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Radial Transport of Ions in Roots[1]

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Abstract. Measurements were made of the relative amounts of ^{86}Rb, ^{36}Cl, and ^{32}P accumulated in the cortex and stele of intact roots of corn (*Zea mays*), either detached or attached to their shoots. Both 4- and 7-day-old roots accumulated as much or more ^{86}Rb in the stele as in the cortex. In experiments with ^{36}Cl, cortex and stele accumulated the same amount, except for 4-day-old and 7-day-old attached roots, in which the cortex contained more ^{36}Cl than the stele after 23 hr. An additional study of ^{32}P uptake showed greater accumulation in the cortex than the stele for a short period of time, but as much in the stele as in the cortex after 8 to 24 hr. Transport of ^{86}Rb, ^{36}Cl, and ^{32}P into the xylem exudate increased with increasing accumulation of these ions in stele and cortex of the root. These experiments show no consistent difference between cortex and stele of intact corn roots with respect to their ability to accumulate several kinds of ions.

One of the most interesting problems in connection with salt absorption is the mechanism by which salt is accumulated and retained in the stele of roots. A widely accepted explanation of this process is that of Crafts and Broyer (5) which is supported by Laties and his co-workers (14, 15, 16). According to this explanation, ions are accumulated in the cells of the cortex and move through the symplast into the stele where they leak out into the xylem. This theory requires the cytoplasm of the living cells of the stele to have a lower capacity to accumulate and retain ions than the cortical cells. Laties and his co-workers reported that stele freshly separated from the cortex has a limited capacity to accumulate chloride, rubidium, and sulfate ions, but this capacity increases some hours after separation from the cortex.

In contrast, Yu and Kramer (23) found that both separated stele and the stele of intact roots accumulated ^{32}P as effectively as the cortex. However, it was suggested that perhaps conclusions based on the behavior of phosphorus may not be applicable to other ions. Additional studies, therefore, were made of the accumulation of ^{86}Rb and ^{36}Cl by the stele and cortex of corn roots and the work with ^{32}P was extended.

Methods

Materials Used. Seeds of corn (*Zea mays* L. Pioneer hybrid, No. 309-B) were soaked in tap water for 22 to 24 hr and were germinated between wet paper towels at 21°. Some seedlings were used for experiments 4 days from the beginning of germination. The roots of these plants, designated as 4-day-old seedlings, were relatively rigid, had numerous root hairs, were 4 to 6 cm long, and had a diameter of at least 1.14 mm. Their manifestation of root pressure exudation, ease of separation of cortex from stele, and infrequency of lateral root primordia made these roots ideal for this study. The shoots of these seedlings were relatively small, and their primary leaves were still folded during the experiments.

The 7-day-old seedlings used in this study were cultured in the same way as the 4-day-old seedlings except that after the first 4 days, seedlings were transferred to 1/10 strength Hoagland solution No. 1 (10). They were grown in solution in a growth chamber at a light intensity of 1800 to 2000 ft-c on a 12-hr photoperiod until 7 days old. Apical segments of 6 cm or less were used in determining the relative accumulation of each radioactive ion in cortex and stele. Such segments show higher rates of root exudation than the older segments along the same root. Preliminary studies indicated that few or no lateral root primordia were present in this region. These roots elongated an average of 0.6 cm in 8 hr and 2 cm in 23 hr. The shoots of the 7-day seedlings were fully expanded, resulting in appreciable transpiration.

Uptake of ^{32}P was measured with 6-day-old corn seedlings. The culture method was the same as that described by Yu and Kramer (23) except that in the culture solution and in the experimental solution KH_2PO_4 was replaced by $NH_4H_2PO_4$ and the final concentration of $H_2PO_4^-$ was 1 mM. Only the apical 6 cm segments of roots were used for the experiments.

Experimental Methods. Four-day-old intact seedlings and excised roots were suspended in 2 liters of experimental solution for 2, 8, and 23 hr. The activity of ^{86}Rb in the solution was 50 μc/l. Other ionic concentrations were: K^+ slightly less than

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0.2 mM; $H_2PO_4^-$, 0.1 mM; NO_3^-, 1.0 mM; Ca^{2+}, 0.5 mM; Mg^{2+}, 0.2 mM; and SO_4^{2-}, 0.2 mM. This solution was used for all ^{86}Rb uptake experiments. The ^{86}Rb had a specific activity of 0.635 mc/mg Rb. It was received in HCl which was neutralized with 1 N KOH for use in the experimental solution.

