

Dual Mechanisms of Ion Uptake in Relation to Vacuolation in Corn Roots

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Summary. Absorption isotherms for chloride and rubidium ions have been determined through a wide concentration range for nonvacuolate root tips, and for vacuolate subapical sections of corn root. In the range 0 to 0.5 mM, chloride absorption is hyperbolic with concentration in both tips and proximal sections. At high concentrations, 1 to 50 mM, a second multiple-hyperbolic isotherm for chloride is noted in vacuolate tissue, while the isotherm for nonvacuolate tips rises exponentially. A linear to exponentially rising isotherm is taken to signify diffusive permeation.

The same distinction between tip and subapical tissue characterizes Rb absorption. Rb uptake is indifferent to the nature of the counterion at all concentrations in the tip, while the counterion exerts a predictable influence on Rb absorption in proximal tissue. The effect of a poorly absorbable anion on Rb uptake is greater in the high concentration range. Evidence is presented for the metabolic nature of ion transport into nonvacuolate root tips. Verification is offered that ion uptake is mediated by dual mechanisms, and the thesis is developed that the high-affinity (low K_s) system mediates ion passage through the plasma membrane while the low-affinity (high K_s) system implements transport through the tonoplast.

In the usual range of concentrations employed experimentally, isotherms for ion uptake by plant tissues have been found to be hyperbolic. The latter observation has led to the concept of carrier-mediated transport (10, 33), which concept has subsequently been extensively and fruitfully explored (3, 10, 11, 12, 13, 14). Until relatively recently, however, salt absorption studies have been performed predominantly at concentrations from 1 to 50 mM, a range which may be considered unnaturally high in relation to the concentration of soil solutions (5, 11; see Discussion). Following the discovery by Epstein and Hagen (10) that 2 distinct transport systems may be involved in the absorption of a given ion, the relative contribution of each depending on the concentration, Hagen and Hopkins (14) defined 2 systems for phosphate absorption by barley roots which differed in their affinity for phosphate by some 3 orders of magnitude, while Fried and Noggle (13) reported a similar duality respecting the absorption of Na, K, Rb and Sr. In recent years Epstein and his coworkers have examined dual absorption mechanisms in depth for both cations (11, 12) and anions (8). For each of the major ions there is a high-affinity, low K_s system (system 1), saturated at approximately 0.1 mM, and a low affinity, high K_s

system (system 2), which operates maximally at concentrations from 100 to 500 times the latter. Although at high concentrations the absorption isotherm has subsequently been shown to be multiple (8, 11, 12), the principal distinction remains that between the low K_s and the first high K_s system, and in this connection it remains useful to speak of a dual isotherm or dual mechanism (11).

A dual isotherm for the absorption of a given ion points to more than one transport system, and raises the question of the location of each system. Where 2 demonstrable systems exist, they may be thought to operate in parallel at the same membrane, or may be considered to function consecutively in the passage of an ion through the plasma membrane and tonoplast respectively. In the latter instance it follows that to be separately observable, the high-affinity system must be at the plasma membrane, and the low affinity system at the tonoplast. It further follows that at high concentrations, i.e. at concentrations in excess of those which saturate system 1, ion passage through the plasma membrane must exceed that mediated by the high-affinity system, the absorption isotherm in the high range thereby reflecting ion movement from the cytoplasm into the vacuole. Evidence to be presented suggests that in the range of system 2, ion movement through the plasma membrane by diffusion is rapid enough to fulfill the requirement, i.e. not to be rate-limiting. To the extent that a correction is made for the

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contribution of system 1 in the estimation of the contribution of system 2 to the total, the systems are tacitly considered in parallel, albeit they may be thought to deliver ions to separate compartments (11). If the systems are in fact in series, and if the cytoplasm is filled rapidly compared with the vacuole, as is to be expected (27, 29), steady-state uptake in the high range will describe solely the rate of ion movement into the vacuole, and the contribution of system 2 need not, and indeed should not, be corrected for the activity of system 1.

In the following study, predominantly nonvacuolate corn root tips are compared with vacuolate sub-apical, or proximal, root sections with respect to the absorption isotherms for both chloride and rubidium. On the assumption that the low K_s system mediates ion passage across the plasma membrane, while the high K_s system implements transport across the tonoplast, absorption in root tips, which lack a vacuole and tonoplast, may be expected to present a predictably different isotherm from that evinced by vacuolate proximal sections when uptake is examined through a wide concentration range.

