with *Vallisneria* leaves. It will be discussed more extensively in other papers. It is based on the assumption of Münch that the cellular protoplasts interconnected by plasmodesms form a symplast in which substances can be translocated. The general presence of plasmodesms

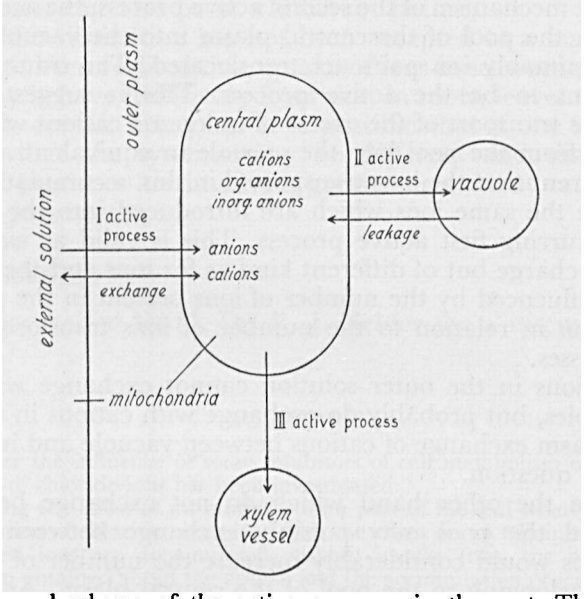


Fig. 31. General scheme of the active processes in the root. The first active process takes the ions into the central plasm. The second active process takes the ions from the central plasm into the vacuole. The third active process takes the ions from the central plasm to the xylem.

has been stressed by Schuhmacher and Lambertz these last few years. The physiological investigations communicated in this and some previous papers have elucidated the role of the symplasm in the plasmatic transport in root and leaf.

In *Vallisneria* leaves beside the active processes of uptake and secretion a migration of salt ions is found from cell to cell in the symplasm. The migrating ions withdrawn from the pool of ions in the central plasm need not be the same ions which are concurrently absorbed from the outer solution.

In the root a third active process seems to be present (Arisz 1945, 1956) which takes the ions from the pool in the central plasm into the xylem vessels (exudation process). The actual mechanism of the active processes is unknown. It suffices that the cation or the anion alone is actively transported for moving both salt ions but it may be that both salt ions are actively transferred. The same problem arises

for the three active processes in the root. The ions may be absorbed free or bound to carriers, the bonding may be reversible or irreversible while oxidation reduction processes may be involved in the binding to or the setting free from the carrier.

In the general scheme the representation given is based on the results with *Vallisneria* that in the first active process the anions are transported by an active process from the surface into the central plasm. In the central plasm there are cations which can exchange for cations in the medium.

About the mechanism of the second active process, the accumulation of ions from the pool of the central plasm into the vacuoles, little is known. Presumably ion pairs are translocated. The transport of the cations seems to be the active process. This is suggested by the simultaneous transport of the excess of inorganic cations with organic acid anions from the pool into the vacuole in equivalent amounts.

It is apparent that the ions transported in this accumulation process need not be the same ions which are introduced into the cytoplasm by the concurring first active process. This is valid as well for ions of the same charge but of different kind as for ions and their isotopes. It will be influenced by the number of ions present in the pool of the central plasm in relation to the number of ions translocated in the active processes.

Since cations in the outer solution cannot exchange with cations in the vacuoles, but probably do exchange with cations in the pool of the inner plasm exchange of cations between vacuole and inner plasm is out of the question.

Anions on the other hand which do not exchange between the medium and the pool may possibly exchange between pool and vacuole. This would considerably increase the number of the anions potentially belonging to the pool of the central plasm. An exosmosis of salt from the vacuole or from the central pool to the medium is in this way ruled out.

In several investigations excised roots have been used. This gives an apparent simplification of the system since the effect of the third active process the secretion into the xylem is eliminated. From the general scheme it is apparent that the secretion into the xylem is going on during the absorption, continually removing ions present in the pool of the central plasm. There is no necessity that the same ions which are absorbed are also secreted into the xylem. In studying the accumulation process into the vacuoles the consequence of this change in the composition of the pool in the inner plasm may not be neglected.

A simplification of the general scheme by assuming only one process that takes the ions from outside into the vacuole seems to me to be unwarrented for the present as it excludes all the possibilities mentioned above. If for instance in the central plasm beside the absorbed cations and anions also organic salt ions are present, in the accumulation process an organic anion from the pool can be taken into the vacuole together with a cation, while when taken up from the medium the

cation was accompanied by a hydroxyl or bicarbonate ion. It would surely lead to a simpler scheme if the carrier and the nature of the binding of ion to carrier were the same for all active processes, so that the same complex could be carried from the outer layer of the cytoplasm to the vacuoles and to the xylem vessels and from cell to cell over long distances. This supposition, however, is not yet justified by experimental data. It is necessary that for the present we start from a general scheme that leaves the possibility that the carriers and the processes of complex formation can be different, so that the mechanism of the processes can also be of a different nature.