In experiments with excised roots, the cut end of each root was fitted with a small polyethylene tube to collect the exudate. At the end of each experimental period, the roots were washed in aerated 1/10 streng.h Hoagland's solution at 0° to 5° for 5 min. The apical 0.5 to 1 cm of each root was removed and the cortex was separated from the stele of the remaining root. Samples of the separated stele and cortex were washed a second time in aerated 1/10 streng.h Hoagland solution at 0° to 5° for 25 to 30 min. The fresh weight of each sample was determined after the second washing. The total time used in washing. stripping, and weighing was about 1.5 hr. Root tissues were kept between wet filter paper or in cold wash solution between operations. Samples were dried for ^{86}Rb determinations. The radioactivity of ^{86}Rb was measured either by a gas flow counter or by a scintillation counter.

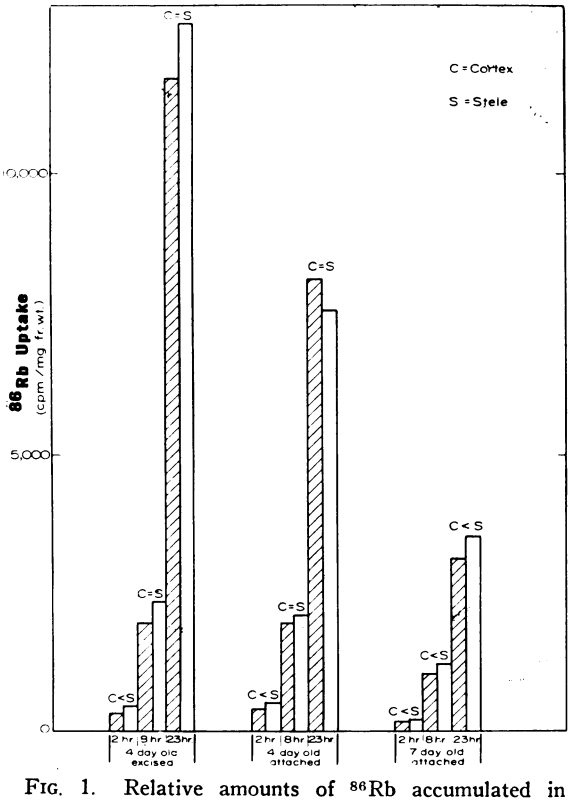

FIG. 1. Relative amounts of ^{86}Rb accumulated in the cortex and stele of excised and attached corn roots after 2, 8, and 23 hr. The cortex was separated from the stele and the activity of the 2 tissues was counted separately. Data for 4-day roots are averages of 10 replications, those for 7-day roots are averages of 4 or 5 replications. C = cortex, S = stele. Differences are significant at the 5% level and are indicated as $C < S$, $C > S$, or $C = S$.

The 7-day-old seedlings were used in experiments to study the effect of a transpiring shoot on the radial transport of ^{86}Rb. Seedlings were held upright in a humid chamber with only the apical 6 to 8 cm of root immersed in radioactive solution. The shoots of the experimental plants were exposed to a light intensity over 3000 ft-c and a gentle breeze was provided to insure conditions favorable for transpiration. The uptake times were 2, 8, and 23 hr. At the end of each uptake period, the treated segments were washed as described in the preceding paragraph. After the first washing, the apical segments of the roots were excised and were treated as above to determine the relative accumulation of ^{86}Rb in cortex and stele.

The experimental procedures used for ^{36}Cl were the same as for ^{86}Rb. The nutrient solution used for the ^{36}Cl experiment contained 0.1 mM of KCl; the addition of the isotope (specific activity 1.05 mc/g) resulted in a final KCl concentration of 1.45 mM. The activity of ^{36}Cl used was 50 μc/l. Other ionic concentrations were the same as for experiments with ^{86}Rb. The ^{32}P uptake study presented in this paper was performed in the same manner as reported in the previous paper of Yu and Kramer (23). Only the apical 6 cm segments of the roots were used in the present work. and the uptake periods were 1, 4, 8, 16, and 24 hr. The experimental solution was not buffered. Preliminary studies showed no significant difference in uptake between buffered and unbuffered experimental solutions.