Vacuolation is not sharply delineated in developing roots. Handley et al. (15) indicate that the first 1.8 mm of corn-root tips are free of visible vacuoles, while Brown and Cartwright consider that the cells of the apical 1.5 mm are 68% nonvacuolate (6). Electron microscopic studies reveal that minute vacuoles are in the making just a few cells behind the root apex (36). Although this study seeks to compare vacuolate with nonvacuolate cells, it is recognized that experimentally the distinction cannot be absolute, and that furthermore subapical cells differ from vacuolate cells for reasons which transcend vacuolation (6, 17). Thus, the validity of the working hypothesis will to a considerable degree depend on the results, and it can be said that predictions are met.

Materials and Methods

Hybrid starchy corn (*Zea mays*), Hy 2/07, from Illinois Foundation Seeds, Incorporated, was soaked and germinated as previously described (7) in 0.5 mM CaSO_4 . Freshly harvested primary roots of 3-day-old seedlings, 9 to 10 cm long, were rinsed with distilled water for 1 hour and cut into tip and proximal sections. Tips comprised the apical 2 mm, while 13 mm proximal sections were taken between 2 and 15 mm from the apex. For each absorption measurement about 0.2 g tissue was placed in 500 ml of appropriate salt solution at low concentrations, or into 50 ml of solution at high salt concentrations. Absorption isotherms for chloride and for rubidium were determined for both tip and proximal sections through a wide concentration range. Salts were labeled with ^{36}Cl or ^{86}Rb , final specific activities being approximately 2 μc per mmole for Cl, and 5 μc per mmole for Rb. The experimental flasks were

gently shaken in a reciprocal water-bath shaker at 24°. The pH of the solutions was 5.8 to 6.0. Absorption periods were from 1 to 3 hours, it having first been established that uptake was linear with time for at least 8 hours. Experiments were discontinued by first rinsing the root sections 3 times with cold water for 30 minutes in chloride absorption experiments, and with either 1 mM or 10 mM cold nonradioactive rubidium chloride solution following rubidium absorption at low and high concentrations, respectively. Sections were then blotted dry and, following fresh weight determination, arranged on an aluminum planchet with 0.2 ml of 3% polyvinyl alcohol (Du Pont Elvanol grade 51-05) as adhesive. Tissue was dried at room temperature, following which radioactivity was determined with a gas-flow micromil window detector. Self-absorption was negligible. Calculations of the amount of ion uptake were based on the determined specific activity of the original experimental solutions.

Ca ion has been shown to influence membrane integrity (30), to affect passive flux rates and transport rates (16, 17, 19) as well as specificity of transport of the alkali cations (9), and to influence the K_s for monovalent cation uptake as well (17). At low pH (30) or in the presence of relatively high concentrations of monovalent cation (9), Ca must be present at all times to insure tissue integrity. However, when the pH is close to 6.0 and the salt concentration is low, there is no marked effect of the absence of Ca during relatively short experimental periods when roots have been grown in dilute CaSO_4 (16, 30). Under the latter conditions, the influence of Ca is noted only after some hours (16). On the basis of the foregoing considerations Ca was omitted for simplicity in the experiments which follow. The similarity of our isotherms in preliminary experiments to those of Elzam et al. (8) lent further assurance of the reliability of our experimental procedure. Nevertheless we have recently compared the isotherms for Cl absorption by corn root tips in the absence and presence of CaSO_4 (0.5 mM) in both concentration ranges. For a given range the isotherms were virtually superimposable. In work stemming from that reported herein, Ca has been present routinely, and experiments have verified and extended the conclusions drawn below.

