Potassium Transport in Corn Roots

I. RESOLUTION OF KINETICS INTO A SATURABLE AND LINEAR COMPONENT

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ABSTRACT

Influx isotherms were obtained for 86Rb+ uptake into 2-cm corn (Zea mays [A632 × [C3640 × Oh43]) root segments for both low- (0.2 millimolar CaSO4) and high-salt (0.2 millimolar CaSO4 + 5 millimolar KCl) grown roots. Unlike the discontinuous curves usually presented for K+ influx, our isotherms were smooth, nonsaturating curves that approached linearity at K+ (Rb+) concentrations above 1 millimolar. The kinetics for K+ transport could be resolved into saturable and linear components. The saturable components yielded K_m values of 16 and 86 micromolar for low- and high-salt roots, respectively, while V_max values were 5.62 and 1.85 moles per gram fresh weight per hour. Results of experiments with the penetrating sulphydryl reagent, N-ethyl maleimide (NEM), and the impermeant reagent, p-chloromercuribenzenesulfonic acid (PCMBS) indicated that the saturable and linear components were independent mechanisms of K+ transport.

Short-term NEM exposures (30 seconds to 5 minutes) selectively inhibited the saturable system, but had little effect on the linear component. Increasing NEM exposures resulted in further inhibition and subsequent abolition of the saturable component; the linear component exhibited limited NEM sensitivity. PCMBS elicited the same general inhibitory trends, although it was less effective as a saturable component inhibitor. The effects of NEM and PCMBS on K+ efflux were also studied. Short NEM exposures had no effect on cytoplasmic efflux, while inhibiting vacuolar efflux significantly. From these data, it is unclear at which site(s) NEM is acting. A more complex response was obtained with PCMBS, where a monophasic efflux curve was observed. Analysis indicated that the vacuolar efflux was stimulated, while the cytoplasmic component was abolished.

The nature of the linear component is discussed, and it is proposed that the mechanism may be more complex than simple facilitated diffusion.

It is generally accepted that K+ uptake isotherms for roots of higher plants are complex (11, 25). The appearance of discontinuities in influx isotherms led to Epstein's formulation of the now well-known dual-isotherm hypothesis (12), in which it was proposed that two carriers were located in the plasmalemma, each carrier having different Michaelis-Menten parameters. In opposition to this hypothesis, Nissen has argued that such kinetics are due to a single, complex transporter which undergoes transitions in response to changes in external ion concentration (29).

It has also been hypothesized that a diffusion limitation of ions exists at low external concentrations, such that their availability for uptake by root cortical cells is significantly reduced (2, 10). Göring and coworkers have argued that because of this diffusion limitation, the cells of the root periphery play a dominant role in uptake. Therefore, they propose that the commonly observed dual isotherms may be an artifact of this diffusion barrier. Uptake saturation observed at low substrate concentrations (Epstein's Mechanism I) would be due to transport into epidermal cells, while uptake at higher concentrations (Epstein's Mechanism II) would be the result of absorption by identical carriers located on cortical cells within the root. This hypothesis is supported by studies of glucose uptake by yeast cells (14). Uptake in cell suspensions yielded isotherms consisting of one saturable curve, while uptake into cells packed in an agar matrix (to approximate a tissue) yielded influx isotherms that were biphasic. Although the above results are consistent with Göring's hypothesis (14), it should be recognized that complex kinetics have been observed in a wide range of tissues (26 and references therein) and, therefore, other interpretations may be valid.

Borstlap (4) has also presented a critique of the multiphasic interpretation of uptake. He argues that many of the frequently cited discontinuous uptake isotherms could be explained by models consisting of one or more Michaelis-Menten terms plus a linear term, particularly if experimental error and biological variability are considered. The existence of this linear term has often been ignored, despite the fact that transport kinetics consisting of Michaelis-Menten and linear terms have been well documented in both plant and animal systems (3, 5, 8, 9, 13, 20, 22, 27, 28, 32). Such kinetics have generally been associated with the transport of organic solutes, the Michaelis-Menten terms are thought to represent carrier-mediated transport, while the linear term is usually considered to represent passive diffusion.

In the present study, we investigated K+ (86Rb) influx into excised root segments of Zea mays seeds. Our influx isotherms were smooth and nonsaturating. Based on experiments in which sulphydryl modifiers were employed, we present evidence that these isotherms are due to the functioning of a saturable and a linear component. Furthermore, from the results of studies on both the influx and efflux of K+, we suggest that the linear component is more complex than simple facilitated diffusion.

MATERIALS AND METHODS

Plant Material. Zea mays seeds (A632 × [C3640 × Oh43], Crow's Hybrid Corn Co., Milford, IL) were surface sterilized in 1% NaOCl and imbibed overnight in aerated deionized H2O. The seeds were then germinated on stainless steel screens placed over 4-L beakers that contained aerated solutions of either 0.2 mM CaSO4 (low-salt conditions) or 0.2 mM CaSO4 + 5 mM KCl (high-salt conditions). Seedlings were dark-grown in a high humidity growth chamber at 21°C. The primary root of 5-d-old seedlings was used for all influx and efflux experiments.

