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Potassium Transport in Corn Roots[1]

II. THE SIGNIFICANCE OF THE ROOT PERIPHERY

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ABSTRACT

The relative transport capabilities of the cells of the root periphery and cortex were investigated using a variety of experimental techniques. Brief (30 seconds to 1 minute) exposures with the penetrating sulfhydryl reagent, *N*-ethyl maleimide (NEM), and the impermeant reagent, *p*-chloromercuribenzene sulfonic acid (PCMBS), dramatically reduced $^{86}Rb^+$ (0.2 millimolar RbCl) uptake into 2 centimeter corn (*Zea mays* [A632 × (C3640 × Oh43)]) root segments. Autoradiographic localization studies with [^{3}H]NEM and [^{203}Hg]PCMBS demonstrated that, during short term exposures with either reagent, sulfhydryl binding occurred almost exclusively in the cells of the root periphery.

Corn root cortical protoplasts were isolated, and exhibited significant K^+($^{86}Rb^+$) influx. The kinetics for K^+ uptake were studied; the influx isotherms were smooth, nonsaturating curves that approached linearity at higher K^+(Rb^+) concentrations (above 1 millimolar K^+). These kinetics were identical in shape to the complex kinetics previously observed for K^+ uptake in corn roots (Kochian, Lucas 1982 Plant Physiol 70: 1723–1731), and could be resolved into a saturable and a first order kinetic component.

The existence of a hypodermal apoplastic barrier was investigated. The apoplastic, cell wall binding dye, Calcofluor White M2R, appeared to be excluded from the cortex by the hypodermis. However, experiments with damaged roots indicated that this result may be an artifact resulting from the binding of dye to the epidermal cell walls. Furthermore, [^{203}Hg] PCMBS autoradiography demonstrated that the hypodermis was not a barrier to apoplastic movement of PCMBS.

These results suggest that although cortical cells possess the capacity to absorb ions, K^+ influx at low concentrations is limited to the root periphery. Cortical cell uptake appears to be repressed under these conditions. At higher concentrations, cortical cells may function to absorb K^+. Such a model may involve regulation of cortical cell ion transport capacity.

The pathway of ion movement from the soil solution to the stele is thought to involve both the symplast and apoplast of the root cortex. Both epidermal and cortical cells may be able to absorb ions from the cell wall solution. Thus, ions are believed to move to the endodermis via either diffusion through the root free space, or by symplastic movement, presumably through the plastmodesmata, or by a combination of the two routes. Influx across the plasmalemma of any of the cells lining the free space would allow entry into the symplast; subsequent flux across the endodermis would proceed exclusively via the symplast. Although this model has general acceptance (5, 7, 15, 27), dissension does exist in the literature.

The ability of root cortical cells to absorb ions from the apoplastic solution has been questioned. Vakhimistrov (28) has suggested that the cortical cells play an insignificant role in ion uptake, with most transport occurring at the epidermal cells. Using a theoretical model for Rb^+ uptake in barley roots, Bange (3) found that the model only generated reasonable uptake kinetics when transport was restricted to the epidermis. The recent work of van Iren and Boers-van der Sluij (29) also supports this hypothesis. Their work was based on the assumption that plasmolysis severs plasmodesmata, and therefore would symplastically isolate each cortical and epidermal cell of a barley root. Autoradiographic localization of $^{86}Rb^+$, accumulated following plasmolysis, was generally limited to the root periphery.

Some workers have also suggested that the kinetics usually presented for ion uptake into roots may be explained as the basis of a complex interplay between diffusion and uptake within a multilayered tissue (4, 6, 10, 21, 25, 26). In the model presented by Göring and coworkers (6, 10), uptake at low concentrations would approximate the saturation kinetics of carriers located at the root periphery, whereas uptake at higher concentrations would be complicated by diffusion-limited availability of ions for carriers located within the cortex. In support of this hypothesis, Grunwaldt *et al.* (12) found that in corn roots, osmotic shock selectively damaged the cells of the root periphery. Following such a treatment, phosphate uptake was severely inhibited only at low external phosphate concentrations (13).

In the older roots of most monocots and some dicots, the presence of a hypodermis has been demonstrated (8, 9, 22). In detailed work involving fluorescence microscopy of cell walls and studies on the movement of apoplastic dyes in corn and onion roots, Peterson and coworkers have demonstrated that the hypodermis appears to be an apoplastic barrier to the diffusion of high mol wt dyes which bind to cell walls (22–24). Furthermore, they found that all radial and transverse walls of the hypodermis contained what appeared to be a Casparian band. Although most of this work has been done with the older root regions, the possibility exists that such an apoplastic barrier could be present in the regions engaged in ion uptake. Such a barrier could cause uptake to occur primarily at the root surface, even though cortical cells have the ability to absorb ions.

Any attempt to integrate anatomical and physiological evidence to produce a coherent model for ion movement through the root gives rise to a complicated scheme. In part, this complexity is due to the ambiguity which exists concerning the basic mechanism(s) by which ions are transported across the plasmalemma. In a recent study of the kinetics of K^+ transport into corn roots, we presented data consistent with uptake being due to the combined effect of a saturable and first order kinetic process (14). Brief exposure (30 s) of the root to sulfhydryl reagents caused a dramatic inhibition of K^+ uptake. This indi-

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cated that these sulfhydryl reagents could be used to further resolve the question concerning the site of entry of K^+ into the root symplast. In the present paper, we have used these reagents, along with a wide range of other experimental techniques, to investigate the relative contribution of the root periphery and cortex to K^+ uptake in corn roots. These studies indicate that individual cells of the epidermis and cortex have the ability to transport K^+. However, under physiological conditions, the epidermis appears to be the main site of K^+ entry into the root symplast.

MATERIALS AND METHODS

Plant Material. *Zea mays* seeds (A632 × [C3640 × Oh43], Crows Hybrid Corn Co., Milford, IL) were imbibed, germinated, and grown by the methods previously outlined (14). Seedlings were either grown on a 0.2 mM $CaSO_4$ solution (low salt conditions) or 0.2 mM $CaSO_4$ + 5 mM KCl (high salt conditions). Primary roots of 5-d-old seedlings were used for all experiments.

Influx Experiments. Short term (10 or 30 min) $^{86}Rb^+$ influx experiments were performed using 2-cm root segments as detailed previously (14). Briefly, experiments were performed with 2-cm root segments cut from the 1st through 8th cm of the primary root. Uptake was initiated by the addition of $^{86}Rb^+$ (as RbCl; New England Nuclear) and terminated by the vacuum withdrawal of radioisotope solution. Free space radiolabel was removed by two 8-min washes in ice cold 0.5 mM $CaSO_4$ + 1 mM RbCl.