Results

Chloride Isotherms. Figure 1 depicts the rate of chloride absorption as a function of the external chloride concentration over a 2500-fold range. Between 0.02 and 0.5 mM KCl, chloride is absorbed by a system which is virtually saturated at 0.1 mM in nonvacuolated tips, and at 0.3 mM in vacuolated proximal sections. Increasing the concentration of chloride in the low range causes no further increase in the rate of absorption. At considerably higher concentrations, from 1 to 50 mM, a second hyperbolic absorption isotherm is manifested by proximal sec-

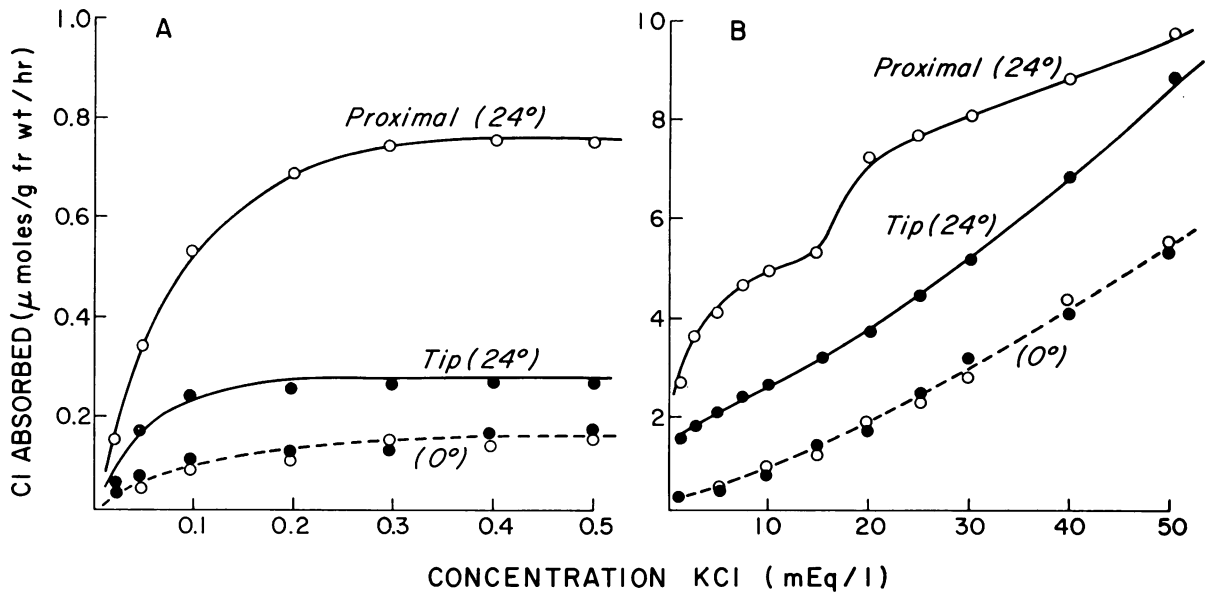


FIG. 1. Chloride absorption by tip and proximal corn root sections as a function of KCl concentration at 24 and 0°. Solid symbols, tip sections. Open symbols, proximal sections. Absorption period, 3 hours.