Influx Experiments. Short-term (10 or 30 min) Rb+ influx experiments were performed using 2-cm-long root segments excised from the 1st through 8th cm of the primary root. Root segments were washed (4 h) in solutions of identical composition...
to their growth solutions to allow recovery from excision. High-salt roots were subsequently washed for 10 min in 0.2 mM CaSO₄ immediately before initiation of influx, to remove free space K⁺. Root segments were then transferred to Plexiglas vials (0.1 g root segments/vial). The base of these vials consisted of stainless steel screen to allow free movement of solution (Fig. 1). The vials (with roots) were then placed into the Plexiglas uptake wells of the experimental system shown in Figure 1. These wells contained 20 ml of aerated uptake solutions which consisted of various concentrations of RbCl, plus 0.2 mM CaSO₄ and 1 mM Mes buffer (pH 6.5). Solution temperature was held at 23 ± 0.5°C by performing all experiments in a temperature-controlled room. The ratio of root weight to solution volume was such that isotope depletion was minimal during the 10- or 30-min uptake periods. Uptake was initiated by the addition of ⁴²⁴⁰Rb⁺ (as RbCl; New England Nuclear Corp.) to a final concentration of approximately 0.1 µCi/ml and terminated by the vacuum withdrawal of the radioactive solution into the chamber below the uptake wells. Free space radiolabel was removed by two 8-min washes in ice-cold 0.5 mM CaSO₄ + 1 mM RbCl. The Plexiglas vials (with roots) were then centrifuged for 15 s to remove surface water, and the roots weighed into scintillation vials. To rupture membranes, roots were incubated in 5 ml of 95% ethanol and then 10 ml of 5 mM ANDA² was added to each vial. Radioactivity was quantified via detection of Cerenkov radiation in a Beckman LS 9800 scintillation system.

To verify that transport discrimination of Rb⁺ versus K⁺ was not occurring, certain influx experiments were repeated using ⁴¹K⁺ (as KCl; New England Nuclear Corp.).

Efflux Experiments. Roots of intact seedlings or 2-cm-long root segments were allowed to accumulate ⁴²⁴⁰Rb⁺ from a solution containing 0.5 mM RbCl, 0.2 mM CaSO₄, 1 mM Mes buffer (pH 6.5), and ⁴⁰⁰RbCl (approximately 0.25 µCi/ml) for about 24 h. Periodic additions of RbCl and ⁴⁰⁰RbCl were made to maintain a constant specific radioactivity. By the end of the 24-h loading period, cpm/ml of the external solution had ceased declining, indicating that isotope flux equilibrium had been achieved.

Roots were washed briefly to remove surface radioactivity and 2-cm segments were placed into experimental Plexiglas vials. The vials were placed into beakers containing 15 ml of aerated solution containing various concentrations of RbCl, 0.2 mM CaSO₄, and 1 mM Mes buffer (pH 6.5). At predetermined times, the vials were removed from the efflux medium, adhering solution was blotted, and the vials were placed into fresh efflux media. Aliquots of each efflux solution, at each time point, were analyzed for radioactivity. At the end of the efflux experiment, radioactivity remaining in the root segments was determined. The efflux data were analyzed according to the methods of Pitman (31) and Cram (6), and interpreted according to the model presented by these workers; i.e. two intracellular compartments, the vacuole and cytoplasm, arranged in series.

Efflux experiments were performed which ensured that leakage of ions from the cut ends was not occurring. The terminal 10 cm of a root preloaded with ⁴²⁰Rb⁺ was excised, and the cut end of the root was threaded through a hole in a Plexiglas plate and sealed with vacuum grease. The cut end protruded about 0.5 cm above the surface of this plate. The 9.5 cm of root below the plate was lowered into a burette containing 10 ml of efflux solution. Therefore, efflux from the root surface could be measured without the complication of leakage from the cut end of the root.

Certain efflux experiments were repeated, monitoring the efflux of radiolabeled from ⁴²K⁺-loaded roots into solutions containing unlabeled KCl. Sulphhydryl Modifiers. The effects of the permeant (NEM) and impermeant (PCMBs) sulphhydryl reagents on Rb⁺ influx and efflux were examined. Solutions containing 0.2 mM CaSO₄, 1 mM Mes buffer (pH 6.5), and either 0.3 mM NEM or 2 mM PCMBs were prepared immediately before use. For experiments involving NEM, either root segments or intact roots were placed into 0.3 mM NEM for various times. The NEM solution was then aspirated and the roots were washed in an aerated solution of 1 mM DTE, 0.2 mM CaSO₄, and 1 mM Mes buffer (pH 6.5) for 10 min. Prior to influx or efflux experiments, roots were washed in 0.2 mM CaSO₄ briefly to remove DTE.