In experiments examining the effects of various exposures of the permeant (NEM[2]) and impermeant (PCMBS) sulfhydryl reagents on Rb^+ influx, the protocol was identical with that previously described (14). Additionally, $^{86}Rb^+$ uptake kinetic studies were performed on segments cut from intact low salt roots and from roots which had been split in half. A special apparatus was constructed which enabled us to position a root in place, pierce the longitudinal axis of the root with a surgical blade, and pull the root past the edge of the blade. Thus, we were able to split roots cleanly and precisely into 10-cm long half roots. These half-roots were cut into 2-cm segments prior to being used in uptake experiments.

NEM Effects on Respiration. Root respiration was followed by measuring O_2 consumption with a Clark O_2 electrode and monitor (Rank Bros.; Cambridge, England). Two-cm root segments were washed for 4 h and then 0.1 g of root tissue was placed in 4 ml of air-saturated solution (0.2 mM $CaSO_4$). O_2 consumption was measured for 20 min at 25°C. NEM was added to a final concentration of 0.3 mM, and after various exposures, DTE (1 mM final concentration) was added to bind unreacted NEM. Stable O_2 consumption was measured for at least 20 min following NEM treatment.

[^{3}H]NEM Microautoradiography. The degree of movement of NEM into the root cortex was measured by performing microautoradiography on corn roots exposed to [^{3}H]NEM. Two-cm root segments from the 3rd through 5th cm of the main root of 5-d-old low salt roots were placed in a solution consisting of 0.2 mM $CaSO_4$, 1 mM Mes (pH 6.5), and 0.3 mM [^{3}H]NEM (200 μCi/ml; New England Nuclear) for periods of 10 s, 30 s, 1, 2, or 5 min. At the end of each exposure, the roots were washed in an excess volume of 1 mM DTE. Two-mm-long sections were excised from the center of each segment and fixed in 1.25% gluteraldehyde for 5 h at 4°C. The sections were rinsed and postfixed in 1% OsO_4 for 2 h at 25°C. The tissue was then dehydrated in a graded acetone series and embedded in Spurr's resin. Thick sections (3 μm) were cut and glued onto glass slides. The slides were then dipped in Kodak Nuclear Track Emulsion NTB3 at 40°C, and allowed to dry. The slides were exposed for up to 17 d at −20°C and developed in Kodak Dektol developer. Control sections (no [^{3}H]NEM treatment) were processed in an identical manner.

To quantify the distribution of autoradiographic grains in the sections, the microscopic images were analyzed with an Imanco Quantimet 720 Image Analyzing Computer (Cambridge Instruments; Cambridge, England).

[^{203}Hg]PCMBS Microautoradiography. Two-cm root segments from the 3rd through 5th cm of the main root of low and high salt-grown roots were placed in a solution consisting of 0.2 mM $CaSO_4$, 1 mM Mes (pH 6.5), and 2 mM [^{203}Hg] PCMBS (40 μCi/ml; Amersham Corp.) for 1, 2, 10, or 20 min. At the end of each PCMBS exposure, the roots were briefly rinsed in 25 ml of 0.2 mM $CaSO_4$ + 1 mM Mes (pH 6.5), and subsequently washed (10 min) in 25 ml of identical solution. Two segments were used per treatment.

Unlike NEM, PCMBS forms reversible linkages with sulfhydryls. Therefore, it was necessary to freeze-substitute the PCMBS-treated tissue to prevent movement of radiolabel. Following the wash, root segments were rapidly frozen in slushy liquid N_2 and then placed into vials containing dry acetone at −70°C for 7 d to allow for dissolution of tissue ice. The vials were then warmed to 25°C, dry acetone was changed twice, and then segments were embedded in Spurr's resin. Sections were cut (1 μm) dry from the central region of the root segments and placed on a drop of ether on a glass slide. The evaporation of the ether caused the adherence of the section to the slides. The slides were then dipped in Kodak Nuclear Track Emulsion NTB2, and exposed at 4°C for up to 32 d. Slides were developed with Kodak D-19 developer and analyzed for autoradiographic grain distribution as in the preceding section. Because the freeze-substituted tissue was unstained, the outline of each cell was poorly resolved during microscopic examination. Therefore, cell walls were stained by placing a drop of 0.2% Azur-B stain in 0.1% Na_2CO_3 over the sections following development of the photographic emulsion. The dye was washed off after 20 s, and resulted in a light staining of cell walls.

Investigation of Root Hypodermis. The fluorescent, impermeant dye, Calcofluor White M2R (Polysciences, Inc., Warrington, PA), which binds to cell walls, was used to investigate the potential existence in the root periphery of an apoplastic diffusion barrier. The methods of Peterson *et al.* (23) were employed. Briefly, either (a) intact root systems, (b) roots in which the hypodermis was ruptured by scoring with a needle along the terminal 10 cm, or (c) roots with the terminal 10 cm split longitudinally (using apparatus previously described) were immersed in a solution consisting of 0.1% Calcifluor, 0.2 mM $CaSO_4$, and 2 mM Mes-Tris (pH 7.0) for 18 to 24 h. The roots were then washed in running tap water for 1 to 2 h to remove unbound dye. Freehand cross sections were taken from the region approximately 5 cm behind the root tip and placed in a drop of nonfluorescent immersion oil. The sections were immediately observed with a Zeiss photomicroscope fitted with epifluorescence optics. Zeiss UGl excitation and barrier 53 filters were used.

Corn Root Protoplast Isolation. Protoplasts were isolated from the cortex of *Z. mays* roots by the methods of Lin (17) and Gronewald and Leonard (11), with minor modifications to minimize the release of epidermal protoplasts. Briefly, the terminal 10 cm of 5-d-old low salt roots were used for all experiments. The root apex was removed and the stele was pulled from the root, leaving the root cortex and epidermis intact. About 12 g of root cortex was collected, cut into 2-mm sections, and placed into digestion solution consisting of 0.6 M mannitol, 1 mM $CaCl_2$, 0.1% (w/v) Pectolyase Y-23 (Seishin Pharm., Chiba-Ken, Japan),

[2] Abbreviations: NEM, *N*-ethyl maleimide; PCMBS, *p*-chloromercuribenzene sulfonic acid; DTE, dithioerythritol; ANDA, 7-amino-1,3-naphthalene disulfonic acid; PTS, trisodium, 3-hydroxy-5,8,10-pyrenetrisulfonate.

