Radial Salt Transport in Corn Roots[1]

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Summary. Primary roots of solution-grown, 5-day-old or 6-day-old seedlings of corn (*Zea mays* L.) 10 to 14 cm in length were used to study radial salt transport. Measurements were made of the volume of root pressure exudation, salt concentration of the exudate, and rate of salt movement into the xylem exudate. The ^{32}P uptake, O_2 consumption, and dehydrogenase activity of the root cortex and stele also were studied.

These roots produced copious root pressure exudate containing 4 to 10 times the concentration of ^{32}P in the external solution. Freshly separated stele from 5-day-old roots accumulated ^{32}P as rapidly as the cortex from which it was separated and the stele of intact roots also accumulated ^{32}P. Separated stele has a higher oxygen uptake than cortex. It also shows strong dehydrogenase activity with the tetrazolium test. The high oxygen consumption, ^{32}P uptake and strong dehydrogenase activity indicate that the cells of the stele probably play a direct role in salt transport.

These data raise doubts concerning theories of radial salt transport into the xylem based on the assumption that the stele is unable to accumulate salt vigorously.

It is generally agreed that root pressure exudation is the result of accumulation of salt in the xylem elements which brings about inward movement of water along a gradient of water potential (5, 14, 15). Such an explanation requires an active transport mechanism to move the salt into the stele and an ion barrier to cause it to be accumulated within the xylem vessels. However, in spite of extensive study, little is known about the mechanism by which salt is moved into the xylem vessels. According to the most often cited theory of radial salt transport in roots, salt is accumulated by the cortical cells and moves inward through the symplast by diffusion, perhaps aided by cytoplasmic streaming, to the stele where it leaks out of the stelar parenchyma cells into the xylem (4). This theory was strengthened by observations of Laties and Budd (10) who reported that cells of the stele are both leaky and ineffective in salt absorption when first removed from the stele and only accumulate salt effectively after 24 hours of incubation in solution. Other investigators have proposed that the endodermis may actively secrete salt into the stele (1, 2, 13) and some have proposed that the adjacent parenchyma cells may secrete salt into the xylem vessels (1, 11, 13, 16).

This study was undertaken in an attempt to learn more about the radial transport of salt in roots, and to search for an adequate theory to explain salt accumulation in the xylem vessels of a copiously exuding root. It also provided a test of the general applicability of the hypothesis of Laties and Budd that the stele of intact roots has a very limited capacity to accumulate salt.

Methods

Materials Used. Seeds of corn (*Zea mays* L., Pioneer hybrid, No. 309-B) were soaked in tap water for 5 to 6 hours and germinated between wet paper towels. After 3 days, the roots were inserted through holes in a cover on a container filled with half strength Hoagland's solution (7). Three days later, primary roots were ready for use. Lateral root primordia were present in the older regions of these roots as indicated in figure 5. While part of the experiments were performed by using the above roots, designated throughout the paper as 6-day-old roots, part of the data were obtained from roots grown in solution for 2 days instead of 3 days. Use of the 5-day-old roots eliminated the complications that may be caused by the presence of numerous lateral root primordia in older roots.

Exudation Rate, Salt Concentration, and Rate of Salt Movement. Apical segments of the 6-day-old corn roots about 10 to 14 cm in length were excised. The basal end of each segment was wiped dry and coated with a layer of high vacuum silicone grease. This provided a water tight seal when the basal end of the root was slipped into a piece of

polyethylene tubing having a bore nearly the same as the diameter of the root. The tubing served to collect the exudate. The root surface was calculated from the diameter and length of the portion of each root immersed in the experimental solution.

The salt concentration of the root exudate was determined by measuring the electrical conductivity of the exudate with a conductivity bridge, and a specially constructed well-type conductivity cell requiring only 10 μl per sample. The cell was calibrated with known concentrations of KCl solution and the conductivity of the root exudate was expressed as KCl equivalents. The rate of salt movement was calculated from the volume and salt concentration of the exudate.