For influx experiments involving PCMBs, root segments were placed in 2 mM PCMBs for various times. PCMBs solution was then aspirated, and root segments were washed in an aerated solution of 0.2 mM CaSO₄ plus 1 mM Mes buffer (pH 6.5) for 10 min. PCMBs (2 mM) was often included in the experimental solution for the course of the uptake. For efflux experiments involving PCMBs, 1 mM PCMBs was added to the isotope loading solution for the last 30 min of loading; the same concentration was used throughout the course of the efflux experiment.

When it was desirable to remove any free inorganic mercury from stock PCMBs (Sigma), the method of Will and Hopfer was employed (36). Free Hg²⁺ was removed by passing stock PCMBs solution through a column (0.5 x 4 cm) of Dowex chelating resin (Sigma, in H⁺ form). The pH of eluted PCMBs was adjusted to 7.5 and the concentration was determined spectrophotometrically.

Abbreviations: ANDA, 7-amino-1,3-naphthalene disulfonic acid; NEM, N-ethyl maleimide; PCMBs, p-chloromercuribenzenesulfonic acid; DTE, dithioerythritol.

Fig. 1. Apparatus used for uptake experiments (A). Corn root segments were placed into Plexiglas vials (C) which were then placed into aerated uptake wells, as shown in B. To terminate an experiment, the Teflon valve was rotated and the uptake solution pulled, under vacuum, into the chamber below the uptake wells.
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at 265 nm (ε = 611 M⁻¹ cm⁻¹ at pH 7.5). Purified PCMBs was prepared immediately prior to use.

RESULTS

Rb⁺ Influx Isotherms. Rubidium influx isotherms for low- and high-salt roots were smooth, nonsaturating curves (Figs. 2 and 3). As Rb⁺ concentration increased above approximately 1 mM, the curves approached linearity; the slopes of the linear portion were almost identical for both low-salt and high-salt root isotherms. In experiments studying influx from a wider range of Rb⁺ concentrations (0–50 mM RbCl), the observed linearity was maintained, with no evidence of discontinuities or saturation. Figure 3 focused on the concentration range associated with Mechanism I in the Epstein model. The isotherms were smooth, and although they more closely approximated Michaelis-Menten kinetics, they still did not approach saturation.

There were several problems in experimental design which had to be dealt with in these studies. Experiments were designed which generated large numbers of data points and replicates, while minimizing variability, in order to establish accurately the shapes of uptake isotherms. Through the design, construction, and subsequent use of the uptake apparatus shown in Figure 1, we were able to obtain the necessary data from our experiments. With this apparatus, we could handle large numbers of root segments rapidly with a minimum of damage, and could quickly manipulate a wide range of substrate concentrations. Reproducibility from replicate experiments was excellent.

In the present experiments, we found that uptake periods of 10 or 30 min produced isotherms that were almost identical in shape and influx value. Because a larger amount of radioisotope was accumulated during the 30-min uptake, which reduced counting variability, most experiments were run for 30 min. From efflux experiments, it was determined that the 16-min wash at 2°C removed all but about 5% of the free space ⁸⁶Rb⁺, while removing only 5% of the ⁸⁶Rb⁺ accumulated in the cytoplasm (data not shown). Thus, we thought that the 30-min uptake/16-min wash regime yielded influx values which represented transport across the plasmalemma.

The final problem concerned the potential discrimination of the transporter(s) for K⁺ versus Rb⁺. K⁺ uptake is often measured from ⁸⁶Rb⁺-labeled KCl solution, with the assumption that discrimination is minor. However, in some systems, such as Chara (18) and Phaseolus leaf slices (16), significant discrimination occurs in the transport of K⁺ versus Rb⁺. Läuchli and Epstein have demonstrated that the transport discrimination between K⁺ and Rb⁺ in corn roots is only about 20% (21). Nonetheless, in order to have total confidence in our influx data, we studied radioisotope influx from ⁸⁶Rb⁺-labeled RbCl solutions. Therefore, it was necessary to repeat key flux experiments in which we followed ⁴²K⁺ uptake from KCl solutions to ensure that our interpretations derived from Rb⁺ flux data applied to K⁺ transport. In experiments that were the same as those of Figure 2, K⁺ influx isotherms were nearly identical in shape and rates to those for Rb⁺.