2.0% (w/v) Cellulysin (Calbiochem), 0.05% (w/v) BSA (Sigma), and 0.5 mM DTT (pH 5.5). All solutions used for isolation were sterilized by passing through a 0.45-μm Millipore filter prior to use. Root material (3 g) was incubated in 20 ml of digestion solution for 2 h at 29°C with gentle shaking (50 cycles/min).

Following digestion, the resultant mixture was filtered through two layers of cheesecloth saturated with suspension solution [0.7 M Mannitol, 25 mM Mes-Tris (pH 5.5), 0.2 mM $CaCl_2$, 0.5 mM DTT]. The undigested material was resuspended in 20 ml of suspension solution and gently agitated to release cortical protoplasts. Very gentle agitation was used to minimize release of epidermal protoplasts.

The filtrates were combined and centrifuged at room temperature for 8 min at 350*g*. The protoplast pellet was gently resuspended in suspension solution and recentrifuged (8 min, 350*g*). The pellet was again gently resuspended, in 7 ml of 10% (w/v) Ficoll (type 400; Sigma) in suspension solution, and overlaid with successive layers consisting of 7 ml of 8, 5, and 0% Ficoll in suspension solution. The step gradient was centrifuged at 360*g* for 18 min and protoplasts collected at the interface of the 0 and 5% Ficoll layers. The protoplasts were diluted with suspension solution and pelleted at 360*g* for 8 min. The final pellet was resuspended in a solution consisting of 0.7 M mannitol, 0.2 mM $CaCl_2$, and 2 mM Mes-Tris (pH 6.5).

The root tissue which remained following digestion was examined using Nomarski microscopy with a Zeiss Photoscope III to determine the degree to which the epidermal cells retained their protoplasts.

$^{86}Rb^+$ Protoplast Influx Experiments. The kinetics of $K^+(^{86}Rb^+)$ influx were studied following the methods of Mettler and Leonard (20) and Gronewald and Leonard (11), with minor modifications. Briefly, influx at 23°C was measured from solutions which contained 0.2 mM $CaCl_2$, 2 mM Mes-Tris (pH 6.5), RbCl (0.05–10 mM), and approximately 0.7 M mannitol. The osmotic concentration of each uptake solution was measured with a Wescor 5100C vapor pressure osmometer (Wescor, Inc., Logan, UT) and osmotically adjusted to 720 ± 5 mOsm/kg. Protoplasts were added to a final concentraton of 500,000/ml and uptake was initiated by the addition of $^{86}Rb^+$ (0.4 μCi/ml). Final uptake volume was 1 ml. The protoplasts were allowed to accumulate radioisotope for 15 min with agitation (50 cycles/min). Uptake was terminated by pipetting the entire 1 ml volume onto a step gradient consisting of 5 ml of 0.7 M mannitol, 2 mM Mes-Tris (pH 6.5), 1 mM $CaCl_2$, and 10 mM KCl, laid on top of 3 ml of 2% Ficoll in 0.7 M mannitol. The step gradients were contained in 12-ml plastic conical centrifuge tubes. The protoplasts were pelleted by centrifugation at 1000*g* for 10 min, the gradient solutions removed by vacuum aspiration, and the pellet resuspended in 1 ml of water. A 100-μl aliquot was used for Lowry protein determination; the remaining 0.9 ml were added to 10 ml of 5 mM ANDA and radioactivity determined using a Beckman LS9800 scintillation counter.

RESULTS

Influence of Sulfhydryl Modifiers on $K^+(^{86}Rb^+)$ Influx and Respiration. The effect of increasing exposures of the permeant sulfhydryl reagent, NEM, on influx from a 0.2 mM RbCl solution was investigated. NEM rapidly inhibited influx; a 30-s exposure to 0.3 mM NEM inhibited Rb^+ uptake by 75 to 95% (Fig. 1A). Such a short NEM exposure would be expected to influence primarily the cells of the root periphery. Since NEM has been shown to inhibit a number of cellular processes, including respiration (31), we examined its effect on root respiration (Table I). The 30-s NEM pulse, which dramatically reduced Rb^+ uptake, had no effect on root respiration; significant inhibition was not observed until the tissue was exposed for 2 min or longer.

Because NEM can influence a number of processes, both at the plasmalemma and inside the cell, the effect of the impermeant sulfhydryl reagent, PCMBS, on $K^+(^{86}Rb^+)$ uptake was also studied (Fig. 1B). Again, a rapid inhibition of influx was observed. A 1-min exposure with 2 mM PCMBS inhibited uptake from 0.2 mM RbCl by 70 to 85%. Inhibition by sulfhydryl modification (both NEM and PCMBS) was reversed by DTE treatment (2 mM). Significant recovery occurred only after a period of 4 h: under 0.2 mM K^+ conditions, influx returned to 65% of the control value; a value of 80% of control was obtained for 10 mM K^+ conditions.

At low K^+ concentrations, where uptake may be limited to the epidermis because of diffusion limitations and ion depletion by the root surface, sulfhydryl modification rapidly abolished K^+

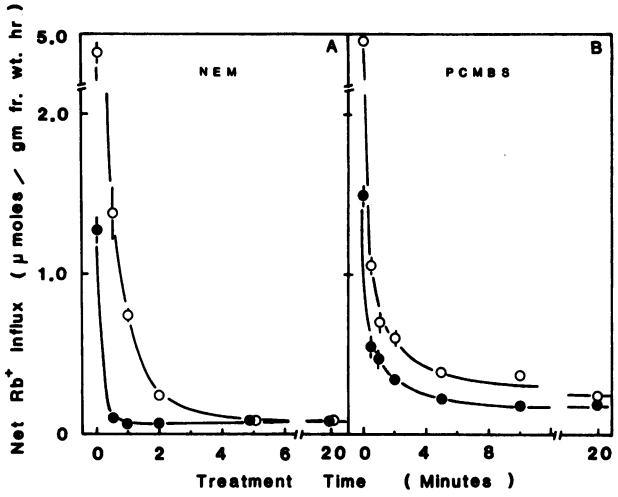

FIG. 1. NEM- and PCMBS-induced inhibition of $^{86}Rb^+$ influx (0.2 mM RbCl) for corn root segments grown in 0.2 mM $CaSO_4$ (low salt status [○]) or in 5 mM KCl + 0.2 mM $CaSO_4$ (high salt status [●]). The sequence of experimental procedures was as follows. A, Root segments were pretreated in 0.3 mM NEM for indicated time, then given a 10-min wash in 1 mM DTE. Root segments were then washed in 0.2 mM $CaSO_4$ + 1 mM Mes (pH 6.5) prior to uptake to remove DTE. B, Root segments were pretreated in 2 mM PCMBS for indicated time, then given a 10-min wash in 0.2 mM $CaSO_4$ + 1 mM Mes (pH 6.5) prior to uptake. Pretreatment solutions contained 0.2 mM $CaSO_4$ + 1 mM Mes (pH 6.5) in addition to NEM, PCMBS, or DTE. Uptake solution contained 0.2 mM RbCl, 0.2 mM $CaSO_4$, and 1 mM Mes (pH 6.5). All data points in this figure and subsequent ones (except Fig. 7) are the average of four replicates; the error bars are ± SE. Points which lack error bars do so because the SE were smaller than symbols used.

