Lateral Transport of Ions into the Xylem of Corn Roots

II. EVALUATION OF A STELAR PUMP[1]

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ABSTRACT

When an excised corn (*Zea mays*) root pretreated with chloride was exposed for 10 minutes to pulse labeling with ^{36}Cl and then transferred to unlabeled chloride, the activity in the xylem exudate reached a maximum about 4 minutes after pulse labeling was discontinued and then declined sharply. The rate at which labeled chloride was transported across the root into the xylem and basipetally therein was on the order of 75 to 250 centimeters per hour. Consequently, symplasmic movement of chloride in corn roots is fast and may not be rate-limiting in transfer from the root surface to the xylem. Experiments on pulse labeling with ^{22}Na gave similar results. A large fraction of the absorbed ^{22}Na was not translocated into the exudate but was tightly sequestered in a cell compartment, probably the vacuole.

Electron probe analysis was used to reveal the pattern of potassium distribution in cross sections taken 10 to 11 millimeters from the tip. The cytoplasm and vacuoles of the xylem parenchyma cells accumulated potassium to a much greater extent than cortical and other stelar cells. Ultrastructural studies showed that the cytoplasm of the xylem parenchyma cells contains numerous membrane systems. It was concluded that the xylem parenchyma cells secrete ions from the symplasm into the conducting vessels, and it was suggested that this secretion is driven across the plasmalemma by a carrier-mediated transport.

According to the classical Crafts-Broyer hypothesis on lateral transport of ions in roots (11), the stele does not participate actively in transfer from the symplasm to the xylem vessels. The membranes of the stelar cells are deemed permeable to ions, and hence ions simply leak into the vessels. The Casparian strip of the endodermis functions to prevent ions from diffusing backwards into the outer space of the cortex.

Laties (25), Laties and Budd (26), and Lüttge and Laties

(33) found that freshly isolated steles of corn roots do not accumulate ions. They concluded that the stele *in situ* probably is ineffective in salt absorption, and hence leaky in intact roots (25). In contrast, Yu and Kramer (45, 46) demonstrated that steles of intact roots do accumulate ions. Therefore, it is doubtful that the physiological state of freshly isolated steles reflects that of steles of intact roots. Much evidence cited in the "Discussion" of the present paper lends support to the concept that the stele has an active role in salt transfer to the xylem. Moreover, the use of microautoradiography and electron probe analysis has produced direct evidence that certain cells in the stele, mainly parenchymatous, are able to accumulate ions and may be involved actively in transport to the vessels (29 and references therein).

In this report, experiments are described using pulse labeling and electron probe analysis which support the view that stelar parenchyma cells accumulate ions and secrete them into the xylem vessels.

MATERIALS AND METHODS

Eight-day-old seedlings of corn (*Zea mays*, DeKalb 805) grown as described in the preceding paper (31) were used in the experiments.

Pulse Labeling. Three kinds of pulse labeling experiments using ^{36}Cl and ^{22}Na were conducted. In the experiments on the effect of preloading roots with unlabeled Cl, the roots of an intact plant were first exposed for 3 hr at 30 C to an aerated solution containing 0.5 mM KCl (unlabeled) and 0.5 mM CaSO$_4$ (preloaded roots, cf. 31). This pretreatment was omitted in the unpreloaded run. Thereafter, the experimental regime was identical in both runs. A root approximately 20 cm in length was excised, rinsed for 1 min with 0.5 mM CaSO$_4$, and submerged for 10 min in an aerated solution at 30 C which contained 0.5 mM KCl, the Cl being labeled with ^{36}Cl, and 0.5 mM CaSO$_4$, for pulse labeling. The root was then rinsed for 1 min with 0.5 mM CaSO$_4$, and a length of polyethylene tubing was attached to it and trimmed to leave a 2-mm sleeve. A 10-µl microcapillary tube was inserted into the upper end of the sleeve. The root was then transferred into an aerated solution of 0.5 mM KCl (unlabeled) and 0.5 mM CaSO$_4$ at 30 C to obtain serial collections of 10-µl volumes of xylem exudate. The depth of immersion of the root was about 18 cm. The exudate was radioassayed for ^{36}Cl as described in the preceding paper (31).

When the activity of the exudate dropped to very low readings, the root was rinsed for 1 min with deionized water, weighed, and dried in a polyethylene vial. Fifteen milliliters of 5 mM 7-amino-1,3-naphthalenedisulfonic acid were added, and ^{36}Cl was assayed by Cerenkov counting (31).