The kinetic profiles presented in Figure 2 can be interpreted in various ways. Because of supporting evidence which will be reported later, we have chosen to interpret these isotherms as the sum of a saturable and a linear component, which can be expressed as:

\[ v = V_{\text{max}} S/(S + K_m) + kS \]  

where \( v \) is the rate, \( S \) is the cation concentration, \( V_{\text{max}} \) and \( K_m \) are the Michaelis-Menten constants, and \( k \) is the first-order rate coefficient. Using the method of Denbham and Levin (8), the linear component (\( kS \)) was computed and subtracted (Fig. 4). The resulting data approximated a saturable curve. Eadie-Hofstee and Lineweaver-Burk transformations yielded identical kinetic constants for the saturable system. Apparent \( K_m \) values were 16 μM for low-salt roots and 86 μM for high-salt roots, while \( V_{\text{max}} \) values were 5.61 and 1.85 μmol g fresh weight⁻¹ h⁻¹, respectively, for low- and high-salt roots. The linear components were identical for isotherms from either root salt status. In order to ensure that the linear component was not an artifact of incomplete washing of radiolabel from the apoplast, the 30-min uptake 16-min wash regime was performed on root segments that had been repeatedly freeze-thawed to rupture the syndam. These roots did not accumulate any measurable radioisotope, indicating that the linear component was due to Rb⁺ uptake into the syndam.

Influence of Sulfhydryl Reagents on Influx Isotherms. The effect of the penetrating, covalently binding sulfhydryl reagent, NEM, on Rb⁺ influx isotherms was studied. Increasing exposures to NEM selectively inhibited the saturable component in both low- and high-salt roots, while inhibiting the linear component much less dramatically (Fig. 5). In high-salt roots, a 10-s pulse with 0.1...
mm NEM, prior to uptake, reduced the saturable component \( V_{\text{max}} \) by 60%, while inhibiting the linear component only slightly. A 30-s exposure to 0.3 mM NEM totally abolished the saturable component, while reducing the linear component by 30%. Low-salt roots required longer exposures to NEM to elicit similar degrees of saturable component inhibition. A 30-s NEM pulse inhibited the saturable component \( V_{\text{max}} \) by 70%, while leaving the linear component unaffected. A 5-min NEM exposure totally inhibited the saturable component. Identical responses to NEM were found for \( K^+ \) influx isotherms in experiments involving \( ^{42}\text{K}^- \) uptake from KCl solutions.

Table I summarizes the effects of different NEM exposures on both the linear and saturable components of \( \text{Rb}^+ \) uptake. The basic response is a selective inhibition of the saturable component with increasing NEM exposure. As NEM exposures lengthened, the linear component was also reduced, but much less significantly. It is of interest to note that increasing NEM exposures not only reduced the \( V_{\text{max}} \) of the saturable component, but also increased the apparent \( K_m \), indicating that the apparent affinity of the transporter for \( \text{Rb}^+ \) was reduced.

The influence of the impermeant sulphydryl reagent, PCMBs, on \( \text{Rb}^+ \) influx was also investigated. Although the resulting kinetic curves were more complex than found for NEM experiments, the same general inhibitory trends were seen (Fig. 6). Uptake in the concentration range dominated by the saturable component (0–1 mM) exhibited a linear response to increasing \( \text{Rb}^+ \) concentrations, thus indicating that PCMBs caused some form of transition in the transport mechanism responsible for saturable uptake. A family of curves resulting from increasing exposure to PCMBs could be obtained (data not shown). However, even with prolonged exposure to PCMBs (40 min), it was never possible to abolish the saturable component. We also found that the linear component in low-salt root isotherms was always stimulated by PCMBs. Exposure of 1 min or longer with PCMBs (2 mM) always stimulated the linear component by about 40% over control values.

Table I. Effect of Various NEM Exposures on the Saturable and Linear Components of \(^{86}\text{Rb}^+\) Uptake into Corn Root Segments

| Salt Status | Length of NEM Exposure | Saturable Component | Linear Component (k)
|-------------|------------------------|---------------------|-----------------
| Low salt    | 0 (control)            | 16 \( \mu \text{M} \) | 5.62 \( \mu \text{mol g fresh weight}^{-1} \text{h}^{-1} \) | 0.30
|             | 30 s                   | 29                  | 1.73             | 0.29
|             | 2 min                  | 81                  | 0.70             | 0.20
|             | 5 min                  | ND                  | ND               | 0.16
| High salt   | 0 (control)            | 86                  | 1.85             | 0.31
|             | 10 s\(^a\)             | 153                 | 0.76             | 0.27
|             | 18 s\(^b\)             | ND                  | ND               | 0.25
|             | 30 s\(^c\)             | ND                  | ND               | 0.21

\(^a\) 0.1 mM NEM.
\(^b\) First-order rate constant for linear component of \( \text{Rb}^+ \) transport in mol g fresh weight \(^{-1} \text{h}^{-1} \).
\(^c\) Saturable component not detectable following NEM treatment.