Results

Rubidium Uptake and Transport. Accumulation of ^{86}Rb by the cortex and the stele of intact roots is presented in Fig. 1. For excised roots of 4-day seedlings, the relative accumulation of ^{86}Rb per unit fresh weight was greater in stele than in cortex after 2 hr, but the 2 were not significantly different after 8 and 23 hr of uptake. The same appeared to be true in roots of 4-day-old intact seedlings. In 7-day-old seedlings, the stele accumulated significantly more than the cortex in all 3 periods of uptake tested. However, the total amount accumulated was less than in younger roots.

The rate of accumulation of ^{86}Rb by the cortex and stele is shown in table I. There was a continuous increase in rate of uptake over the entire experimental period in stele and cortex of both excised and attached roots of 4-day-old seedlings. In apical segments of roots of 7-day-old seedlings the rate of uptake increased for the first 6 hr, then remained about the same over the remainder of the experimental period. Table I also shows the rate of transport to the xylem sap as indicated by the activity in the root xylem exudate. The concentra-

Table I. *Rate of ^{86}Rb Uptake in Root Cortex and Stele, Rate of Transport to the Exudate, and Accumulation Factor*

Period of uptake	Rate of ^{86}Rb accumulation: 4-day-old excised cortex	4-day-old excised stele	4-day old intact cortex	4-day old intact stele	7-day-old intact cortex	7-day-old intact stele	4-day-old, excised roots: Rate of transport to the xylem sap	Accumulation factor ^{86}Rb activities Exudate/Root medium
hr			*cpm•mg fw^{-1} • hr^{-1}*				*cpm•hr^{-1}•root^{-1}*	*ratio*
0–2	130.5	229.5	182.0	258.4	88.6	106.0	248	3.4
2–8	281.7	323.1	269.0	269.0	169 6	195.8	2810	15.1
8–23	648.0	684.0	406.1	361.3	156.5	173.7	6140	53.4

Table II. *Rate of ^{36}Cl Uptake in Root Cortex and Stele, Rate of Transport to the Exudate, and Accumulation Factor*

Period of uptake	Rate of ^{36}Cl accumulation: 4-day-old excised cortex	4-day-old excised stele	4-day-old intact cortex	4-day-old intact stele	7-day-old intact cortex	7-day-old intact stele	4-day-old, excised roots: Rate of transport to the xylem sap	Accumulation factor ^{36}Cl activities Exudate/Root medium
hr			*cpm•mg fw^{-1} • hr^{-1}*				*cpm•hr^{-1}•root^{-1}*	*ratio*
0–2	9 5	**10.3**	13.9	13.5	18.3	17.3	. . .[1]	. . .[1]
2–8	14.9	10.6	12.0	10.1	18.2	20.5	199.5	2.6
8–23	14.9	13.5	13.9	7.7	16.3	10.2	964.2	4.7

[1] Amount of exudate insufficient for assay.

Table III. *Rate of ^{32}P Accumulation in Cortex and Stele and Rate of ^{32}P Transport Into the Root Exudate*

Period of uptake (hr)	0–1	1–4	4–8	8–16	16–24
Avg rate of accumulation (cpm hr^{-1} mg fw^{-1}):					
cortex	8.6	8.9	8 5	6.6	— 0.4
stele	2.2	7.9	10.2	8.9	— 1.14
Avg rate of transport (cpm hr^{-1} root^{-1})	4.5	31.3	74.2	122.7	56.0

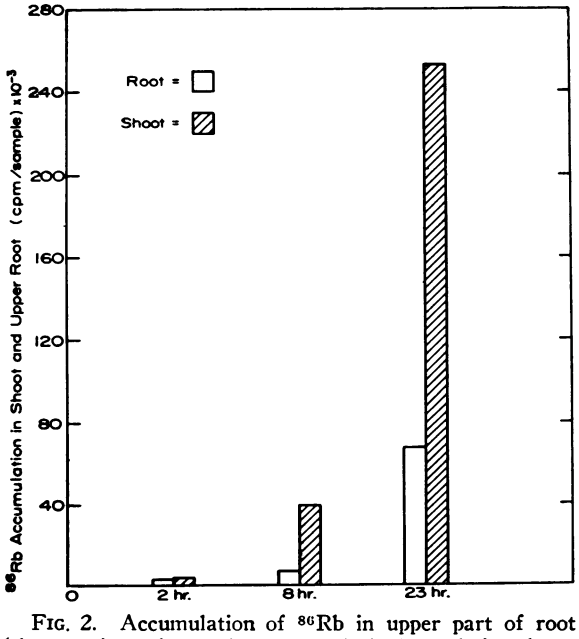

FIG. 2. Accumulation of ^{86}Rb in upper part of root (the portion above the test solution) and in shoot, expressed as counts per min per sample for the various periods of time indicated on the abscissa.

tion of ^{86}Rb in the xylem sap increased over the entire experimental period, as indicated by the increasing accumulation factor.