A second series of pulse labeling experiments with ^{36}Cl was

conducted as follows. Only preloaded roots were used. The root was excised, rinsed for 1 min with 0.5 mM CaSO₄, and attached to the polyethylene sleeve, and a 2-μl microcapillary tube was inserted. The root was exposed for 10 min to an aerated solution containing 0.5 mM KCl, the Cl being labeled with ^{36}Cl, and 0.5 mM CaSO₄ for pulse labeling. The labeled solution was again kept at 30 C. Pulse labeling was terminated by washing the root, still attached to the exudate collection assembly, with five changes of a chemically identical but unlabeled solution for a total period of 1 min. The root was then placed in a large volume of an identical, unlabeled solution. Serial collections of 2-μl volumes of exudate were obtained throughout the periods of pulse labeling and exposure to unlabeled solutions. Exudates and roots were radioassayed for ^{36}Cl as described above.

In the pulse labeling experiments with ^{22}Na, the procedure was similar. Preloading was done in an aerated solution containing 5 mM NaCl (unlabeled) and 0.5 mM CaSO₄ at 30 C for 2 hr. After excision, rinsing, and attachment of a 10-μl microcapillary tube, the root was exposed for 10 min to an aerated solution containing 5 mM NaCl, the Na being labeled with ^{22}Na, and 0.5 mM CaSO₄ for pulse labeling. This was done also at 30 C. Pulse labeling was terminated by washing the root with five changes of a chemically identical but unlabeled solution for a total period of 1 min. The root was then placed in a large volume of an identical, unlabeled solution. Serial collections of 10-μl volumes of exudate were obtained throughout pulse labeling and exposure to unlabeled solutions. The exudates were transferred quantitatively to planchets, dried, and counted with a gas flow detector. The root samples also were transferred to planchets, dried, ashed at 500 C, and counted with a gas flow detector.

Electron Probe Analysis. Some experimental root material which was grown to study transport of ions into the exudate under steady state conditions (31) was used to determine the distribution pattern of ions through electron probe analysis. The root of an intact plant was pretreated for 3 hr at 30 C with an aerated solution containing 0.2 mM KCl and 0.5 mM CaSO₄. The root was then excised and a length of polyethylene tubing was attached to it. The root was allowed to exude fluid from its basal end for 1 hr from an identical solution. Finally, the root was rinsed for 1 min with deionized water and processed for electron probe analysis.

A new technique was developed to prepare plant specimens for electron probe analysis (32). Briefly, root segments 1 mm in length were cut in the cold (4 C) 10 to 11 mm from the root apex and immediately frozen at the temperature of liquid N₂. For dehydration, the freeze-substitution technique involving anhydrous ether at −30 C was utilized (cf. 35). Krichbaum et al. (24) have demonstrated with microautoradiographs that freeze-substitution with ether and the more frequently used technique of freeze-drying yield identical results. The dehydrated specimens were gradually infiltrated with Spurr's low viscosity epoxy resin embedding medium E (40) at temperatures below 0 C. Final embedding and curing were done as described by Spurr (40).

Anhydrous sectioning was accomplished with a Cambridge ultramicrotome, using hexylene glycol in the microtome trough. One- and 2-μ sections were cut with a diamond knife and mounted on the polished surface of a Be disc (American Beryllium Corp., Inc., Sarasota, Fla.). Beryllium was used as supporting material because its background readings in electron probe analysis are low and excellent discharge of current and heat is achieved while the section is exposed to the electron beam (32). The sections on the Be discs were vacuum coated with 150 to 200 Å of Al.
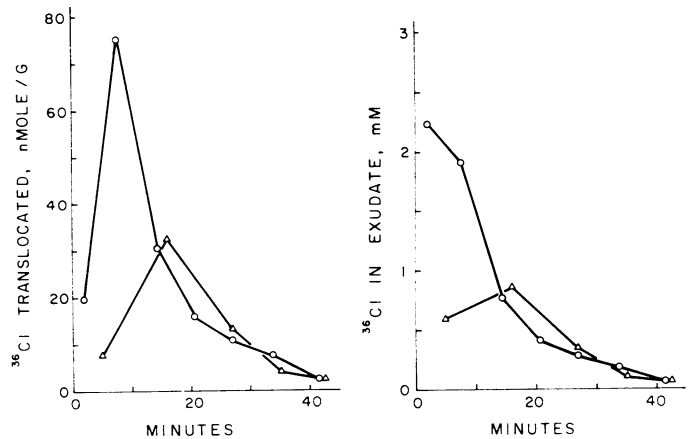
Measurements were conducted with an Applied Research