\( \text{Rb}^+ \) Efflux Experiments. Because sulphydryl reagents dramatically inhibited \( \text{Rb}^+ \) influx, experiments were performed to determine if these modifiers also influenced efflux. Control efflux data, when plotted as the log of tissue radioactivity versus time, yielded curves consisting of three approximately linear components (Fig.
were 37.3 control and NEM-treated curves. These data suggest the NEM-treated component (vacuolar) in the tissue and achieved.

FIG. 6. Influence of PCMBs on $^{86}$Rb$^+$ influx isotherms. Corn root segments were given a 20-min pretreatment in 2 mM PCMBs + 0.2 mM CaSO$_4$ + 1 mM Mes (pH 6.5) prior to uptake. Two mM PCMBs was also included in the uptake solution. First-order rate coefficients following PCMBs exposure were 0.42 and 0.30 $\mu$mol g fresh weight$^{-1}$ h$^{-1}$ mm$^{-1}$, for low- and high-salt roots, respectively.

FIG. 7. Influence of NEM on efflux of $^{86}$Rb$^+$ from high-salt roots. Roots were loaded for 24 h with $^{86}$Rb$^+$ until isotopic flux equilibrium was achieved. NEM was added to the isotope loading solution (0.3 mM NEM) and roots were exposed to it for 30 s prior to commencing the $^{86}$Rb efflux experiment. The efflux solution contained 0.2 mM RbCl + 0.2 mM CaSO$_4$ + 1 mM Mes (pH 6.5). Data was plotted as the log of cpm remaining in the tissue ($Q_i$) normalized for root weight versus time. The slowest effluxing component (vacuolar) was extrapolated to zero and subtracted from the overall curve. Insert shows a plot of the resulting data ($Q =$ cpm in slow component). These data represent cytoplasmic efflux for control (○) and NEM-treated (■) corn roots. Vacuolar half-times were 27.5 and 70 h for control and NEM-treated root tissue, respectively. Cytoplasmic half-times were 37.3 and 36 min for control and NEM-treated root tissue, respectively.

FIG. 8. Influence of PCMBs on $^{86}$Rb$^+$ efflux in high-salt-grown corn roots. Following a 24-h $^{86}$Rb$^+$ loading period, 1 mM PCMBs was added to the isotope solution for 30 min prior to initiation of efflux. One mM PCMBs was also included in the efflux solution through the first 9 h. Data was analyzed as in Figure 7. Insert depicts control cytoplasmic efflux (○). Efflux curve resulting from PCMBs exposure could not be resolved into cytoplasmic and vacuolar components. Vacuolar half-times were 42 and 11.5 h for control and PCMBs-treated root tissue, respectively. Following removal of PCMBs, the half-time increased to 48 h. The control cytoplasmic half-time was 38 min. PCMBs appeared to abolish the cytoplasmic component.

7). Each component was determined by the sequential subtraction of the slower-exchanging from the subsequent faster-exchanging component of the curve. We have chosen to interpret such curves as the product of efflux from three compartments in series, the vacuole, cytoplasm, and cell wall, while recognizing the fact that such an interpretation has been subject to some criticism (26, 35). These criticisms will be dealt with in the discussion.

The effect of a 30-s NEM pulse on $^{86}$Rb$^+$ efflux from high-salt roots is also shown in Figure 7, with the insert representing cytoplasmic efflux. NEM had no effect on efflux across the plasmalemma ($t_{1/2}$ [control] = 37.3 min; $t_{1/2}$ [NEM] = 36 min). However, NEM had a significant effect on vacuolar efflux, reducing it by a factor of about 2.5 ($t_{1/2}$ [control] = 27.5 h; $t_{1/2}$ [NEM] = 70 h). Similar results were obtained for $^{42}$K$^+$ efflux into solutions of various KCl concentration.

PCMBs had a more complex effect on $^{86}$Rb$^+$ efflux (Fig. 8). Following washout from the cell walls, efflux was linear throughout the remainder of the experiment, provided PCMBs was included in the efflux solution. Hence, it was impossible to separate efflux into cytoplasmic and vacuolar components. The half-time for efflux in the presence of PCMBs was 11.5 h, while in control tissue the value for the vacuole was 42 h. Thus, it appeared that the cytoplasmic component was somehow abolished, yet vacuolar efflux was stimulated. The observation that the actual flux of $^{86}$Rb$^+$ from PCMBs-treated roots exceeded that of control roots throughout the experiment supports the hypothesis that PCMBs stimulated vacuolar efflux. Once PCMBs was removed from the efflux solution, a rapid recovery back to control efflux values was seen (Fig. 8).