Exudation rates and salt concentrations were measured at 12 hour intervals with 10 roots in aerated or nonaerated external root media. The total experimental period was 48 hours. Roots were bathed in half strength Hoagland's solution during the experiments. The rate of exudation is shown in figure 1A, the salt concentration of the exudate in figure 1B, and the rate of salt movement in figure 1C. The volume of root pressure exudate and the amount of salt transported indicate that the corn roots used in this study are suitable material for investigating radial transport of salt in roots. The lack of response to aeration indicated that it was unnecessary under the conditions of this experiment.

Measurements of [32]*P Uptake by Separated Cortex and Stele.* The apical 1 or 2 cm were removed from 5-day-old roots 10 to 12 cm in length and the cortex was stripped from the stele. The separation occurred at the endodermis, leaving the stele intact. The strips of cortex were broken into segments 2 to 8 cm long and split longitudinally once or twice to ensure a thickness of tissue similar to that of the stele. Both kinds of tissue were suspended in half strength Hoagland's solution buffered at pH 6.1 by "Mes" buffer, 2-(N-morpholino)-ethanesulfonic acid (6) until used in experiments. Samples of stele or cortex were blotted dry with filter paper and weighed. The fresh weight per sample ranged from 50 to 150 mg. The samples of tissues were then enclosed in tiny cheesecloth bags which were suspended in experimental solution by means of wax-coated wire, looped within the bags to prevent their collapse. The experimental solution used was half strength Hoagland's solution (pH 6.1), with enough ^{32}P added to produce an activity of 20 μc per liter. The maximum final phosphorus concentration of the solution was 0.5032 m$\scriptstyle\rm M$. Samples of fresh stele and cortex were used 2 to 4 hours after beginning separation. Samples of aged stele and cortex were used after 24 hours' incubation at 30° in buffered half strength Hoagland's solution. The ^{32}P uptake period was 4 hours for all samples. At the end of each uptake period the samples were washed in a large volume of half strength Hoagland's solution at 0 to 5° for a total of 10 minutes to remove

[32]P from the tissue surfaces. The wash solution was changed 3 times during this period. The ^{32}P activity was then determined from dried samples of tissue with a gas flow counter at a counting efficiency of about 45 %. The count rates were not corrected for sample self-absorption, which amounted to about 2.5 to 3 % for [32]P. Experiments were performed at a constant room temperature of approximately 24°.

Uptake of [32]*P by Cortex and Stele of Intact Roots.* Sixteen to 20 excised intact roots similar to the ones used in the previous experiment were suspended for 4 or 8 hours in the experimental solution prepared as in above experiment. The cut end of each root was fitted with a small polyethylene tube to collect the exudate. At the end of each experiment, the roots were washed in half strength Hoagland's solution at 0 to 5° for 10 minutes and the cortex separated from the stele. The roots and the separated stele and cortex were kept between sheets of wet filter paper while not in solution. Half of the samples of separated stele and cortex were washed a second time in half strength Hoagland's solution at 0 to 5° for 30 minutes, while the other half were not. The second washing did not remove any additional [32]P. The fresh weights of the stele and cortex separated from each individual root were determined soon after separation or after the second washing. Samples were then placed on planchets and were dried for ^{32}P determination. The total amount of exudate collected from each root was recorded and the exudate was put onto disks of filter paper in planchets for determination of the total ^{32}P contained in the xylem exudate.

O_2 *Uptake and Dehydrogenase Activity.* The respiratory activity of 5-day-old root tissue was measured in terms of O_2 uptake with a Warburg apparatus. Pieces of cortex or stele similar to those used in the ^{32}P uptake studies were suspended in 3 ml of buffered half strength Hoagland's solution in Warburg flasks. About 0.2 ml of 20 % KOH was placed in the center well of each flask to trap the CO_2 evolved during respiration. The flasks were suspended in a 30° water bath and the O_2 consumption was measured approximately 4, 5, and 6 hours after the stele and cortex were separated. Measurements were made again after 24 hours of aging in the experimental solution at 30°.