FIG. 1. Translocation of ^{36}Cl across corn roots and ^{36}Cl accumulation in the xylem exudate *versus* time after ^{36}Cl pulse labeling for 10 min. The root of an intact plant was either pretreated for 3 hr with 0.5 mM KCl (preloaded root, ○) or pretreatment was omitted (unpreloaded root, △). Pulse labeling solution: 0.5 mM KCl labeled with ^{36}Cl. Unlabeled solution: 0.5 mM KCl. All solutions contained also 0.5 mM CaSO₄, 30 C, aerated. The volumes of the first exudate sample were 2.2 μl (○) and 3.5 μl (△), respectively. Volumes of all other exudate samples were 10 μl. Data are values obtained with single roots. See "Results" for significance of "^{36}Cl translocated" and "^{36}Cl in exudate."

Laboratories electron probe analyzer, model EMX-SM, with scanning capabilities. The instrument was operated at 15 KeV accelerating voltage, 0.5 μ electron beam diameter, and 0.023 μa sample current on Be.

Epoxy resins based on epichlorohydrin contain small amounts of Cl (32). The measurements were therefore confined to K. As had been found earlier (28), the "line scanning" technique was most suitable to evaluate the pattern of K distribution as related to K transfer into the xylem. Line scans are obtained by moving the specimen along a line of traverse under a static electron beam and recording the corresponding intensity of x-ray radiation of a particular chemical element.

Transmission Electron Microscopy. Monitor castings for transmission electron microscopy were obtained by a procedure slightly altered from that described above. Tissue segments were freeze-substituted with ether as described, washed with acetone at −30 C, and fixed overnight with 1% OsO₄ in acetone at −70 C. The fixed segments were washed with acetone, warmed to −30 C, and infiltrated and embedded. Standard thin sectioning procedures were used. Thin sections were contrasted with uranyl acetate and lead citrate and examined in an RCA EMU G electron microscope at 50 KeV.

RESULTS

Pulse Labeling. The effect of pretreating roots for 3 hr with 0.5 mM KCl on the exudation of ^{36}Cl after pulse labeling is shown in Figure 1. On the left side, the amounts of ^{36}Cl are plotted *versus* collection times. The corresponding concentrations of ^{36}Cl in the exudate are given on the right side of Figure 1. The first exudate collection comprised only a fraction of 10 μl, i.e., 2.2 μl for the preloaded root and 3.5 μl for the unpreloaded root. This permitted an estimate of the ^{36}Cl concentration in the exudate at a time shortly after pulse labeling was discontinued. Thereafter, each collection represents 10 μl of exudate. Amounts of ^{36}Cl exuded are calculated on the basis of 1.00 g fresh weight of root, and on the basis of the specific radioactivity of the chloride solution used in the pulse labeling. That is, only that Cl is included in the measurement
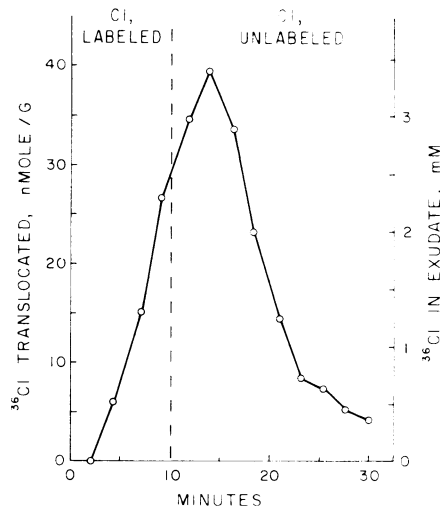
FIG. 2. Kinetics of transfer of a ^{36}Cl pulse across corn roots. Root of the intact plant pretreated for 3 hr with 0.5 mM KCl. Pulse labeling solution: 0.5 mM KCl labeled with ^{36}Cl. Unlabeled solution: 0.5 mM KCl. All solutions contained also 0.5 mM CaSO₄, 30 C, aerated. Volumes of exudate samples: 2 μl. Data are values obtained with a single root. See "Results" for significance of "^{36}Cl translocated."
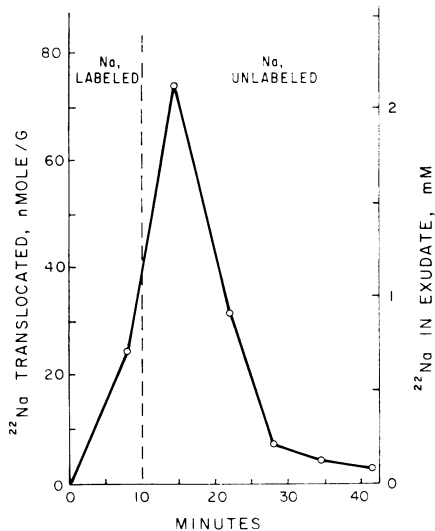