Fig. 2 shows the amount of ^{86}Rb in the upper part of the roots and in the shoots of intact 7-day seedlings after 2, 8, and 23 hr. The upper part of the root refers to the portion above the region immersed in test solution.

Chloride Uptake and Transport. Fig. 3 shows that the accumulation of ^{36}Cl in the cortex and stele of excised roots from 4-day seedlings was equal for all 3 uptake periods, but in attached roots accumulation in the cortex exceeded that in the stele after 23 hr as it also did in attached roots of 7-day seedlings. However, in contrast to the results with ^{86}Rb, the rate of accumulation of ^{36}Cl in cortex and stele leveled off or declined toward the end of the experiment (table II). This difference may be related to the fact that the external concentration of tracer was considerably higher in the ^{36}Cl experiment than in the ^{86}Rb experiment. There was considerable increase in ^{36}Cl transport to the xylem sap and in the accumulation factor over the experimental period, as with ^{86}Rb.

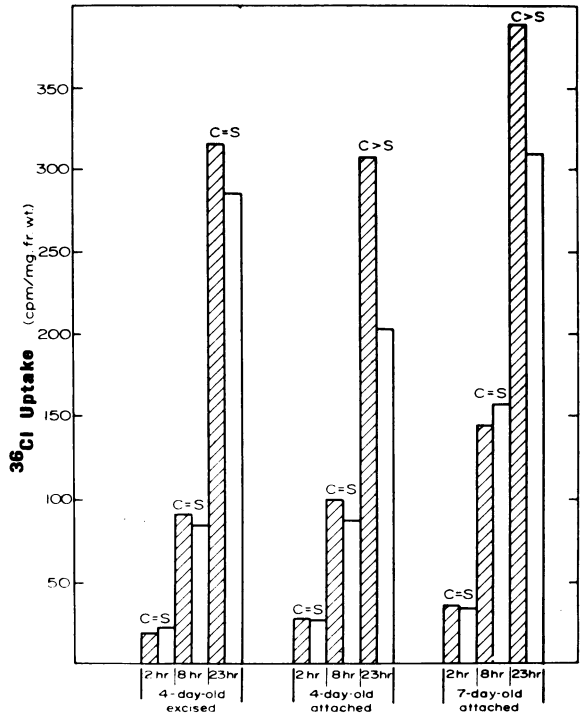

FIG. 3. Relative amounts of ^{36}Cl accumulated in the cortex and stele of excised and attached corn roots after 2, 8, and 23 hr. The cortex was separated from the stele and the activity of the 2 tissues was counted separately. Data for 4-day roots are averages of 10 replications, those for 7-day roots are averages of 4 or 5 replications. C = cortex, S = stele. Differences are significant at the 5 % level and are indicated as C < S, C > S, or C = S.

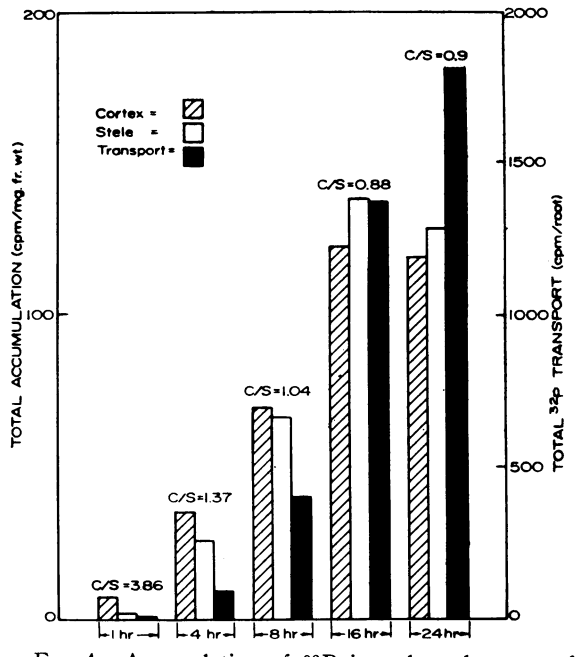

FIG. 4. Accumulation of ^{32}P in stele and cortex of excised 6-day-old corn roots and rate of transport out of roots. Accumulation is expressed as counts per min per mg of fresh weight; transport is in counts per min per root, based on the activity in the xylem exudate.