The effects of NEM and PCMBs on efflux (cytoplasmic and vacuolar half-times) are summarized in Table II. The general responses to NEM and PCMBs hold true for both low-salt and high-salt roots over a range of RbCl concentrations in the efflux solution. In efflux experiments utilizing excised root segments, the leakage of ions from the cut ends of the xylem could contribute significantly to the overall measured efflux (35). In order to ensure that the NEM effect on vacuolar efflux involved cortical tissue...
and was not due to inhibition of xylem efflux, experiments were performed with a closed system consisting of the terminal 10 cm of the root (cut end sealed and not in contact with bathing medium). The control cytoplasmic and vacuolar half-times for this system were similar to those obtained using 2-cm root segments. One possible explanation for this would be that the half-times for cytoplasmic and vacuolar xylem efflux are similar to those for cortical tissue efflux. This conclusion is supported by the observation that the kinetics of cortical and xylem "Na" efflux in barley roots were similar (17). The more important observation in the sealed root system was that the NEM response was identical with that in root segments, indicating that NEM was acting on vacuolar efflux from the root cortical and epidermal cells. Another point of interest is the observation that, as the external RbCl concentration was increased from 0.2 to 10 mM there was no significant change in either cytoplasmic or vacuolar efflux in either low- or high-salt roots. Thus, it appears that exchange diffusion of K⁺ across the plasmalemma is minimal, unlike that of Cl⁻ (6). Finally, efflux across the plasmalemma and tonoplast appears to differ substantially in low-salt compared to high-salt roots. In high-salt roots, efflux from the cytoplasm is about twice that observed in low-salt roots, while vacuolar efflux is about twice as rapid in low-salt roots.

**DISCUSSION**

In the present study, we focused on the concentration range from 0 to 10 mM to stay somewhat near the range of physiological relevance. Studies on soil K⁺ concentrations indicate the majority of the soil K⁺ is below 2 mM (33). As clearly shown by the results presented in Figures 2 and 3, the concentration dependence of K⁺ influx into corn roots followed a smooth, non-saturating profile. We were unable to detect discontinuities in the concentration region from 1 to 10 mM. These results are consistent with the recent theoretical analysis of root ion transport kinetics presented by Borstlap (4), but are at variance with the interpretations presented by many earlier workers. As is evident from Borstlap's analysis, verification of discontinuities in the kinetic profiles of other systems may require further and more detailed experimental documentation.

**Evidence for Saturating and Linear Mechanisms.** The kinetics observed for K⁺ (Rb⁺) influx in corn roots can be interpreted as the operation of a single complex, allosterically regulated enzyme. The binding of an effector, such as K⁺, to a subunit of the enzyme, could induce a conformational change which would reduce the affinity of the binding site for substrate. Systems demonstrating such negative cooperativity often exhibit kinetics similar to those presented here. Hodges has argued that the kinetics observed for ion uptake in plant roots could be due to negative cooperativity, while the 'bumps' observed in most influx isotherms could be explained by an additional component exhibiting positive cooperativity (15).

An alternative interpretation would attribute the observed kinetics to the operation of a saturable mechanism in conjunction with a first-order kinetic process. The results from our sulfhydryl reagent studies offer support for this interpretation, in that it was possible to reduce, sequentially, and finally abolish the saturable component with increasing NEM exposure. Under these conditions, the linear component was reduced much less dramatically. It would appear that as NEM exposure increased, more of the saturable carriers were titrated until they were all inhibited. Additionally, the saturable carriers which continued to function following NEM exposure exhibited a large increase in their apparent Kᵅm (see Table 1), which could be the result of biochemical modification of the carrier. Alternatively, NEM could be inhibiting transport into the cells of the root periphery, while cortical cells would continue to function under the influence of diffusion limitation.

The saturable component appears to be Epstein's Mechanism I. It is dominant in the low concentration range (0–1 mM) and approximates Michaelis-Menten kinetics. Furthermore, it is sensitive to inhibitors such as the sulfhydryl reagents used for these experiments or uncouplers such as CCCP (32). K⁺ uptake in this concentration range is believed by many workers to be active, accumulating K⁺ against its electrochemical potential gradient,
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although this is still subject to debate.