Relative dehydrogenase activities of cortex and stele were determined by suspending freshly prepared central longitudinal sections of root tissue (from 6-day-old seedlings) in a phosphate buffer (pH 7.6) to which was added enough neotetrazolium phosphate to make a 0.05 % solution. The tissue was incubated at 37° for 2 hours. In a control experiment, the neotetrazolium phosphate was replaced by distilled water. The red precipitate resulting from reduction of tetrazolium salt was used as an indication of the site of dehydrogenase activity (3).

Results

The rate of exudation, salt concentration of the exudate, and rate of salt movement of aerated and unaerated 6-day-old corn roots are shown in figure 1. Apparently the oxygen supply to these roots was adequate, because aeration with a stream of air did not result in any significant increase in salt uptake or exudation.
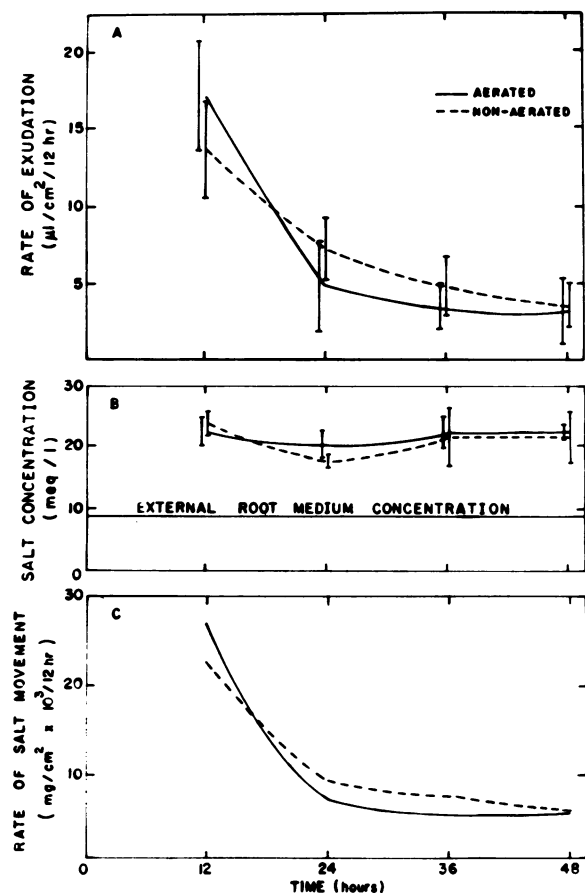

FIG. 1. A) The rate of root pressure exudation per cm² of root surface area; B) The salt concentration of the exudate expressed as KCl equivalents; C) The rate of salt movement per cm² of root surface; all for 12 hour periods for 6-day-old roots.

The results of the measurement of uptake of ^{32}P by separated cortex and stele are presented in table I, column (1). Accumulation of ^{32}P was about the same for cortex and stele, and was much greater for fresh than for aged tissue. This is contrary to the finding of Laties and Budd (10) that stele tissue separated from the cortex for 24 hours showed increased uptake of chloride.

The results of measurements of uptake of ^{32}P by cortex and stele of intact roots which subsequently were separated for measurement are shown in table I, columns (2) and (3), and in table II. Considerably more ^{32}P was accumulated in the cortex than in the stele of intact roots during a 4 hour uptake period. However, when the treatment period was lengthened to 8 hours, the accumulation in the stele and cortex were about the same, suggesting that the capacity for ^{32}P accumulation of stele and cortex are about the same when the supply is not limiting. This also is supported by the data presented in column (1) of table I which indicates that the initially separated stele, in which ^{32}P is not barred by the cortical tissue from entering the stelar cells, accumulates as much ^{32}P as the cortex. The delay in uptake by the stele might result from the longer pathway and greater resistance to movement of ions to stele than to cortex. It also appears from this study that the stele is no leakier than the cortex, because the second washing of the separated cortex and stele did not remove any additional ^{32}P from either the stele or the cortex.