Table I. *Effect of Various NEM Exposures on Root Respiration*

Respiration was followed by monitoring O_2 consumption in a Clark-type O_2 electrode. After control root respiration was measured for 20 min, NEM was added to a final concentration of 0.3 mM. Roots were exposed to NEM for the desired length of time, and then DTE was added to a final concentration of 1 mM. After respiration stabilized at its subsequent value, O_2 consumption was followed for an additional 20 min. The resultant respiration values are listed as per cent of control.

Treatment	Respiration
	% of Control
Control	100[a]
Control + DTE	100
30 s NEM + DTE	100
1 min NEM + DTE	96
2 min NEM + DTE	78
5 min NEM + DTE	57
20 min NEM	39

[a] Average control respiration was 18.0 μmol O_2/g fresh weight·h.

uptake. The time course for NEM inhibition from more concentrated K^+ solutions (1 or 10 mM) was next studied. Under these conditions, diffusion of substrate into the cortex may allow these cells to contribute to K^+ transport into the tissue. In high salt-grown roots, maximal inhibition of uptake was achieved with an NEM exposure of approximately 30-s, irrespective of the substrate concentration (Fig. 2B). However, maximal inhibition of uptake in 10 mM RbCl was 50% of the control and exposures of up to 20 min did not result in further inhibition. This correlates with our earlier work (14) in which we showed that sulfhydryl modifiers influence the kinetics of K^+ uptake by abolishing what was interpreted as a saturable component of transport; the linear component was relatively insensitive to sulfhydryl inhibition. In high salt roots, the linear component was responsible for about 50% of the observed uptake when 10 mM RbCl was present. In low salt-grown roots, the inhibition was slightly different. A 30-s NEM pulse inhibited uptake from 10 mM RbCl by only 30%. Prolonging the exposure to NEM resulted in further inhibition (Fig. 2A). Maximal inhibition occurred after exposures of 6 min or longer, and resulted in a 75% inhibition. This result is also consistent with our previous data (14).

Localization of NEM and PCMBS in the Root. To determine if the brief NEM or PCMBS pulses were binding primarily at the root periphery, autoradiography was performed on roots exposed to [^{3}H]NEM and [^{203}Hg]PCMBS. Figure 3 demonstrates the spatial location of [^{3}H]NEM in the root following a 30-s or 5-min exposure. A 30-s NEM pulse produced a binding pattern localized predominantly in the epidermis, with a smaller amount of label in the hypodermis (Fig. 3A). A relatively diffuse and low level of activity was distributed throughout the rest of the root. To quantify the distribution of radiolabel, the autoradiographs were analyzed using an Imanco Quantimet 720 Image-Analyzing computer. Numerous cross-sections were analyzed and the data averaged and presented in histogram form (see fig. 3). Analysis of the 30-s NEM treatment verified that almost all of the label was localized in the root periphery. The low, fairly constant level of label throughout the rest of the root (4%) was due to computer detection of the cell walls, as verified by analysis of control sections (data not shown). By comparison, both the autoradiograph and image analysis, after a 5-min NEM pulse, showed that significant amounts of [^{3}H]NEM were bound to cells throughout the root (Fig. 3B).

Because NEM can penetrate and move within the symplast, it was necessary to repeat the autoradiographic experiments with the impermeant [^{203}Hg]PCMBS. Following a 1-min [^{203}Hg]

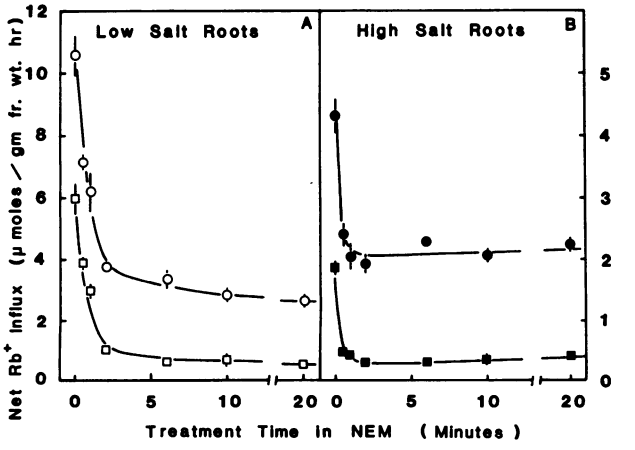

FIG. 2. NEM-induced inhibition of $^{86}Rb^+$ influx for low salt-grown and high salt-grown corn roots. A, Low salt-grown root uptake from either 1 mM RbCl (□) or 10 mM RbCl (○). B, High salt-grown root uptake from either 1 mM RbCl (■) or 10 mM RbCl (●). Experimental pretreatments and solutions are as described in Figure 1.