FIG. 3. Kinetics of transfer of a ^{22}Na pulse across corn roots. Root of the intact plant pretreated for 2 hr with 5 mM NaCl. Pulse labeling solution: 5 mM NaCl labeled with ^{22}Na. Unlabeled solution: 5 mM NaCl. All solutions contained also 0.5 mM CaSO₄, 30 C, aerated. Volumes of exudate samples: 10 μl. Data are values obtained with a single root. See "Results" for significance of " ^{22}Na translocated."

which was furnished by the pulse labeling solution. For the kinetics of steady state Cl transport in such experiments, see the previous paper (31). From the unpreloaded root there was a slow rise in ^{36}Cl exudation after pulse labeling. The radioactivity peak was reached after 16 min, the ^{36}Cl concentration in the exudate being 0.85 mM (calculated on the basis of the specific radioactivity of the pulse labeling solution). Thus, only a slight accumulation of ^{36}Cl took place in the exudate compared with the external concentration. Thereafter, the content of labeled Cl in the exudate declined slowly and steadily. A large fraction (1.5 μmoles/g) of ^{36}Cl which was taken up during pulse labeling was retained in the root and was not translocated into the exudate.

When a root was pretreated with unlabeled KCl solution and then exposed to pulse labeling, a sharp peak of ^{36}Cl translocation was observed about 8 min after the root was transferred to the unlabeled solution. The amount of ^{36}Cl translocated decreased thereafter very rapidly. This was reflected more clearly in the concentration of ^{36}Cl in the exudate, where the ^{36}Cl had accumulated about 5-fold when pulse labeling was discontinued (at 0 min on time scale). Thereafter, the ^{36}Cl concentration in the exudate dropped below 0.5 mM within 20 min. The preloaded root retained only 0.5 μmole of ^{36}Cl per g. In other words, rapid and efficient transport of ^{36}Cl across the root from a short pulse treatment occurred only when the vacuoles of the root cells were preloaded with Cl (cf. 31). With pulse labeling of a preloaded root, it should then be possible to obtain an estimate of the rate of transport of Cl from the root surface to the cut basal end of the excised root. The results of such an experiment are presented in Figure 2.

Since exudate was collected throughout this experiment, including the period of pulse labeling, and exudate samples as small as 2 μl were collected, an accurate picture of the appearance of the ^{36}Cl pulse in the exudate was obtained. Two minutes after the beginning of the radioactive pulse, labeled Cl appeared in the exudate, and its concentration rose rapidly, reaching a maximum about 4 min after the pulse was discontinued. At that time the concentration of ^{36}Cl in the exudate exceeded that in the medium by 7-fold. The content of labeled Cl then declined sharply to a low level of about 5 nmoles of ^{36}Cl per g within the next 15 min. The labeled Cl retained in the root amounted to 0.35 μmole/g, which agrees fairly well with 0.5 μmole/g retained in the preloaded root of Figure 1.

From the time course of the appearance of the pulse in the exudate, which has the shape of a wave (Fig. 2), the rate of translocation of labeled Cl across the root into the xylem and basipetally therein was estimated. Four minutes after the root was placed in the labeled solution, the ^{36}Cl concentration in the exudate reached that of the external solution, viz., 0.5 mM. Also, the peak of activity was reached 4 min after termination of the pulse. Thus it took the root 4 min to move ^{36}Cl from the surface to the point of excision. This root had a length of 20 cm. On the assumption that the main lateral transfer of ions occurs in the apical 5 cm (2), the rate of transport was on the order of 250 cm/hr. Even on the assumption that the longitudinal distance traveled was only 5 cm, the rate of transport was high, viz., 75 cm/hr.