Phosphate Uptake and Transport. The results of the study of ^{32}P uptake are shown in Fig. 4 and table III. During the first hr ^{32}P uptake was much greater in the cortex than in the stele, probably because little ^{32}P had been transported to the stele. After 8 hr, accumulation in the stele equaled or exceeded that in the cortex. Transport to the xylem, as indicated by amount of ^{32}P in the xylem sap, increased rapidly as ^{32}P began to accumulate in the stele.

Discussion

Rubidium Uptake and Transport. ^{86}Rb was used to simulate potassium because it appears to be absorbed in the same manner (8). With the low external concentration used (slightly less than 0.2 mM), uptake should be by the high affinity mechanism which operates at low concentrations (7, 8). The stele appeared to accumulate more rubidium per unit of fresh weight than the cortex during the first 2 hr in all 3 systems studied (Fig. 1). Perhaps this is because the cells of the stele contain more cytoplasm in proportion to vacuole than those of the cortex (unpublished observations). In roots of 4-day-old seedlings, uptake by cortex and stele was equal after 8 hr. However, in 7-day-old transpiring seedlings, this equality was not reached even after 23 hr of uptake.

After 23 hr of uptake excised roots had accumulated more ^{86}Rb than roots of intact plants (Fig. 1). There are 2 possible explanations. One is that a portion of ^{86}Rb absorbed by the root tissue of intact plants is being transported to the shoot. The total amount of transport in intact seedlings amounted to twice the transport in excised roots after 23 hr of uptake. This greater transport in intact seedlings is believed to be a result of the growth of both roots and shoots during the experimental period. Another possibility is that the uptake of ^{86}Rb (*i.e.* K^+) from the external root medium may be curtailed by the possible internal supply of K^+ from the seeds. Since the 7-day-old experimental roots were cultured differently and belonged to a different group of plants, it is inappropriate to compare their relative accumulation with that of the 4-day-old ones. Probably, the same considerations suggested above for 4-day-old intact seedlings can be applied to the 7-day-old seedlings. As the shoots of the latter group are both expanding and transpiring, the effect of the shoot might be more pronounced. It is also possible that roots of 4-day-old plants contained less K than those of 7-day-old plants at the start of the uptake experiments, the reason being that the former were grown only between paper towels and the latter were

immersed in aerated 1/10 Hoagland solution for 3 additional days. This could also serve to explain the increasing rate of ^{86}Rb uptake with time observed in roots of 4-day-old plants (table I).

Table I shows that the rate of transport and the accumulation factor both increased continuously with the rate of accumulation in cortex and stele of 4-day-old excised roots.

Considerable ^{86}Rb was removed by the upper part of the 7-day-old roots when only the lower 6 cm was immersed in solution (Fig. 2). Some of the rubidium that moved to the upper part of the root doubtless moved into the numerous lateral roots arising at the root-shoot transition and some was translocated to the shoot. Removal of salt from the xylem sap by surrounding cells was suggested by Arisz (2) and Klepper (13) and it apparently occurred in these experiments.

Chloride Uptake and Transport. The uptake and transport of chloride was investigated by the same methods used for rubidium. The concentration of chloride was 1.45 mM, higher than desired, but satisfactory for comparing uptake of chloride by cortex and stele. Presumably, the uptake mechanism for chloride at this concentration also is the low affinity mechanism (7, 8, 21). The data in table II show that the stele of corn roots accumulates ^{36}Cl approximately as actively as the cortex. The use of a relatively high external concentration of chloride hastens equal accumulation by cortex and stele.

In contrast to the results with ^{86}Rb (Fig. 1), ^{36}Cl accumulation in cortex exceeded that in the stele after 23 hr of uptake in roots of 4-day-old and 7-day-old intact seedlings (Fig. 3). This could be a result of the difference in external concentrations of ^{36}Cl and ^{86}Rb used, or a result of difference in the manner in which the 2 ions are accumulated. Further investigation of this point is warranted.