The data of table II on ^{32}P uptake for 4 hours and 8 hours indicate that an increase in total ^{32}P of the exudate accompanies increased ^{32}P accumulation in the stele and cortex. There also is a positive correlation between the ^{32}P content of the xylem exudate and the volume of the exudate.

Figure 2 shows the ^{32}P uptake by separated stele and cortex of 6-day-old roots. The ^{32}P activity of the external solution used was 12 μc per liter and the experimental solutions were not buffered. Azide used at a concentration of 10^{-3} M (9) in some experiments greatly reduced the ^{32}P accumulation. In the absence of azide, the stele appears to accumulate greater amounts of ^{32}P than the cortex, possibly because of the presence of

Table I. ^{32}P Uptake in Cortex and Stele of 5-Day-Old Corn Roots

The data are expressed as counts per minute per milligram of fresh weight. (2) and (3) were obtained from 2 different sets of roots. Data presented in (1) are averages of 8 samples; in (2), 20 samples; and in (3), 16 samples.

| (1) (2) (3) Initially separated* | | (1) (2) (3) Initially intact** | | (1) (2) (3) Initially intact*** | |
Cortex	Stele	Cortex	Stele	Cortex	Stele
Fresh Aged	Fresh Aged				
28.8 10.2	30.3 11.0	23 9	11.6	524.0	497.3
±4.8 ±3.5	±5.9 ±5.9	±6.1	±6.4	±17.2	±22.9

* Separated cortex and stele having 4 hours uptake period.
** Intact roots immersed in ^{32}P for 4 hours and the stele and cortex separated for counting.
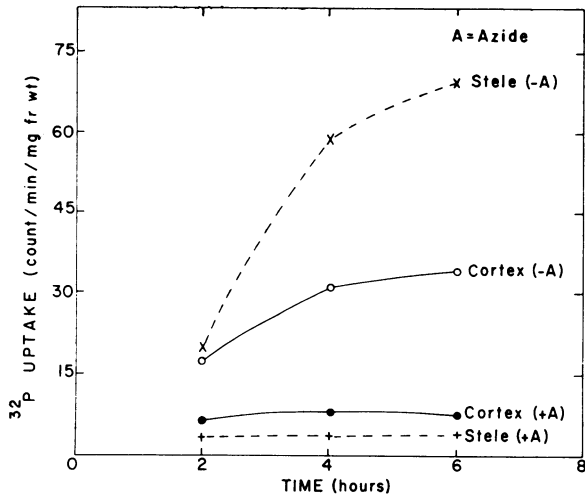*** Same as (2), except the uptake period is 8 hours.

FIG. 2. ^{32}P uptake by separated stele and cortex of 6-day-old roots and the inhibitory effect of azide. Treatments started approximately 4 hours after the beginning of separation of stele and cortex. x, o mean of 4, others mean of 3 replications. The same experiment was repeated with similar results.

lateral root primordia in the stele of these 6-day-old roots. Apparently the cells of root primordia accumulate large quantities of ^{32}P. The strong reduction of ^{32}P uptake by azide indicates that there is metabolic accumulation and not merely isotopic exchange of the radioactive phosphorus with the nonradioactive phosphorus already in the roots.

The oxygen uptake of cortical and stelar tissue of corn roots is shown in figure 3. The points that are connected by lines in the graph are the averages of 6 measurements (fig 3). The individual measurements are plotted around the averages.