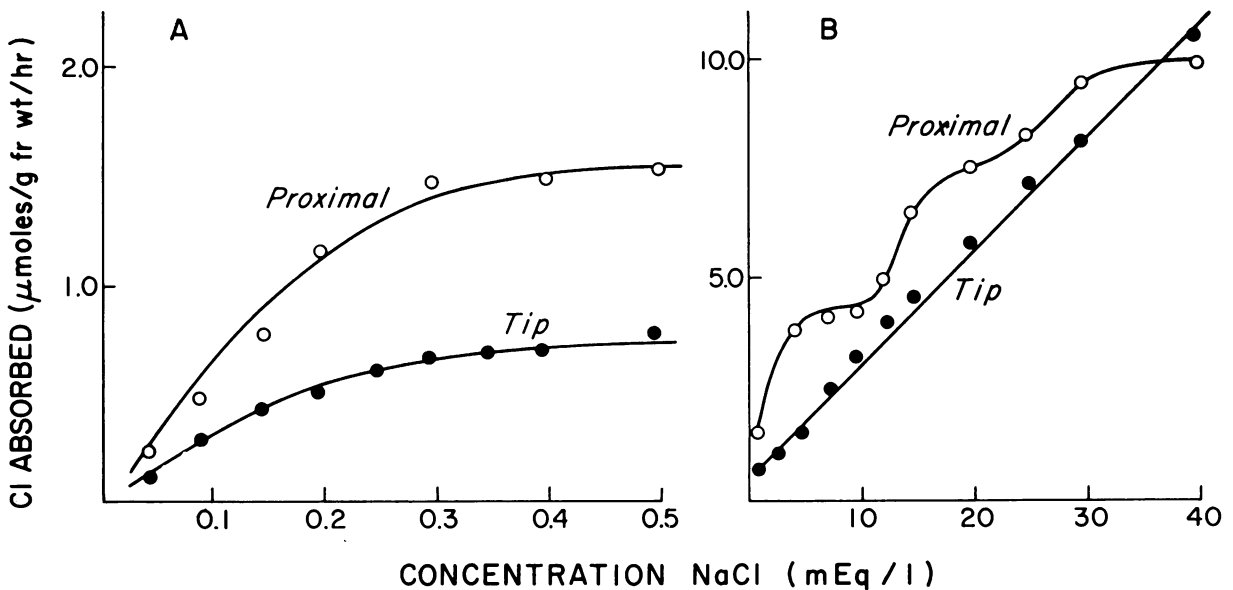


FIG. 2. Chloride absorption by tip and proximal corn root sections as a function of NaCl concentration. Temperature, 24°.

tions, which may be followed by one or more additional inflection points as the concentration is raised (cf. 8). By contrast, nonvacuolated tips show either a straight or an anomalously rising exponential isotherm in the high concentration range. The latter type is associated with diffusive penetration and the suppression of the negative membrane potential with increasing external salt concentration (23, 24). Absorption at low concentrations, hyperbolic with respect

to concentration in both proximal and tip sections, is temperature sensitive, albeit less so on a percentage basis in tips than in proximal sections.

Isotherms for chloride absorption from solutions of NaCl and CaCl₂ respectively are shown in figures 2 and 3. Chloride uptake from NaCl was always a little higher than from KCl (fig 2), and chloride absorption from CaCl₂ was considerably lower than from KCl (fig 3). The isotherms for chloride ab-

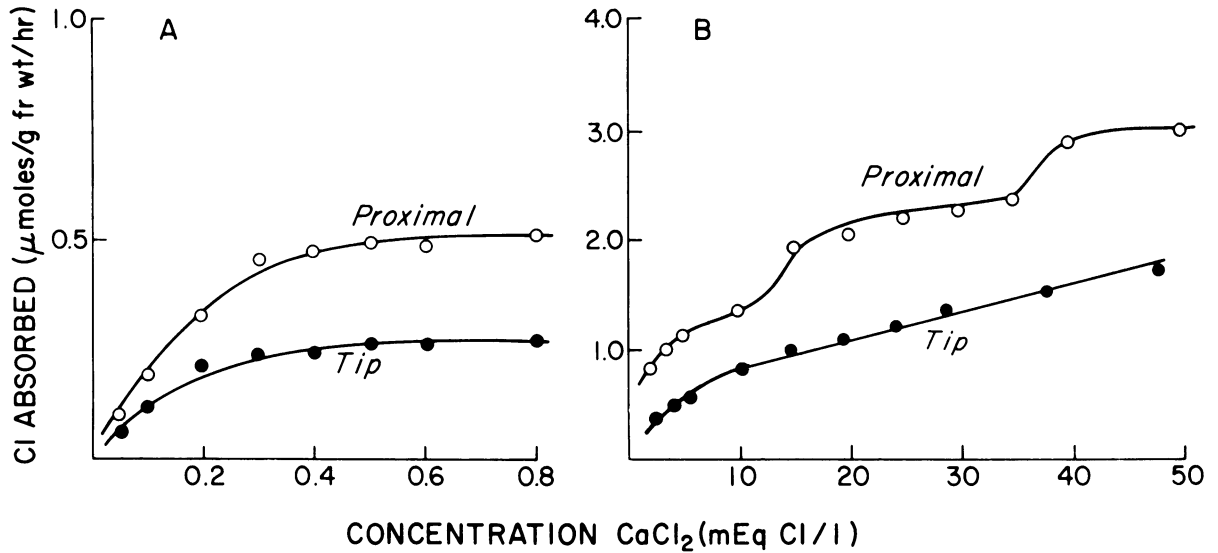


FIG. 3. Chloride absorption by tip and proximal corn root sections as a function of CaCl_2 concentration. Temperature, 24° .

sorption by the tip at high concentrations differ for potassium and calcium salts respectively in precisely the manner predicted on the basis of diffusive entry (24).