Phosphate Uptake and Transport. New experiments with ^{32}P confirm the earlier observations (23) that the stele of intact roots can accumulate phosphate as effectively as the cortex. Uptake of phosphate differs from that of other ions because for short periods more phosphate is accumulated in the cortex than in the stele and the concentration in the stele only equals that of the cortex after 8 hr of absorption (Fig. 4 and table III). This may result from the fact that much phosphate is removed from circulation and used in various organic compounds in the cells of the cortex (6).

The major objective of this research was to extend earlier work and to learn whether the stele of intact roots is capable of accumulating ions other than phosphate. The results indicate clearly that the stele of intact roots accumulates rubidium and chloride as effectively as the cortex. The earlier findings on accumulation of phosphate were also verified.

The Crafts-Broyer hypothesis of salt transport into the xylem is based on the assumption that the cells of the stele have a lower capacity to accumulate and retain salt than those of the cortex. Therefore ions accumulated in the cortical cells are moved into the stele through the symplast where they leak out into the xylem conduits. Crafts and Broyer (5) proposed that the cells of the stele are "leaky" because of a deficiency of oxygen. However, there is increasing evidence of the diffusion of oxygen from shoots to roots (9); this raises questions about the existence of an oxygen deficiency in the stele. Laties and Budd (14) attributed the presumed leakiness of the stele cells to a volatile inhibitor of metabolism. However, we found no evidence of inhibition of accumulation in the stele of intact roots. Work of Biddulph (3) and Branton and Jacobson (4) also indicates that the cells of the stele can accumulate salt as energetically as the cells of the cortex.

As the results of our experiments raise questions concerning the basic assumptions of the Crafts-Broyer-Laties hypothesis we must consider alternative explanations for salt transport into the xylem. Hylmo (11) proposed that the protoplasts of the living xylem elements accumulate ions which are released into the xylem when the protoplasts disintegrate. Considerable numbers of living protoplasts occur in the xylem of the absorbing zone of roots and Anderson and House (1) reported that uptake of salt is closely correlated with the number of living protoplasts. However, it is doubtful if death of protoplasts in the maturing xylem elements would provide enough salt. It was calculated from the rate of exudation and the volume of the xylem vessels in corn roots that the solution in the xylem vessels is being replaced at least 3 times per hr. We therefore question if breakdown of protoplasts releases enough salt to permit escape of salt at the observed rate.

Another possibility is that the living cells adjacent to the xylem vessels secrete salt into them, as proposed by Arisz (2), Sutcliffe (19), van Andel (20), and others. Salt is secreted by glands in leaves and there seems to be no reason why it could not occur in the stele of roots. A related problem is the secretion of organic compounds into the root xylem. For example, a number of organic nitrogen compounds occur in the xylem sap which presumably are synthesized in the root cells and transferred to the xylem. There also is increasing evidence of the synthesis of growth regulators such as cytokinins and gibberellins in the roots and their transfer to the xylem sap (12). These facts should be taken into account in considering mechanisms for transfer of solutes to the xylem elements from the surrounding tissue.

If it is accepted that the protoplasts of the root cells are connected by plasmodesmata to form the symplast, this provides a pathway for movement of ions from the root surface to the living cells adjoining the xylem vessels. There is some debate con-

cerning the mechanism by which ions enter the symplast. Laties and Luttge (15, 16) claim that at salt concentrations greater than 1 mM ions move through the plasmalemma by diffusion and the symplast is a part of free space. However, if the plasmalemma were permeable to ions at moderate or high concentrations it would be impossible for salt to accumulate in the xylem sap to the concentrations often observed. Welch and Epstein (21) recently reported that both the low and the high concentration salt transport mechanisms operate in parallel across the plasmalemma, obviating the difficulties inherent in the proposal of Laties and Luttge.

Probably ions are accumulated in the cytoplasm of the epidermal cells, diffuse inward through the symplast, possibly aided by cytoplasmic streaming, until they reach the cells adjoining the xylem vessels. Possibly these cells secrete ions and organic solutes into the xylem vessels. In addition a small amount of salt is released when the protoplasts of the xylem vessels break down. If it is granted that the protoplasts of xylem vessels of the active absorbing zone of roots are still alive (1, 17, 18), and cells throughout the root are connected by plasmodesmata, then it is reasonable to believe that some of the salts might diffuse passively through plasmodesmata into xylem vessels independently of the permeability of the plasmalemma.

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