The study indicates that the stele has a higher rate of O_2 uptake than the cortex in both fresh and aged tissues. There is a consistent decrease in respiration of both cortex and stele as the tissue

ages. Figure 4 is a comparison of ^{32}P uptake and respiration in separated fresh and aged tissue of stele and cortex. Both ^{32}P uptake and respiration are higher in fresh tissues than in the corresponding aged ones. Despite a significantly greater rate of respiration in both fresh and aged stele than in cortex there were no differences between the stele and cortex in regard to ^{32}P uptake (table I, column 1 and fig 4).
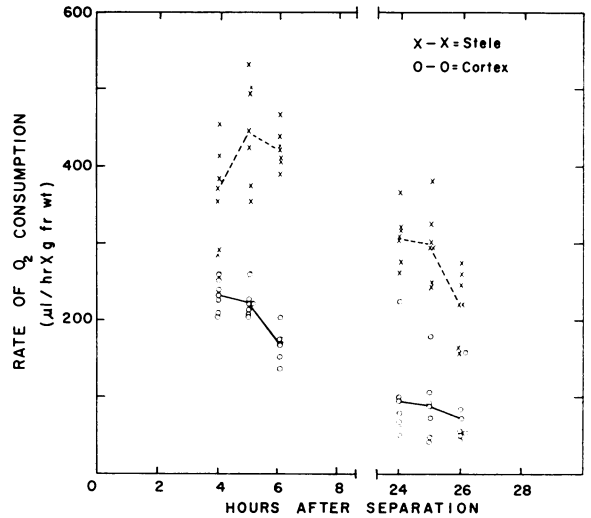

FIG. 3. Change in respiration with time of stele and cortex of 5-day-old roots.

Results of the study on the relative dehydrogenase activities of cortex and stele are shown in figure 5. It is noted that the root tip, lateral root primordia, pericycle and nearby regions displayed high activities. Stele tissue in general showed a higher activity than the cortex.

Table II. ${}^{32}P$ Uptake by Cortex and Stele of Intact Roots

The data for 4 and 8 hours were obtained from 2 different sets of roots.

Four hr uptake			Eight hr uptake				
Cortex	Stele	Exudate	Cortex	Stele	Exudate	Accumulation factor*	Volume of exudate
CPM/mg fr wt		CPM/sample	CPM/mg fr wt		CPM/sample		μl
11 2	8.4	48	613.7	701.5	3352.4	8.2	21.5
24.7	23.7	358	278.1	222.0	916.4	8.9	5.4
21.6	12.7	38	988.0	853.7	1021.1	8.3	6.5
26.7	13.3	57	460 5	481.7	3021.0	9.9	16.0
26.9	8.3	...**	397.7	363.0	3303.6	9.4	18.5
21.6	8.9	...**	294.6	108.3	687.5	3.9	9.2
22.4	8.4	...**	517.7	401.0	1051.0	10 2	5.4
25.8	8.5	57	641 8	380.0	1628.5	5.7	15.0
20.2	7.5	26	577.0	586.8	1604.7	8.9	12.0
37.9	16.5	105	458.7	458.0	4507.0	7.0	20.0

* Accumulation factor = ^{32}P conc. of the exudate/^{32}P conc. of root medium.
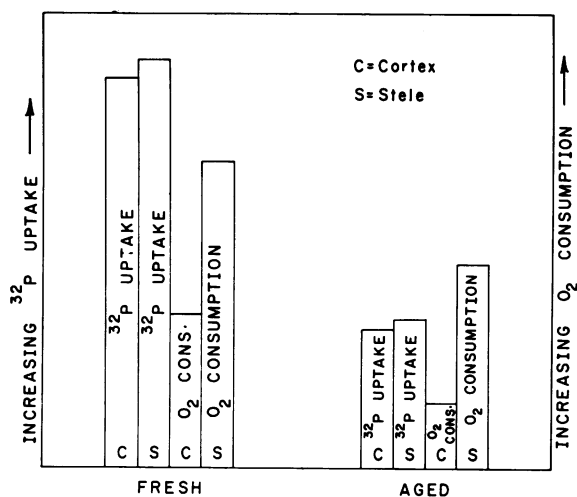** Not measured.