The results of an experiment on pulse labeling with ^{22}Na are shown in Figure 3. Amounts translocated were calculated on the same basis as described for Cl; i.e., they include only that Na which came from the pulse labeling solution. The time course of the appearance of ^{22}Na in the exudate was similar to that of ^{36}Cl (cf. Fig. 2), with the exception that the activity of ^{22}Na in the exudate virtually became nil within about 30 min after termination of pulse labeling, whereas with ^{36}Cl, there was some transfer into the exudate for extended periods of time after the pulse. A large fraction (0.48 μmole/g) of the labeled Na taken up during pulse labeling was not transmitted to the exudate but was tightly sequestered in a cell compartment, presumably the vacuole.

Electron Probe Analysis. Figure 4 presents a transverse section, 2 μ thick, cut 10 to 11 mm from the root apex and mounted on a Be disc. The tissues appeared to be preserved well. A similar section was examined by the line scanning technique on the electron probe analyzer, and the result is shown in Figure 5. As the electron beam impinged upon the epidermis, a high K concentration became evident. Potassium concentrations in the cortex were much lower. However, simultaneous observation of the position of the specimen relative to the beam, and of the strip chart recorder, showed that the

peaks in the cortex corresponded generally with the cell vacuoles. The cytoplasm of the cortical cells did not appear to contain much K. The K content of the endodermis was low. On the other hand, the cells of the pericycle and other stelar parenchymatous tissue accumulated K to a large extent. Note that the peak in the xylem parenchyma cell next to the vessel went off scale. Fine adjustments of the specimen position revealed no significant differences in K concentration between cytoplasm and vacuole of the xylem parenchyma cells. The lumen of the xylem vessel did not contain high amounts of K, a result which was expected because the vessels were open at the cut end of the root. Rinsing the root for 1 min prior to freezing and possible infiltration of the root segment with liquid embedding medium allowed the solutes in the vessels to wash out.
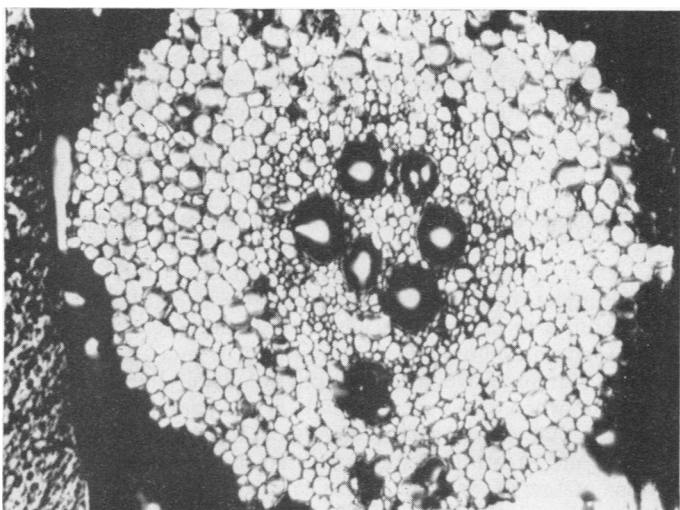

FIG. 4. Transverse section, 2 μ thick, of a corn root taken 10 to 11 mm from the root apex. Tissue frozen at -170 C, freeze-substituted with anhydrous ether and embedded in low viscosity epoxy resin. Section mounted on a Be disc, coated with 150 to 200 Å of Al and photographed by reflected light microscopy. No heavy metal fixation or staining. × 136.
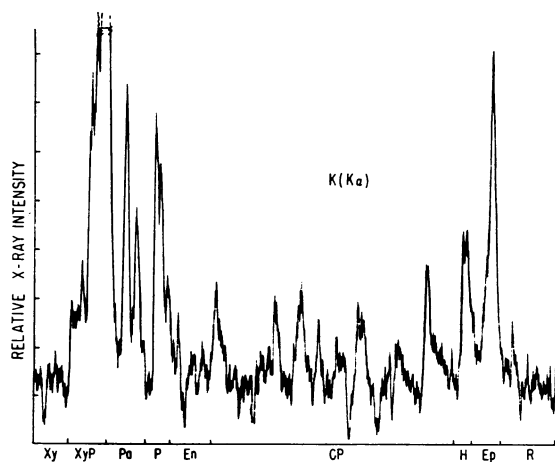