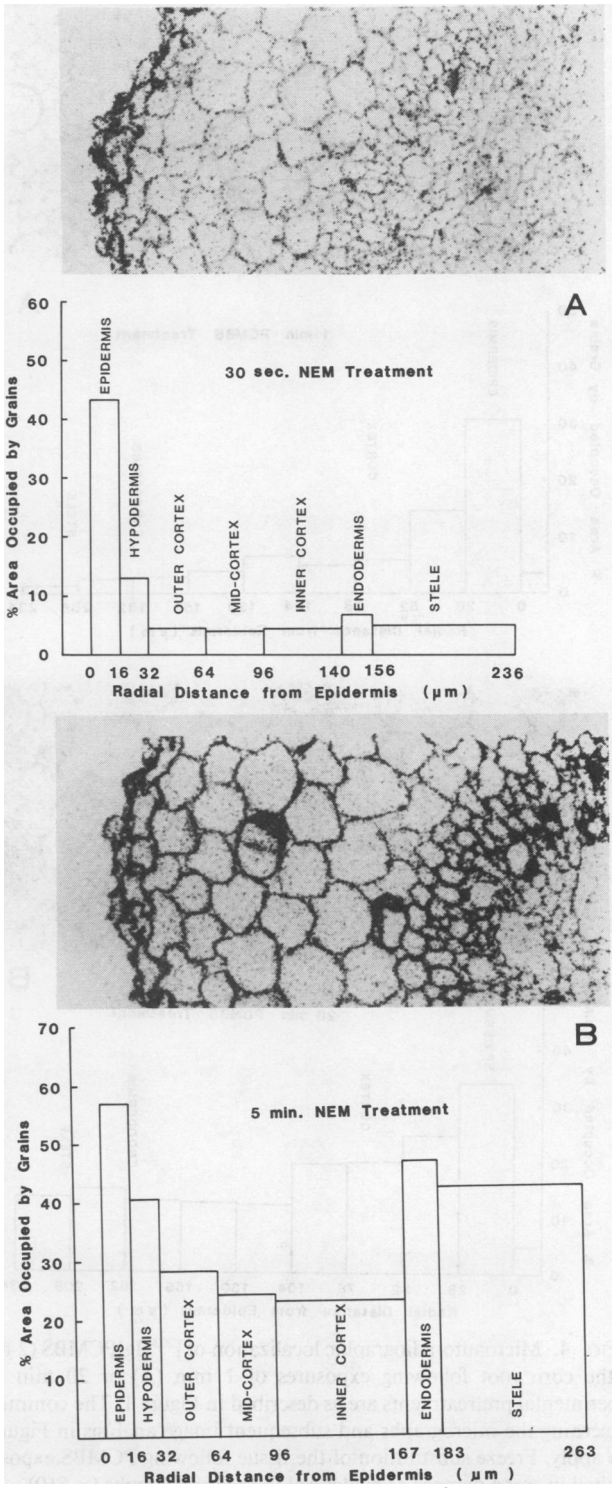

FIG. 3. Microautoradiographic localization of [^{3}H]NEM (0.3 mM) in a corn root following exposures of 30 s (A) or 5 min (B). Experimental pretreatments are as described in Figure 1. Each micrograph is representative of the grain distribution for that particular exposure. Numerous root cross-sections from each NEM treatment were analyzed for grain distribution with an Imanco Quantimet 720 Image Analyzing Computer. The data were averaged and presented in the histograms located below each micrograph. Because the histograms represent an average obtained from many cross-sections, radial distance into the root had to be normalized to account for variations in root diameter. Therefore, the labels above the histograms, which denote various root regions encountered as one moves radially into the root, do not always match exactly with the corresponding micrographs (× 470).

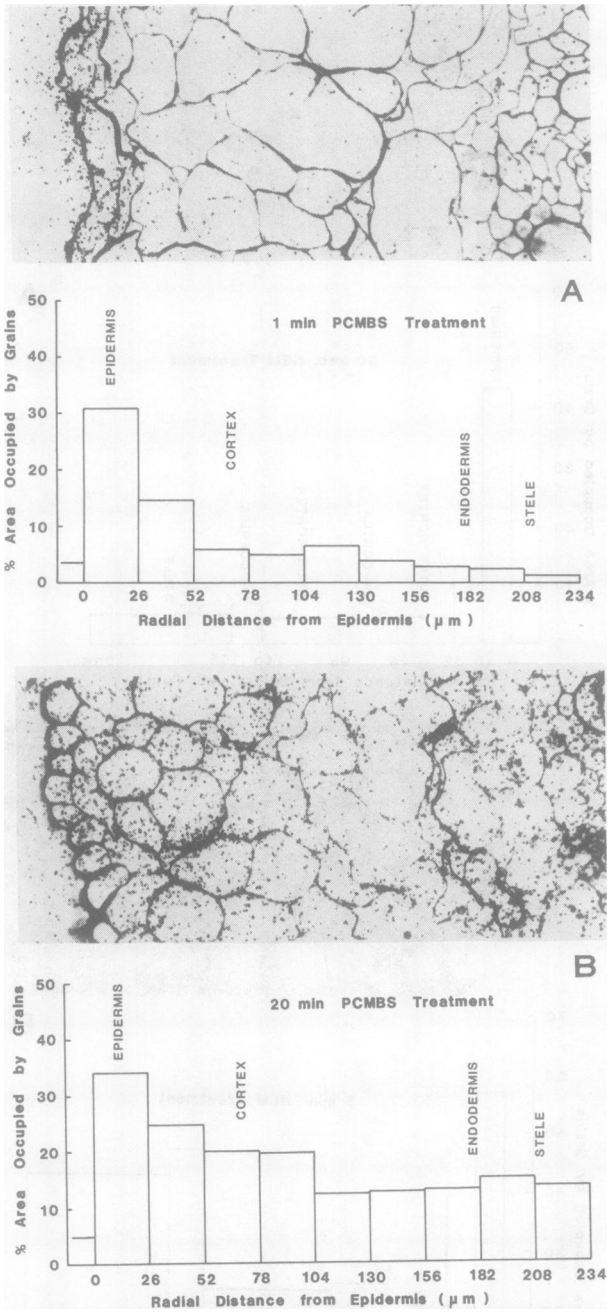

FIG. 4. Microautoradiographic localization of [^{203}Hg]PCMBS (2 mM) in the corn root following exposures of 1 min (A) or 20 min (B). Experimental pretreatments are as described in Figure 1. The comments concerning the micrographs and subsequent image analysis in Figure 3 also apply. Freeze substitution of the tissue following PCMBS exposure resulted in some damage as evidenced in the micrographs (× 510).

PCMBS exposure, label was bound almost exclusively to the cells of the root periphery (Fig. 4A). Unfortunately, there was tissue damage during the freeze substitution, as evidenced in both micrographs. However, the grain distribution was unaffected by this damage. The brief PCMBS exposures always resulted in label localized in the root periphery. The band of autoradiographic grain distribution was wider than we observed for [^{3}H]NEM. This is due to at least two factors. First, because ^{203}Hg is a γ-emitter, exposures can occur some distance from the actual site of decay. Second, during freeze-substitution, there appears to have been some slight movement of label. These observations are supported by the appearance of label beyond the surface of

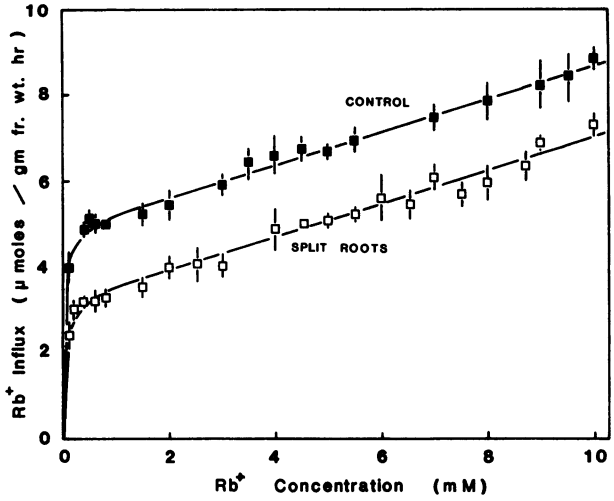

FIG. 5. ^{86}Rb^{+} influx isotherms for either corn root segments (■) or split-root segments (□). Five-d-old low salt-grown seedlings were used for all experiments. To obtain split-root segments, the terminal 10 cm of the main root was split longitudinally through its center with the apparatus described in "Materials and Methods." The root halves were then cut into 2-cm segments and washed prior to uptake.

the root (Fig. 4A). In comparison with the short exposure autoradiographs, a 20-min PCMBS pulse resulted in fairly heavy grain distribution throughout the entire root (Fig. 4B). Image analysis of freeze-substituted control tissue resulted in a low (5%) level of detection distributed uniformly throughout the root (data not shown).