FIG. 4. Comparison of ^{32}P uptake and respiration in separated fresh and aged tissues of stele and cortex of 5-day-old corn roots. The actual values may be seen in table I, column 1 and figure 3.
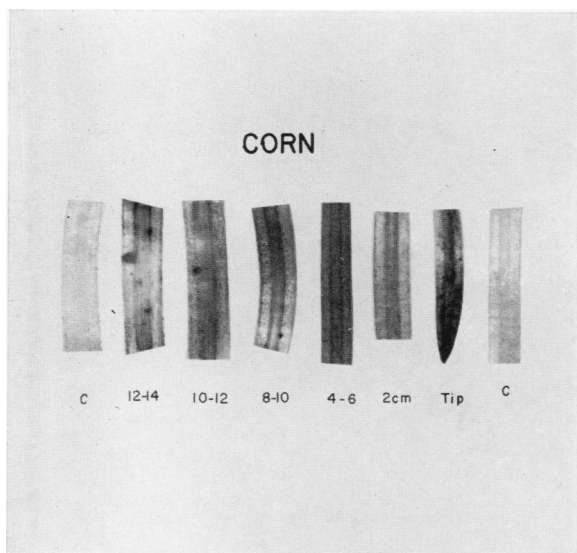

FIG. 5. Photomicrograph of central longitudinal sections of 6-day-old primary root treated with neotetrazolium salt, showing the relatively high dehydrogenase activity located in the stele, root tip and lateral root primordia. Numbers refer to distance from root apex, C refers to untreated control segments.

Discussion

The results obtained from these experiments are interesting when considered in relation to the root pressure theory of Crafts and Broyer (4) and the observation of Laties and Budd (10) on salt uptake by the stele. The results of the present experiments differ from those of Laties and Budd in 2 respects. First, they reported that steles removed from apical 2 cm segments of roots of 4-day-old seedlings initially have a very low salt accumulating capacity, but in the present experiments with older and longer root segments the stele accumulated salt as vigorously as the cortex. Second, they reported that the stele showed a dramatic increase in accumulation of salt after 24 hours of aging, but a decrease in salt accumulation of the aged stele and a corresponding decrease in respiration were observed in the present experiments with 5-day-old roots. Some experiments with 6-day-old roots showed an increase in salt uptake by the stele over the period 6 to 8 hours after separation, but uptake decreased in the 8 to 10 hour period (fig 2). In these experiments uptake of Cl^{-}, and they aged stele and cortex in by the cortex after 6 hours from beginning separation, possibly because of the large number of branch root primordia developing in these roots.

The difference in results obtained by Laties and Budd and by this laboratory may be due to differences in age and physiology of the roots and also different experimental conditions used in the 2 laboratories. Laties and Budd (10) studied the uptake of Cl^{-}, and they aged stele and cortex in 10^{-4}M $CaSO_4$ solution. In contrast to the behavior of corn roots, a gradual increase in salt uptake and respiration was observed in the separated stele of roots of *Vicia faba* L. Roots of this species show little exudation (unpublished results). Thus it is important to realize that what seems to be true of 1 kind of root may not be true of other kinds of roots. Even among roots of the same species, the physiology may differ at different developmental stages.

The high uptake of ^{32}P and O_2, and the high dehydrogenase activity of the cells of the stele of the corn roots used in these studies indicate that they are physiologically very active. They certainly have a high capacity to accumulate salt, whether in situ or when separated from the cortex, contrary to the claim of Laties and his colleagues. Unfortunately, although our data raise doubts concerning the passive leakage of salt out of the cells of the stele into the xylem elements, they do not provide an alternative theory to explain the radial transfer of salt. The high physiological activity observed in the tissue of the stele suggests that it may play an active role in radial transport of salt rather than the passive one proposed by the Crafts-Broyer theory. It is regrettable to conclude that little more is known today than was known 2 or 3 decades ago about the mechanism of radial transport of salt into the xylem vessels, because collecting nutrients from the substrate and subsequently distributing them to the whole plant is one of the most important functions of roots. The

results of this study suggest that a critical re-examination of the theories about the mechanism of radial transport of salt into xylem vessels is needed.

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