FIG. 5. Line scan of K (Kα) radiation across a corn root section similar to that shown in Figure 4. Electron probe x-ray analyzer operated at 15 KeV accelerating voltage, 0.5 μ beam diameter, and 0.023 μa sample current on Be. R: Epoxy resin outside of tissue; Ep: epidermis; H: hypodermis; CP: cortical parenchyma; En: endodermis; P: pericycle; Pa: unspecified stelar parenchymatous tissue; XyP: xylem parenchyma; Xy: xylem vessel.
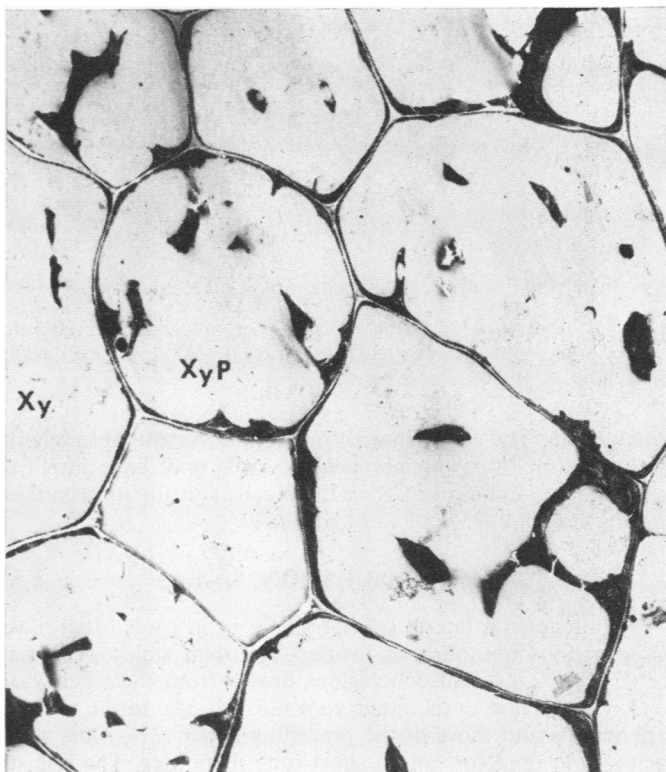

FIG. 6. Micrograph by transmission electron microscopy of corn root prepared by the freeze-substitution technique. Portion of the primary xylem in transverse section. Root segments treated with OsO$_4$ during processing; sections contrasted with uranyl acetate and lead citrate. Xy: Differentiating xylem vessel; XyP: xylem parenchyma. × 1200.

Similar line scans were recorded for many other sections also taken about 10 mm from the root apex. The results thus obtained were qualitatively always the same as those in Figure 5, although some quantitative variabilities were apparent. The epidermis did not always contain large amounts of K, and the K content of the endodermis appeared highly variable. However, the cytoplasm of the cortical cells was consistently low in K, and the amounts of K accumulated in the xylem parenchyma cells just outside the vessels exceeded by many times those of any other cell type.

Ultrastructure of Xylem Parenchyma Cells. Thin sections of freeze-substituted tissue segments embedded in epoxy resin were examined by transmission electron microscopy. A low magnification electron micrograph depicting a portion of the primary xylem in transverse section is shown in Figure 6. Note that the configuration of the cells appears normal despite freeze-substitution with ether, and the cytoplasm is not displaced from the cell walls; but there are minor indications of cytoplasmic shrinkage. The xylem parenchyma cells are much smaller than the cortical cells (see Fig. 4). At this level in the root the vacuoles of the xylem parenchyma cells are traversed by protoplasmic strands. Note also that the xylem vessel (Fig. 6) still is not fully differentiated.