Kinetic Studies. If ion uptake by cortical cells is diffusion limited, then splitting the roots longitudinally should increase the availability of substrate to cortical cells. This would be especially so if the hypodermis of the corn root does function as an apoplastic barrier to the movement of K^+ into the cortex. Furthermore, if the complex K^+ influx kinetics observed previously were due to, or at least partially influenced by, diffusion-limited availability of ions to the root cortex, they would be modified by uptake in split-roots. In Figure 5, the kinetics for K^+ uptake in intact *versus* split-roots are compared. The overall shape of the kinetic curves was identical. However, the influx values in the split-roots were consistently lower than those in whole root segments.

Corn Root Cortical Protoplasts. Corn root protoplasts were next studied for the following reasons. First, if a protoplast preparation could be shown to be purely cortical in origin, the capacity (or lack thereof) for cortical cell ion uptake could be demonstrated. Second, if the complex kinetics found for K^+ uptake in roots were due to two kinetically distinct mechanisms, such kinetics should also be observed in a single-cell system, where significant diffusion limitations and the problems which arise in a complex tissue do not exist.

Our protoplast preparations were free of cellular debris and bacteria and tests using vital stains indicated that the majority of the protoplasts were viable. After cell wall digestion, each root segment remained as a translucent tube. Extensive microscopic examination indicated that these tubes always consisted of intact epidermal cells (Fig. 6). As shown in Figure 6A, the epidermis and hypodermis remained undigested. Often several outer layers of cortex were also left intact. By observing the epidermal cells with Normarski optics, it was determined that they always maintained their cellular contents (Fig. 6B). Thus, since we removed the stele, and the epidermal cells always remained intact, the kinetics presented represent uptake by cortical protoplasts. Average yields of about 700,000 protoplast/g root tissue were obtained and K^+ influx values fell between those presented by

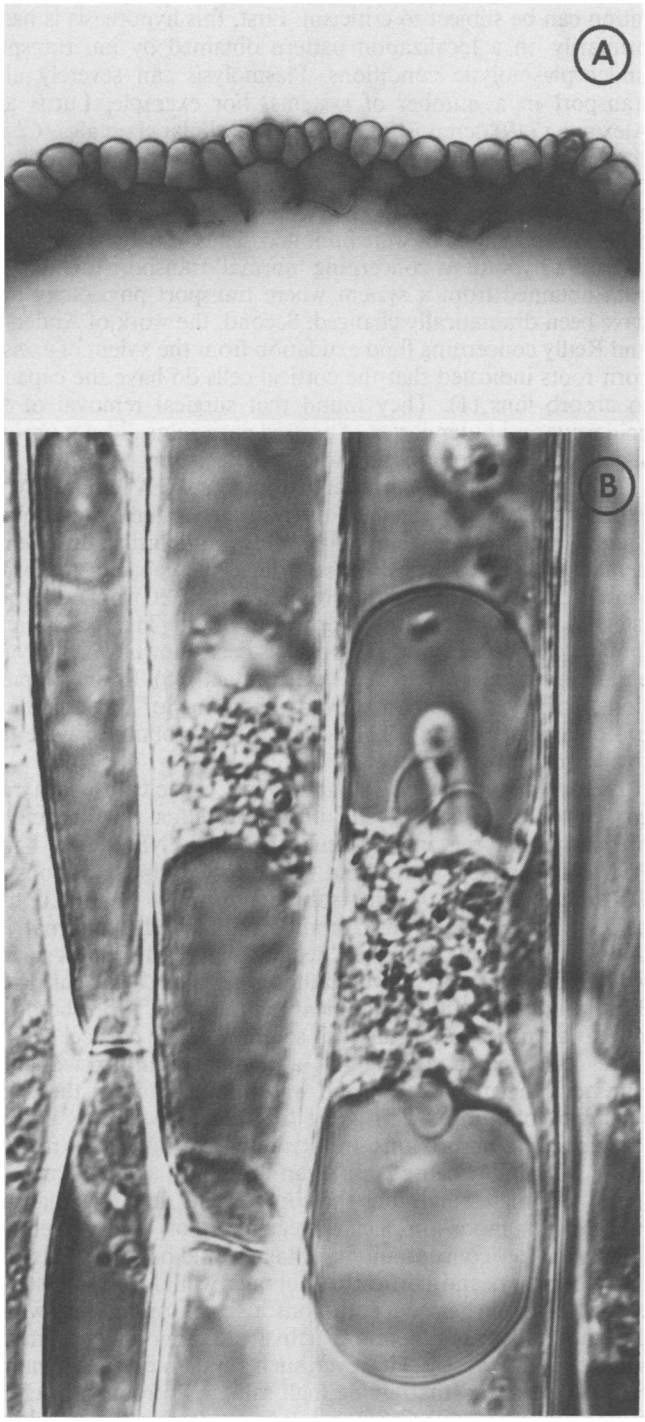

FIG. 6. Micrographs of corn root tissue which remained intact after enzymic digestion had released protoplasts from the cortex. In all sections observed, the epidermis resisted digestion and remained whole. A, Cross-section of corn root tissue observed with bright field optics. The epidermal and hypodermal cell walls were intact following enzymic treatment. Note that although the inner tangential wall of the hypodermis has undergone partial digestion, it is still continuous with the rest of the hypodermal cell wall (× 280). B, Surface view using Nomarski optics of corn root tissue observed in A. Nomarski optics were used to examine the cellular structure of the epidermis (× 1600).