The Linear Component: Simple Diffusion? The nature of the linear component is intriguing, and experiments designed to characterize and identify the linear mechanism are currently being carried out in our laboratory. In the concentration range where the linear component dominates uptake, many workers feel that influx of K⁺ is passive. It is relatively insensitive to inhibitors and shows no signs of saturation at the higher concentrations tested. There have been many examples of transport kinetics in both animals and plants in which a linear component makes a significant contribution. The linear component has usually been dismissed as either passive or exchange diffusion. However, some of our findings suggest that linear uptake may be the product of a more complex mechanism. First, a transporter accumulating radionuclide via exchange diffusion should be rate controlled by either internal or external substrate concentration. In our system, the linear component was identical in roots varying significantly in internal K⁺ concentration (low- versus high-salt roots), an observation that argues against exchange diffusion. Furthermore, changes in the external K⁺ (Rb⁺) concentration from 0.2 to 10 mM had no effect on K⁺ (Rb⁺) efflux. This is in contrast to CI⁻ efflux, which has been shown to be stimulated by increases in external Cl⁻, thereby leading to the postulation of a CI⁻ exchange diffusion system at the plasmalemma (6). Second, some of the sulphydryl reagent effects on the linear component cannot be explained by a simple diffusion-mediated mechanism. An ionic species such as K⁺ should permeate the lipid bilayer of biological membranes slowly; therefore, it is safe to assume that diffusive influx of K⁺ would be carrier-mediated (i.e., a facilitated diffusion, possibly through a proteinaceous channel or pore). Although the linear component was relatively insensitive to sulphydryl reagent inhibition, increasing exposures of NEM did result in a reduction of the linear component. NEM has been shown to depolarize the membrane potential to a level presumed to be the diffusion potential for K⁺ (22, 23). This depolarization would reduce the driving force for passive K⁺ influx. Thus, this NEM-induced reduction in the linear component is still consistent with uptake by facilitated diffusion. However, PCMBs has also been shown to depolarize the membrane potential to the K⁺ diffusion potential (22, 23), and PCMBs had no effect on the linear component in high-salt roots, while consistently stimulating the linear component by 40% in low-salt roots. The linear component of uptake may be due to a more complex mechanism than facilitated diffusion. Baker and Hall (1) have cytochemically detected ATPase activity on vesicles near the plasmalemma of corn root cells and have hypothesized that passive uptake in the higher concentration range could be due to endocytosis. Others have proposed vesicular transport between the plasmalemma and vacuole to account for anomalous 'shoulders' occasionally observed on efflux curves (30). Another explanation is based on the consideration that any carrier-mediated transport (including facilitated diffusion) should eventually saturate. Therefore, it is possible that what we observe as linear uptake is only the linear portion of a saturating kinetic curve for a very high $K_m$ (low affinity) transporter.

Finally, Göring and coworkers have hypothesized that multie sulfhydryl comes from the differential sensitivity of K⁺ uptake to NEM and the impermeant PCMBs. While short NEM exposures abolished the saturable component of uptake, it was never possible to totally inhibit the saturable component with PCMBs (exposures up to 30 min). Therefore, saturable K⁺ uptake appears to involve both sulfhydryls at the exterior of the plasmalemma and within the interior of the membrane (cytoplasm). This situation is similar to the sulphydryl involvement in HCO₃⁻ uptake in Chara (24). Purified PCMBs was a much less effective inhibitor of HCO₃⁻ uptake than the penetrating sulphydryl reagents, Hg²⁺ or NEM.

In addition to being a less effective inhibitor of saturable component uptake than NEM, PCMBs also appears to alter the basic mechanism of saturable uptake. In the presence of PCMBs, the saturable mechanism was converted such that it responded linearly to changes in external ion concentration. It is possible that the carriers, following sulphydryl modification, have undergone a change in conformation to some form of 'open channel' structure. Lucas and Alexander obtained similar results while studying NEM effects on HCO₃⁻ uptake in Chara (24). As exposure to NEM (or Hg⁺) increased, HCO₃⁻ uptake exhibited a reduction in the $V_{max}$, while the $K_m$ was unaffected. Further increases in NEM exposure caused a rapid transition in HCO₃⁻ uptake to a linear mode. The authors speculated that this transition represents either a transport conversion to a facilitated diffusion system or, alternatively, influx could have continued unaffected while a change in the carrier allowed for a dramatic increase in HCO₃⁻ efflux; thus, the transporter would be converted to a 'pump and leak' system.

Will and Hopper (36) have reported that PCMBs can contain as much as 0.5% (by weight) inorganic mercury. Many enzyme systems are extremely sensitive to inorganic mercury, which is a potent sulphydryl reagent and can penetrate into the cytoplasm. Lucas and Alexander (24) found that unpurified PCMBs inhibited HCO₃⁻ uptake in Chara much more dramatically than purified PCMBs. However, in our experiments, purified and unpurified PCMBs had similar effects on Rb⁺ uptake. The possibility exists that any free Hg²⁺ in the PCMBs could have been bound up in the cell walls of the root, and thus was not available to influence Rb⁺ transport.

Efflux Analysis. There are obvious inherent dangers in the treatment of a complex organ, such as a root, as a simple structure composed of three compartments in a series. This danger is underscored by occasional examples of efflux kinetics that deviate from the strict three compartment model (30). However, in all of our efflux experiments, the data agreed well with a system obeying first-order kinetics for efflux from a three-compartment model. Further verification of the model can be obtained by applying certain kinetic analyses to the experimental data. As Cram and Laties have shown (7), the plots of ln content versus time and ln efflux versus time should both yield curves fitted by the same number of compartments, with identical rate constants for the corresponding compartments from each plot. This can be best illustrated by the following analysis. For the sake of simplicity, the initial rapid component due to wash out from the cell walls can be ignored; thus, the radioactive content ($Q_t$) of the resulting two-compartment model would be described by the equation:

$$Q_t = A e^{-K_{r1} t} + B e^{-K_{r2} t}$$  

(2)

where $K_r$ and $K_a$ are the rate constants for the rapidly (cytoplasm) and slowly (vacuole) effluxing compartments. Plots of ln $Q_t$ versus time would yield curves composed of two linear phases with slopes of $K_r$ and $K_a$ and y intercepts $A$ and $B$. Efflux ($dQ/dt$) from this system would be described by the equation:

$$dQ/dt = - [K_r A e^{-K_{r1} t} + K_a B e^{-K_{r2} t}$$  

(3)