Figure 4 depicts the effect of bromide on chloride absorption in tip and proximal sections. The data are plotted in a double reciprocal way, the bromide concentration being set in each case, while the chloride concentration is varied. No correction has been made for system 1 in plotting uptake in the

range of system 2 (see Discussion). In the low concentration range, from 0.01 to 0.2 mM chloride, 2 mM bromide inhibits chloride absorption in tips and proximal sections, with no change in ordinate intercept in both cases. Thus, inhibition is presumably competitive in both types of tissue at low levels of external chloride. On the other hand, at high chloride concentrations, 1.0 to 5.0 mM, 10 mM bromide competitively inhibits chloride absorption in proximal root sections, but exerts a noncompetitive, presumably nonspecific, inhibition in root tips.

Figure 5 describes the concentration relations of rubidium absorption by tip and proximal root tissues, in experiments similar to those demonstrating the duality of the chloride absorption isotherm. The isotherm for rubidium essentially resembles that for chloride absorption. Both tip and proximal sections show a clear hyperbolic isotherm at low concentrations. At high salt concentrations proximal sections show a second hyperbolic isotherm, while tips show an essentially straight isotherm.

The effect of the counteranion on rubidium absorption was investigated in studies of absorption from RbCl and Rb_2SO_4 solutions. As shown in figure 5, Rb uptake in tips proved indifferent to the counterion at all salt concentrations. In vacuolated proximal sections, however, Rb absorption from Rb_2SO_4 was less than from RbCl , the difference being more marked at the higher salt concentration (cf. 11).

Chloride uptake by corn root tips is both temperature (fig 1) and inhibitor (table I) sensitive. Inhibitor sensitivity in the tip is greater at low concentrations, as might be expected. Because tips invariably include vacuolate cells, it is understandable why the tip displays inhibitor sensitivity in the high range.

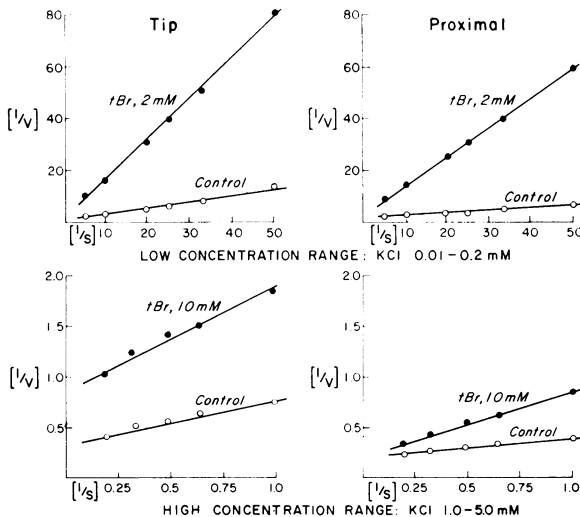


FIG. 4. The influence of bromide on chloride absorption by tip and proximal sections of corn root at 2 ranges of chloride concentration. Double reciprocal plots. K salts in all cases. Bromide 2 mM in the low range of chloride concentrations, and 10 mM in the high range of chloride concentrations. Temperature 24° . Absorption period, 3 hours.