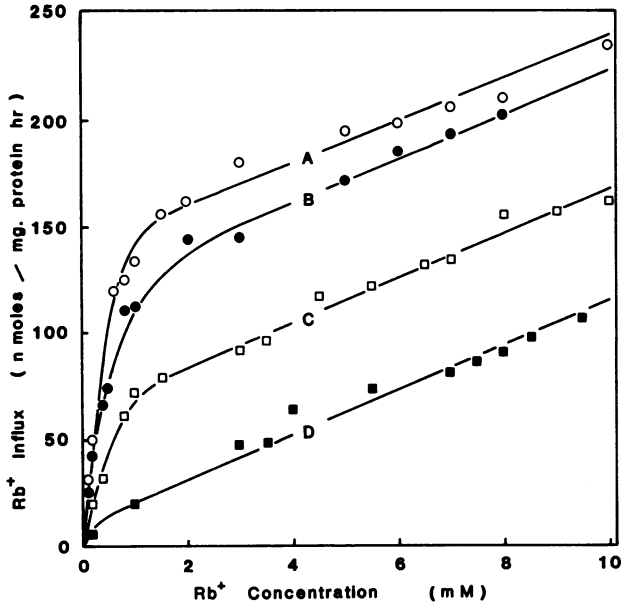

FIG. 7. $^{86}Rb^+$ influx isotherms for protoplasts isolated from the cortex of corn roots. Fifteen-min uptake experiments were run in solutions consisting of RbCl (0.05–10 mM), 0.2 mM $CaCl_2$, 2 mM Mes (pH 6.5), and approximately 0.7 M mannitol. The isotherms labeled A, B, C, and D represent replicate experiments performed on different days. Linear regression performed on data points for Rb^+ concentrations from 1.5 to 10 mM yielded the first order rate coefficient, k (in μmol g fresh weight^{-1} h^{-1} mM^{-1}), for the linear component 9.2 (A), 10.9 (B), 10.7 (C), 10.0 (D). The V_{max} values (in μmol g fresh weight^{-1} h^{-1}) for the saturable component were 143.8 (A), 118.0 (B), 62.0 (C), and approximately 0 (D).

others (11, 17).

To minimize variability in uptake from a range of Rb^+ concentrations (0–10 mM), it was necessary to osmotically adjust each influx solution with mannitol. In experiments in which we examined the effect of osmotic concentration on Rb^+ uptake from 1 mM RbCl solutions, varying the mannitol concentration from 600 to 725 mM caused a 50% increase in Rb^+ influx.

The kinetic curves obtained for protoplasts were quite similar in form to those presented for K^+ influx in corn roots (Fig. 7). The curves presented are from four separate experiments. Unlike influx values obtained from corn roots, we found considerable variability in the measured fluxes obtained on different protoplast preparations. This does not seem unusual considering the fragile nature of the system studied. However, the linear component showed little change in replicate experiments. Note that in curve D of Figure 7, which involved uptake in protoplasts isolated from roots which appeared to be damaged, uptake was almost completely linear.

Is the Hypodermis an Apoplastic Barrier? Most monocots and some dicots exhibit a modification of the cell layer just beneath the root epidermis, the hypodermis, which some workers believe acts as a barrier to apoplastic movement of ions and water. Peterson and coworkers have extensively studied the hypodermis in onion and corn; they have found that it appears to exclude the apoplastic, cell wall-binding fluorescent dyes, Calcofluor and Tinopal, from the cortex (22–24). Because of the importance of such a barrier to our work on the kinetics and site of K^+ uptake in corn roots, it was necessary to repeat these fluorescent dye experiments with our tissue. Our results with intact roots were quite similar to those of Peterson *et al.* (22–24). Calcofluor appeared to be bound exclusively to the epidermis (data not shown). We next ruptured the hypodermis by scoring with a needle, or by longitudinally splitting the terminal 10 cm of the root, prior to a 24-h immersion in Calcofluor. Fluorescence

microscopy demonstrated that the dye penetrated only into the first cell layer along the cut surface (data not shown). We expected that during a 24-h incubation, Calcofluor should be able to diffuse into the exposed cortex of split or scored roots and bind to the cell walls of the entire cortex. Thus, the apparent dye exclusion may be due to the binding of dye to epidermal walls (or walls of cells along the cut surface), which may create a barrier retarding further penetration by free dye molecules. The observation that the apoplastic dye, PTS, which does not bind to cell walls, could penetrate freely to the endodermis of corn roots, further supports this contention (23).

Additionally, from studies concerning the penetration of Na^+, HPO_4^{2-}, and water into corn roots, various workers have concluded that the hypodermis was not an apoplastic barrier unless the oldest (>30 cm from tip) regions of the root were used (1, 2, 8, 9). Our own autoradiographic localization of the impermeant [^{203}Hg]PCMBS also indicated that a hypodermal apoplastic barrier does not exist. After exposures of 5 min or longer, PCMBS appeared to be distributed uniformly throughout the entire cortex.

DISCUSSION

Involvement of the Root Periphery in Ion Uptake. The results of the rapid NEM and PCMBS inhibition of Rb^+ uptake, in conjunction with the autoradiographic localization studies, lend further support to previous work implicating the root periphery in ion uptake from solutions of low concentration. A 30-s NEM or 1-min PCMBS exposure dramatically inhibited Rb^+ uptake, while binding almost exclusively to the cells of the epidermis and hypodermis. The autoradiography following the 30-s NEM (0.3 mM) exposure demonstrated that almost all of the labeled NEM was bound to the root periphery. There was, however, a very low level of more diffusely distributed label throughout the rest of the root cortex and stele. It is not likely that this small amount of NEM would significantly influence ion uptake. This contention is supported by the root respiration data (Table I). A significant inhibition of root respiration was observed only after NEM exposures of 2 min or longer. After a 30-s NEM exposure, binding of NEM to epidermal cells may have resulted in an inhibition of epidermal respiration, along with Rb^+ influx. Longer exposures to NEM may be necessary to allow this chemical to penetrate into the cortical cells and affect respiration. Hence, the low level of NEM found in the root cortex may be insufficient to influence respiration and, quite possibly, it had little or no effect on other cortical cell processes. Furthermore, if the sparse grain distribution in the cortical cells is due to NEM being bound from the cell wall solution, it should represent an extremely low apoplastic NEM concentration. It has been demonstrated that in single cell systems, NEM concentrations of 0.1 mM or higher are usually necessary for significant inhibition of transport. For example, in *Chara*, a 30-s pulse with 0.1 mM NEM inhibited HCO_3^- influx by only 25% (18).

The PCMBS autoradiography is more definitive. PCMBS has been shown to penetrate membranes quite slowly and is considered an impermeant sulfhydryl modifier (30). Therefore, the complications resulting from penetration into the symplast are alleviated. The autoradiograph following a 1-min exposure to PCMBS demonstrated that it was restricted to the root periphery. This result, in conjunction with the dramatic inhibition of K^+ uptake by a 1-min PCMBS exposure, clearly implicates the cells of the root periphery in ion absorption.