Figure 7 represents a portion of a xylem parenchyma cell. Although conventional uranyl and lead contrasting methods were used, a negative staining effect was obtained, apparently owing to the initial processing. The organelles and membrane systems such as the endoplasmic reticulum, though not well defined, are abundant (Fig. 7). Anderson and House (2) studied corn roots by conventional electron microscopy techniques and demonstrated the presence of mitochondria in the xylem
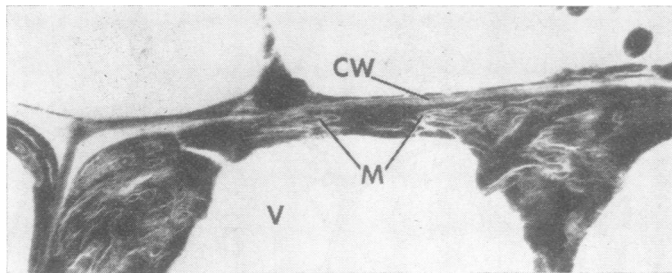
FIG. 7. Xylem parenchyma of corn root prepared as in Figure 6. Membrane systems are lightly contrasted (M). CW: Cell wall; V: vacuole. × 30,600.

parenchyma. The abundance of membrane systems throughout the cytoplasm of xylem parenchyma cells may have implications for accumulation of K in these cells and for its secretion into the vessels.

DISCUSSION

Experiments on lateral transport of ions in roots often have raised serious questions of artifact, as when steles were dissected from roots and conclusions drawn from their behavior were extrapolated to the intact root (26, 33, 34). In the present experiments and those of the preceding paper (31), roots were excised and used for only a short time thereafter. The risk of artifact thus was minimized. Furthermore, Anderson and Allen (1), specifically addressing themselves to this problem, and also using corn seedlings, have shown that the potassium flux through excised roots corresponds very closely with the flux of potassium into the shoots of intact seedlings.

From pulse labeling experiments with roots transporting Cl in the steady state (Fig. 2) it was estimated that the overall rate at which Cl is transported across the root into the xylem and basipetally therein is on the order of 75 to 250 cm/hr. Sodium appears to be transported at approximately the same rate (Fig. 3). It has also been shown that the lateral transport of Cl in roots takes place in the symplasm (31). This symplasmic movement may not be the rate-limiting step in ion translocation from the root surface to the conducting vessels. In view of the high rate at which ions move across the root to the point of excision, the plasmodesmata do not seem to present a high resistance to flow such as has been deduced on the basis of recent ultrastructural studies (38).

If the concept of Crafts and Broyer (11) is valid, active transport of ions across the plasmalemma into the cytoplasm would lead to an accumulation of ions in the cytoplasm of the cortical cells. Furthermore, one would expect the ionic concentration of the cytoplasm to be lower in the endodermis and in the stelar cells than in those of the cortex, since lateral transport in the symplasm to the xylem was thought to occur by diffusion along a concentration gradient (11).

Insofar as the role of the endodermis is concerned, electron probe analysis of K distribution revealed a low K content (Fig. 5). Other evidence also indicates that the endodermis plays only a passive role by interrupting the outer space between cortex and stele by means of the Casparian strip. Bonnett (9) has demonstrated in electron micrographs that the endodermal cells of Convolvulus roots are not likely to be active metabolically, but that the symplasm extends through the endodermal layer. Plasmodesmata have been found also in the endodermis of barley roots (20). With an elegant fluorescent staining technique, the Casparian strip in roots of Avena and Ipomoea has been shown to be complete (36). Such structural evidence is supplemented by the physiological results of Krichbaum et al. (24), who demonstrated that the Casparian strip effectively interrupts the outer space between cortex and stele in corn roots.

When the concentration of K in the cytoplasm of the stelar parenchyma cells is compared with that in the cortical cells, a condition is found that is opposite to that expected from Crafts and Broyer's (11) concept. The stelar parenchyma cells in general, and the cytoplasm and vacuoles of the parenchyma cells of the xylem in particular, contain K at higher concentrations than do the cortical cells (Fig. 5). This cannot be reconciled with the view of other investigators (11, 25, 26, 33), who hold that the stelar cells play only a passive role in ion transfer to the xylem vessels. The rapid response of the rate of exudation to inhibitors (4, 31) also suggests that some process other than ion absorption in the cortex is implicated. Mere passive leakage from stele cells into the xylem vessels would be expected to respond much more gradually to the application of an inhibitor if the effect of the latter were confined to diminishing the rate at which ions are supplied to the stele from the cortex.

Our results are in accordance with those of Yu and Kramer (45, 46), who showed that the stele is able to accumulate ions. Their findings and the present ones do not support the conclusion of Laties and Budd (26) and Lüttge and Laties (33) that freshly isolated steles are physiologically comparable to steles of intact roots. Tissue excision has been reported to cause mitochondria to disintegrate and to become inactive (43) and the endoplasmic reticulum to be reduced to small cytoplasmic vesicles (23). Such structural and physiological alterations may also occur when steles are isolated. Furthermore, there is no unanimity that excised stele is inactive in ion transport (3).