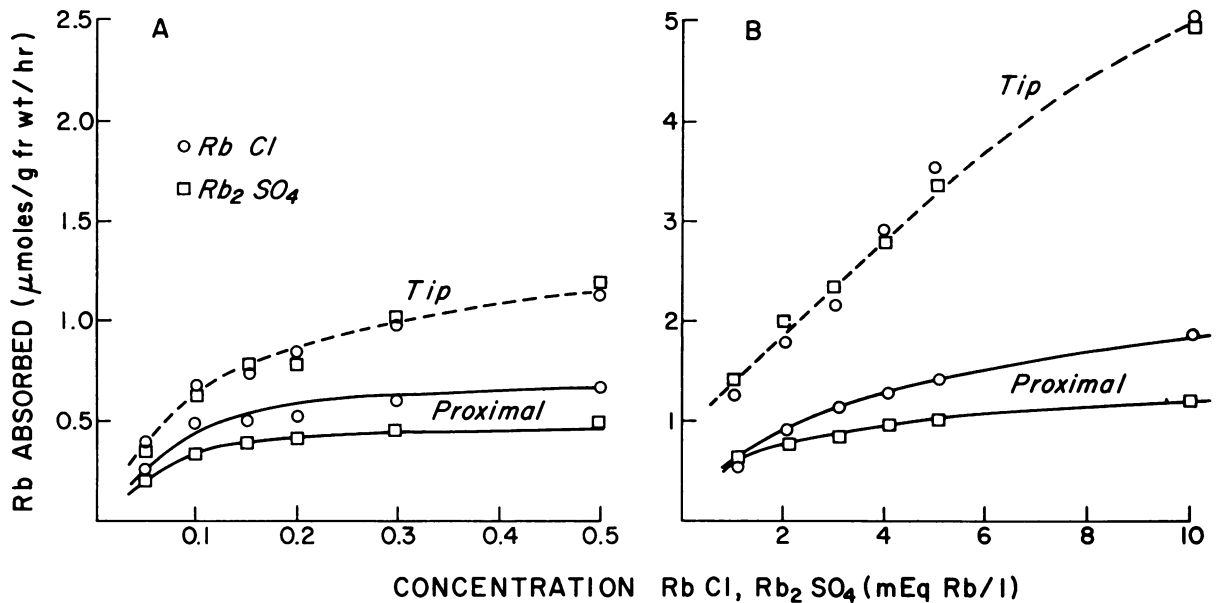


FIG. 5. Rb absorption by tip and proximal corn root sections: the effect of the counterion. Circles, RbCl . Squares, Rb_2SO_4 . Temperature 24° . Absorption period, 3 hours.

Table I. Effect of Inhibitors on Chloride Absorption by Corn Root Segments

| Molarity | | % Inhibition | | | |
|-----------|-------------------|--------------|----------|-------------|----------|
| | | KCl 0.1 mM | | KCl 10.0 mM | |
| | | Tip | Proximal | Tip | Proximal |
| 10^{-4} | 2,4-Dinitrophenol | 84 | 85 | 58 | 78 |
| 10^{-6} | <i>m</i> -Cl-CCP* | 75 | 92 | 52 | 76 |
| 10^{-4} | Cyanide | 10 | 68 | 19 | 48 |
| 10^{-4} | Azide | 75 | 72 | 41 | 55 |

* Carbonyl cyanide *m*-chlorophenylhydrazone.

In summary, both tip and proximal tissue show metabolically implemented, ostensibly carrier-mediated transport in the range 0.02 to 0.5 mM. At concentrations from 1 to 50 mM, only proximal, vacuolate tissue evinces a hyperbolic isotherm both for Cl and Rb absorption. Tip tissue, by contrast, manifests an exponentially rising isotherm for chloride and an essentially linear isotherm for Rb. In each range absorption by vacuolate tissue is ion-specific, apparently carrier-mediated, and responsive to the counterion, while absorption by nonvacuolate tissue displays the first two characteristics only in the low concentration range, and appears to be nonspecific and nonmetabolic in the high concentration range. It is thus deduced that nonvacuolate tissue has the high-affinity system only, while vacuolate tissue has both the high and low-affinity systems.

Discussion

The involvement of more than 1 system in the passage of a given ion from the environment to

the vacuole of a plant cell raises a compelling question regarding the role and location of each system. Two systems have been distinguished both for cation and anion absorption which differ in their ion affinities by roughly 3 orders of magnitude. The high affinity, low K_s system was shown to be considerably more specific than the low-affinity, high K_s system. In addition, the low K_s system for cation uptake was found to be indifferent to the nature of the counterion, while the high K_s system proved responsive to the counterion (11). Verification of dual mechanisms for ion uptake in plant tissues has been widespread (3, 4, 8, 10, 12, 13, 14, 20, 28).

Several considerations have caused us to adopt the hypothesis that the high-affinity mechanism operates at the plasma membrane, while the low-affinity system mediates transport across the tonoplast. On the one hand it is significant that the low K_s system operates in a concentration range found in soil solutions. Extracts from 135 fertile soils show a modal K concentration of approximately 4 ppm, or 0.1 mM, while the modal concentration of phosphorus is roughly 0.06 ppm, or 0.002 mM. Depletion of ions in the immediate environment of the root makes the concentration at the root surface still lower (5). However, so long as the supply of nutrient at the root surface is maintained, phosphate absorption may proceed at a maximal rate from a 0.007 mM solution (34). It seems reasonable therefore that system 1 should implement ion transport across the outer membrane.