A plot of ln $dQ/dt$ versus time would again yield curves with two linear phases. The slopes of the two linear phases would again be...
K_r and K_s. Furthermore, the intercepts of this plot, which we will call I_r and I_s, should be related to the intercepts of the ln Q_r versus time plot by the following equations:

\[ I_r = K_r A \] and \[ I_s = K_s B \]

Therefore, the ratio of the intercepts of the ln dQ_r/dt versus time plot (I_r/I_s) should equal the ratio of the intercepts from the ln Q_r versus time plot multiplied by the ratio of the rate constants

\[ \left( \frac{K_r}{K_s} \right) \times \left( \frac{A}{B} \right). \]

(We emphasize this point because of previous misinterpretations of Cram's and Laities' original work.) These tests were applied to our efflux data (Fig. 7); the rate constants from plots of log Q_r and log dQ_r/dt versus time were approximately equal. K_r values were 4.20 \times 10^{-7} \text{ min}^{-1} and 6.20 \times 10^{-7} \text{ min}^{-1}, and K_s values were 1.93 \times 10^{-3} \text{ min}^{-1} and 1.90 \times 10^{-2} \text{ min}^{-1}. In addition, the ratio of the intercepts from the log dQ_r/dt versus time plot (I_r/I_s) equaled 2.29, while

\[ \left( \frac{K_r}{K_s} \right) \times \left( \frac{A}{B} \right). \]

Therefore, we feel that our efflux data represent, at least as a first-order approximation, efflux from the vacuole and cytoplasm in series, and have chosen to present our kinetic parameters as the half-time for efflux from these compartments.

**Effects of NEM and PCMBS on Efflux.** NEM influenced efflux in a much different manner than influx, while the results involving PCMBS, although difficult to interpret, seem to indicate that its effect on efflux and influx are related. NEM, which significantly inhibited plasmalemma influx, had no effect on efflux across the plasmalemma, while substantially reducing transport from the vacuole. Because NEM is a penetrating molecule, it may be acting directly at the tonoplast to inhibit vacuolar efflux. Alternatively, it could be acting at the plasmalemma to inhibit vacuolar efflux in some unknown fashion. It seems less likely that NEM is acting at a cytoplasmic site, such as the mitochondria, to reduce energy supply for transport, at least following the 30-s NEM pulses in high-salt roots. A 30-s NEM pulse had no effect on root respiration (L. V. Kocian and W. J. Lucas, unpublished results).

The experiments with purified PCMBS demonstrate the effects on efflux of a modifier acting exclusively at the plasmalemma. PCMBS had several significant effects on efflux. First, the basic shape of the efflux curve was changed from the usual biphasic (ignoring cell wall exchange) to a monophasic efflux, presumably representing efflux from one compartment (vacuole). It is difficult from these data to interpret what has happened to efflux from the cytoplasm. As we speculated for influx, it is possible that PCMBS has modified some portion of the carrier for K^+, so that it now functions as an open channel. Other workers have reported that long exposures to PCMBS (up to 1 h) caused transport systems to undergo dramatic structural changes. Trump et al. (34) have demonstrated structural changes of the plasmalemma of Ehrlich ascites tumor cells following exposure to PCMBS, and Will and Hopfer (36) found that PCMBS produced significant increases in the cation permeability of red blood cell membranes. Second, PCMBS significantly increased vacuolar efflux. Since it is acting at the plasmalemma, it appears that this membrane does exert influence on vacuolar transport processes. It is not likely that PCMBS has modified the plasmalemma structure to such a degree that it could penetrate into the cytoplasm. This follows from the observation that the removal of PCMBS from the efflux solution elicited a rapid recovery in efflux, back to approximately control values (Fig. 8).

In conclusion, our results for K^+ transport into corn roots are consistent with the combined operation of saturable and linear components. The saturable component, which may represent Epstein's Mechanism I, appears to be engaged in active transport of K^+ into the root symplast. Nonsaturation transport kinetics are widespread and have been demonstrated in uptake systems ranging all the way from single cells to plant tissues. Although generally considered to represent simple or facilitated diffusion, the first-order kinetic mechanism may be more complex in nature. Therefore, the linear component may be a fairly universal feature of transport, with definite biological significance, and warrants further study.

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