Hitherto this distinction has not been accepted as a matter of fact and it has not been realised that uptake of ions in seaweeds, yeasts and bacteria may behave differently from that in roots, leaves and storage tissue, which contain vacuoles. It is possible that if one is conscious of the fact that the systems may be different and that different biochemical processes are involved, greater agreement can be shown in identical processes.

ACKNOWLEDGEMENT

The author has to thank Dr. R. J. Helder for a discussion of the manuscript.

SUMMARY

In this paper the influence of some inhibitors of cell metabolism on the uptake and transport of chloride ions has been investigated.

By comparing the effect of an inhibitor on a part of the leaf where both uptake and accumulation of chloride take place with the effect on an adjoining part where the transported ions are accumulated without uptake from the outer solution data have been obtained about the uptake and the accumulation process separately. Azide inhibits the accumulation in the vacuoles, cyanide, arsenate and uranyl inhibit the uptake from the outer solution.

The transport through tissue bridges of 4 to 16 mm length was estimated and it was found that azide inhibits the transport through a bundle bridge but not through a parenchyma bridge.

The conclusions to be drawn from these results are exposed and a general scheme is given as a basis for the interpretation of the salt transport in *Vallisneria* leaves and plant roots.

REFERENCES

ARISZ, W. H. 1943. Versl. Ned. Akad. Wet., Afd. Natuurkunde, *52*:639–645.
ARISZ, W. H. 1945. Proc. Kon. Ned. Akad. Wet. *48*:420–446.
ARISZ, W. H. 1947. Proc. Kon. Ned. Akad. Wet. *50*:1019–1032.
ARISZ, W. H. 1948. Proc. Kon. Ned. Akad. Wet. *51*:25–32.
ARISZ, W. H. 1952. Annual review plant physiol. *3*:109–130.
ARISZ, W. H. 1953. Acta Bot. Neerl. *1*:506–515.
ARISZ, W. H. 1954. Nature *174*:223.
ARISZ, W. H. 1954. Acta Physiol. Pharmacol. Neerl. *3*:575–599.
ARISZ, W. H. 1956. Protoplasma *46*:5–62.
ARISZ, W. H. and M. J. SCHREUDER. 1956. Proc. Kon. Ned. Akad. Wet., Series C, *59*:454–461.
ARISZ, W. H. and M. J. SCHREUDER. 1956. Proc. Kon. Ned. Akad. Wet., Series C, *59*:461–470.

ARISZ, W. H. and H. H. SOL. 1956. Acta Bot. Neerl. *5*:218–246.
BUTLER, G. W. 1954. Physiol. Plant. *6*:617–635.
COWIE, D. B. and R. B. ROBERTS. 1935. In Electrolytes in Biological Systems, 1–34. (Shanes, A. M., Ed., The American Physiological Society, Washington, D.C., 243 pp., 1955).
EPSTEIN, E. 1955. Plant Physiol. *30*:529–535.
EPSTEIN, E. 1956. Ann. review plant physiol. *7*:1–24.
HYLMÖ, B. 1953. Physiol. Plant. *6*:333–405.
HOPE, A. B. and P. G. STEVENS. 1952. Austr. J. Sci. Res. B *5*:335.
HOPE, A. B. and P. G. STEVENS. 1953. Austr. J. Biol. Sc. *6*:396–409.
HOPE, A. B. and R. N. ROBERTSON. 1956. Nature: *177*:43.
LOOKEREN CAMPAGNE, R. N. VAN. 1956. Proc. Kon. Ned. Akad. Wet., Series C, 59.
LOOKEREN CAMPAGNE, R. N. VAN. 1957. Acta Bot. Neerl. *6*:543–582.
LUNDEGÅRDH, H. 1954. Symp. Soc. exper. Biol. 8.
LUNDEGÅRDH, H. 1955. Ann. Rev. Plant Physiol. *6*:1–24.
OVERSTREET, R. and L. JACOBSON. 1952. Ann. review Plant Physiol., *3*:189–206.
SCOTT, G. T. and H. R. HAYWARD. 1954. Journ. Gen. Physiol., *37*:601–620.
SOLEREDER, H. 1913. Beitr. Bot. Centr. bl. *30. I*:24–104.