There is no evident logical reason why a second system, with an affinity one thousand times less than the first, should operate in parallel at the same membrane. Carrier-mediated salt movement across the plasma membrane is active and leads to elevated

cytoplasmic concentrations (26, 27, 29). The tonoplast is thus faced with a considerably higher external (i.e. cytoplasmic) concentration than normally occurs in soil solutions, which, as noted, manifest concentrations in the range of system 1. In corn seedlings K is translocated to the shoot very much more readily than Na from 2.0 mM solution, while absorption by the root is similar for both ions. Furthermore, K transport to the shoot is almost as great from 0.1 mM solution as from 2.0 mM (4, cf. 28). In view of the fact that system 1 is virtually indifferent to Na while system 2 strongly favors Na (11), the beginning of a case can be made for the particular association of system 1 with the ultimate movement of ions into the xylem and to the leaves. In this connection we have examined the isotherm for Cl and for Rb transport (in the presence of 0.5 mM Ca) from the external solution to the leaves of corn seedlings (Lüttge and Laties, unpublished) and find it in each case to be that for system 1. By contrast total absorption by the root evinces the operation of both system 1 and system 2. Furthermore, we have shown (Osmond and Laties, unpublished) that when the cytoplasm is filled with K in a preincubation period, the demonstrable contribution of system 1 dwindles, only to be restored when upon further incubation in dilute CaSO_4 the cytoplasm is depleted of K by transport thereof to the vacuole (cf. 22, 35). Thus, the evidence suggests that system 1 serves to transport salt from the external solution to the cytoplasm, and hence to the symplasm (1), while system 2 is involved in salt movement into the vacuole from the cytoplasm. Since the systems are in series, once the cytoplasm receives ions more rapidly than can be effected by system 1, as is the case in the high concentration range, system 1 will no longer be separately observable, and in a short time, when the cytoplasm is filled, net uptake will reflect the operation of system 2. It is for this reason in our view that historically, double reciprocal plots of absorption against concentration in the range of system 2 have frequently yielded straight lines without any correction for system 1 (cf. fig 4).

In line with our hypothesis, for both systems to be perceived in the form of a dual isotherm it is imperative that at relatively high concentrations, i.e. above 1 mM, ions enter the cytoplasm more rapidly than when transported solely by system 1; else uptake would reflect the isotherm for system 1. Although the plasma membrane is definitely a barrier to free diffusion between the cytoplasm and the milieu, diffusive permeation nevertheless takes place (16, 26). It is to be expected that diffusive penetration will gain importance at higher concentrations. With diffusion through the plasma membrane sufficiently rapid, transport across the tonoplast will be rate-limiting and the system 2 isotherm will characterize absorption. When with respect to the diffusion of a given salt through a plant cell membrane the permeability coefficient of the cation exceeds that of the anion, the normal negative mem-

brane potential may be depressed by an oppositely directed diffusion potential, with the consequence that anion absorption increases exponentially with increasing concentration. Thus, both in fresh potato slices (24) and in freshly isolated steles of corn root (23), tissues which manifest little or no active transport, absorption of chloride is characterized by an exponentially rising isotherm. The observed isotherm fits theoretical expectations relating to passive diffusion (24), and the manifestation of an exponentially rising isotherm for anion uptake from an appropriate salt solution is considered to be a specially sensitive indication of permeation by diffusion.

In the low concentration range (0-0.5 mM) both tip and proximal sections display hyperbolic isotherms for chloride absorption, with similar K_s values. At high concentrations (1-50 mM), by contrast, the vacuolate proximal tissue manifests a second complex-hyperbolic isotherm, while the nonvacuolate tip shows a linear to exponentially rising isotherm (figs 1-3). The multiple isotherm for chloride at high concentrations in proximal tissue is much as described by Elzam et al. (8). It suffices to confine attention to the range 1 to 10 mM. With respect to Rb absorption (fig 5), again tip and subapical tissue resemble each other at low concentrations, to the extent that both exhibit hyperbolic isotherms. At high concentrations absorption by the tip is largely linear with concentration through most of the range examined, while uptake by proximal tissue is more nearly hyperbolic. It is noteworthy that Rb absorption by the tip is indifferent to the counterion, while uptake by subapical sections is responsive to the counterion. Rb absorption by the tip may exceed or fall short of uptake by proximal tissue, depending on the corn variety. That proximal sections respond to the nature of the counterion even in the range of 0 to 0.5 mM reflects the likelihood that in 3-hour periods there is some vacuolar absorption even at low concentrations.