Involvement of the Root Cortex in Ion Uptake. Vakhmistrov (28), Bange (3), and Van Iren and Boers-Van der Sluij (29) have argued that ion uptake capacity is restricted to the epidermis. The strongest evidence in support of this hypothesis comes from the localization of absorbed $^{86}Rb^+$ in the epidermis and outer cortex of plasmolyzed barely roots. However, such an interpretation can be subject to criticism. First, this hypothesis is based primarily on a localization pattern obtained by ion transport under plasmolytic conditions. Plasmolysis can severely alter transport in a number of systems. For example, Lucas and Alexander (19) demonstrated in the unicellular giant alga, *Chara corallina*, that when turgor was reduced to near the plasmolytic point with an osmoticum, localized HCO_3^- influx and OH^- efflux were unaffected. However, if the cells were plasmolyzed, both transport systems were inhibited. Thus, it may be erroneous to base a hypothesis concerning 'normal' transport function on data obtained from a system where transport physiology may have been dramatically changed. Second, the work of Anderson and Reilly concerning fluid exudation from the xylem of excised corn roots indicated that the cortical cells do have the capacity to absorb ions (1). They found that surgical removal of the epidermis and outer cortex of excised corn roots did not prevent significant fluxes of ions and water to the xylem.

It is impossible to determine from our NEM and PCMBS experiments whether ion uptake is restricted to the root periphery under all physiological conditions. Our protoplast studies clearly demonstrate that cortical cells do have the capacity to absorb ions. We determined that the protoplasts isolated from corn roots (stele removed) were purely cortical in origin. The technique of 'pulling' the stele from corn roots has been shown to destroy the endodermis while leaving the cortex intact (16). In our preparations, care was taken to prevent liberation of epidermal cell protoplasts (see Fig. 6). Thus, the K^+ fluxes obtained were due to absorption by cortical cells.

Despite demonstrated K^+ absorption capacity by corn root cortical protoplasts, it is still unclear whether this capacity is operational in the intact tissue. The 30-s NEM and 1-min PCMBS pulses, shown to bind to the root periphery, abolished Rb^+ influx from a 0.2 mM solution during a 30-min uptake period. If these sulfhydryl modifiers inhibited epidermal ion uptake, then the hypothesized 'depletion layer' at the root surface would no longer exist. It would then be expected that during the 30-min uptake period a significant amount of $^{86}Rb^+$ should be able to diffuse into the cell wall solution of the cortical cells. If these cells were absorbing K^+, a significant influx would be expected. Inasmuch as uptake was minimal under these low substrate conditions, we suggest that the cortical cells are not functioning to absorb ions. In the presence of high substrate (10 mM RbCl), NEM caused less of an inhibition and so it may be that under these conditions the cortical cells function to absorb K^+. Such a model implicates regulation of cortical cell ion uptake. At low concentrations, uptake would be restricted to the root periphery, and cortical cell uptake would be repressed or 'shut off.' At higher concentrations a signal, originating within the epidermis, may function to activate existing transport mechanisms in cortical cells. However, such a system must be dynamic because we found that giving high salt-grown roots a brief (10 min) wash in 0.2 mM $CaSO_4$ solution resulted in the cells being very sensitive to NEM inhibition (cf. Figs. 1A and 2B).

Kinetic Studies. Our data could also be explained according to the kinetic model presented in an earlier paper (14), which would not necessarily involve cortical uptake. It was previously shown that the kinetic curves for K^+ influx were smooth, nonsaturating, and approached linearity at higher substrate concentrations. We proposed that the observed kinetics were the result of two separate transport mechanisms, one exhibiting saturation and the other following first order kinetics. Through the use of the sulfhydryl modifiers, NEM and PCMBS, it was possible to separate these two systems kinetically. A 30-s NEM exposure was shown to abolish the saturable component of K^+ influx in high salt-grown roots, while the linear component was unaffected. Approximately 50% of total influx of 10 mM Rb^+ was contributed by the linear component. Hence, it is possible that

the NEM-insensitive K^+ uptake at high K^+ levels may be due to a sulfhydryl-insensitive mechanism operating in the epidermis.

These studies have also enabled us to gain further understanding concerning mechanistic interpretations of ion uptake kinetics. Göring and coworkers have argued that such complex kinetics could still be the result of a single transport system following Michaelis-Menten kinetics; the kinetics would appear complex due to the influences of diffusion-limited ion availability for transport in cortical cells (6, 10, 12, 13). However, our kinetic experiments with both split-roots and protoplasts indicate that this is not the case. If ion diffusion into the cortical free space were limited, particularly by a hypodermal barrier, then the splitting of roots should increase the availability of ions for cortical cell uptake. This should significantly increase K^+ influx, especially at low concentrations, and cause an alteration of the kinetics. However, the kinetics observed for split roots were similar to those for intact root segments. The moderate reduction in uptake could be attributed to tissue damage resulting from the root-splitting procedure.

The K^+ uptake kinetics observed for cortical protoplast were quite similar to those previously detailed for roots (Figs. 5 and 7). Therefore, these results clearly indicate that such complex, nonsaturating kinetics are due to uptake by single cells. The K^+ influx kinetics varied from experiment to experiment only in the shape and size of the saturable component (Fig. 7). The linear component remained unchanged; in fact, in one experiment it appears that the saturating transport mechanism was not operating, while linear component uptake continued unaltered (Fig. 7, curve D). These data strongly support the contention that the linear component does indeed represent a cellular transport mechanism, which presumably operates across the plasmalemma in conjunction with a separate saturable component of uptake.

CONCLUSIONS

The pathway for ion flux from the external solution to the stele is still not clearly defined. The root periphery appears to play an important role in ion uptake, possibly as the primary site for uptake at low ion concentration. It was clearly demonstrated that cortical cells have the capacity to absorb ions. However, in the intact tissue, it is unclear whether cortical cells express this capacity. At low ion concentrations, the uptake capacity by cortical cells appears to be repressed, whereas under higher substrate levels it is possible that the ability to absorb ions is stimulated or 'turned on.' Whether the present findings can be extended to other root systems awaits further research.

The complex kinetics obtained for K^+ uptake in split-roots and isolated root protoplasts strongly indicate that such kinetics are not due to diffusion-limited uptake by a complex tissue. Instead, the results support the hypothesis that K^+ uptake results from two kinetically distinct mechanisms. Thus, it appears that saturable and linear uptake are due to separate transport mechanisms, both located on the plasmalemma of epidermal and cortical cells of corn roots.

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