Crafts and Broyer (11) suggested that the stele may leak ions because of low O_2 tension. Fiscus and Kramer (15) now have evidence that the stele of roots operates at O_2 tensions which are suboptimal for respiration. These authors, however, discussed the possibility that the internal root tissues are able to adapt so that they can function adequately with a low supply of O_2. Moreover, in intact plants O_2 moves from the shoot to the root through continuous gas spaces (6).

Studies using methods for localizing radioisotopes and chemical elements in tissues and cells, i.e., microautoradiography and electron probe analysis, strongly favor our concept that stelar cells accumulate ions and pump them into the xylem vessels. Earlier work with the electron probe analyzer (28, 29) revealed that the stele of corn roots had higher concentrations of K and P than did the cortex and indicated that there was an upward concentration gradient from the endodermis to the xylem (29). With the modified technique used in this investigation, it appears that the parenchymatous cells of the xylem just outside the vessels are involved directly in the secretion of ions to the vessels (Fig. 5). Studies involving microautoradiography have disclosed that the stele of roots can accumulate P (13) and Na (39) and that the cells of the xylem parenchyma have the ability to accumulate ions such as SO_4 (16, 17, 44), Ca (8), and Fe (10). Even in isolated steles the xylem parenchyma was more labeled with $^{35}SO_4$ than the adjacent vessels (34).

The question of whether the accumulation of K in the epidermis, as shown in Figure 5 of this investigation and in earlier studies with electron probe analysis (28, 29), points to an active function of the epidermis in lateral ion transport cannot be answered conclusively. Krichbaum et al. (24) observed that ions which accumulated in the epidermis of corn roots could be washed out almost completely with deionized water.

This finding needs to be reevaluated by using washing solutions which contain Ca (cf. 30). The high concentration of P in the epidermis (28, 29) may be the result of bacterial contamination (7, 12). In the case of K, however, microbial contamination does not appear to affect its absorption by roots (14). Hall (19) demonstrated a high ATPase activity in the epidermis of roots of corn, barley, and broad bean and tentatively related this to active ion transport. It is not clear, however, if Hall's ATPase was salt-stimulated. This enzyme may be involved in differentiation, as has been suggested for the unspecific enzyme acid phosphatase (27).

It is concluded that findings of other investigations can be reconciled with the present evidence which assigns to the xylem parenchyma cells an important function in the transfer of ions from the symplasm to the vessels. Interestingly, House and Findlay (22) placed the chief permeability barrier to the transport of water also within the cellular layer surrounding the vessels. Subsequent studies from the same laboratory (2, 3) were interpreted to indicate that the cytoplasm and membrane systems of the living differentiating vessels may be the final sites of salt secretion into the exudate. Those results, however, may be interpreted in our terms as well.

One can only speculate as to possible mechanisms by which ions are secreted from a xylem parenchyma cell into a vessel. Are there transport sites in the cytoplasm of stelar cells comparable with those which have been suggested by Hodges and Vaadia (21) or similar to microvacuoles in salt glands (42)? Are the numerous membrane systems found in the cytoplasm of xylem parenchyma cells (Fig. 7) related to the secretion of ions? In any event, it is likely that the plasmalemma facing the common wall between the vessel and the xylem parenchyma cell mediates ion secretion; hence, a xylem parenchyma cell of a root may function as do the transfer cells described by Gunning and Pate (18). Two kinds of evidence suggest, though they do not prove, that the plasmalemma is the site of ion secretion into the vessels. First, transfer of K into the xylem is selective against Na (5, 37, 41), and selectivity is explained best by means of carrier mechanisms which reside in the plasma membrane. Second, it was shown in the preceding paper (31) that the kinetics of the lateral transport of Cl across corn roots resemble the kinetics of absorption by the root cells. Consequently, it is conceivable that a root has two principal sites for active transport mechanisms—one at the plasmalemma of the cortical cells at which the carrier-mediated transport is directed inward, and the other at the plasmalemma of the xylem parenchyma cells facing the vessels where transport is directed outward, i.e., from the cytoplasm into the walls and the vessels. The phenomenon of root pressure would then be the direct consequence of this carrier-mediated secretion of ions into the conducting vessels.

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