Chloride absorption by the tips is temperature-sensitive, although less so than uptake by subapical sections (fig 1). Furthermore, tip absorption is sensitive to uncouplers of oxidative phosphorylation, more so in the low than in the high concentration range. The disparate response to cyanide and azide may reflect the effectiveness of azide as an uncoupler of oxidative phosphorylation as well as an inhibitor of electron transport (25, see 18). The relative resistance of tip absorption to cyanide is noteworthy but unexplained. Arisz has long contended that transport across the plasma membrane is a metabolically linked event different and distinguishable from transport across the tonoplast (1, 2). MacRobbie offers evidence to the same effect (27). Although we are emphatically of the same view, the inhibitor data of figure 1, beyond indicating metabolic implementation of chloride transport in both nonvacuolate and vacuolate tissue, cannot be explicitly related to the work of either Arisz or MacRobbie.

It has been maintained that cation uptake by the non-vacuolate apical 1.8 mm of maize roots is non-

metabolic. The deduction was made on the basis of both the low temperature coefficient for uptake and loss of alkali cations by corn root tips (15), and the noticeable retardation of absorption and efflux thereof by very low levels (0.002 mM) of Ca (16). It is pertinent to note that the above-cited studies were carried out at a concentration, 5mM, well in the range of system 2. It is to be expected that tip absorption will display a large nonmetabolic component at this concentration (as will movement into the cytoplasm of proximal sections), and there is not necessarily a contradiction between the work of Handley et al. (16) and that reported herein. It should be noted however that cation movement into the cytoplasm of barley roots, distinguishable from transport into the vacuole, has been shown by Hooymans to be metabolically implemented and carrier mediated (19). The time course of ion absorption into the cytoplasm in the latter instance is very similar to the time course reported by Handley et al. for cation uptake by non-vacuolate corn root tips (15, 16). Essentially the same time relations apply to cytoplasmic equilibration in *Nitella* (16, 27) and in beet disks (29). Metabolically implemented absorption is not prevented at low temperatures (19, 22).

While the absorption of cations by system 1 appears independent of the counterion, cation uptake by system 2 ostensibly depends upon the nature of the associated anion (11). When the cation of a salt is more readily absorbed than the anion, roots respond by synthesizing a quantity of organic acid stoichiometrically equivalent to the excess cation taken up (21, 32). System 2 is responsible for this accommodation (31). Although the literature is replete with examples wherein cation absorption is influenced by the counterion (specifically where K or Rb uptake is greater from chloride than from sulfate salts) there are instances where cation uptake is independent of the counterion even in the range of system 2 (20). However, where there is little or no effect of the counterion on cation absorption, the type of salt continues to influence organic acid synthesis, i. e. H^+ formation (21). Thus, we wish to propose that in the range of system 2, cation uptake depends on concomitant anion absorption in inverse proportion to the ability of the tissue to synthesize organic acid in response to excess cation uptake. The exchange of a cytoplasmic H^+ ion for an external cation offers no obvious signal for organic acid synthesis. However, if an absorbed cation is delivered to the vacuole together with a cytoplasmic organic anion, at the same time that an H^+ ion from the cytoplasm moves to the external solution, the loss of organic acid in the cytoplasm will be sensed, and will result in organic acid synthesis to reestablish equilibrium. For this reason also it is proposed that cation uptake by system 2 be placed at the tonoplast.

Finally, since it is being proposed that the low K_s system functions at the plasma membrane, and the high K_s system at the tonoplast, it is appropriate to recall observations made upon excised steles from corn roots, wherein the absorption isotherm at con-

centrations in the range of system 2 changed with aging from exponentially rising to hyperbolic (23). The contention was made that the plasma membrane became transformed from a relatively leaky membrane to a more rigorously differentially permeable one. This contention is at odds with the position taken here that the high concentration range isotherm relates to passage across the tonoplast. Suffice it to say that the observations made on excised steles would equally well apply to a situation in which a relatively impervious tonoplast in which system 2 is largely inoperative is transformed with aging to one in which system 2 is very active (cf. 35). If, with the latter transformation, tonoplast transport rather than passage through the plasma membrane becomes rate-limiting in the high concentration range, the tonoplast-related hyperbolic isotherm which describes transport would supplant the exponential isotherm associated with diffusion through the plasma membrane.

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