Plant Physiol. (1968) 43, 893–901

Electrostatic Association and Donnan Phenomena as Mechanisms of Ion Accumulation[1]

A. J. Hiatt

Department of Agronomy, University of Kentucky, Lexington, Kentucky 40506

Received January 11, 1968.

Abstract. Excised roots of barley (*Hordeum vulgare*, var. Campana) were incubated for periods up to 24 hours in salt solutions of various concentrations and ion accumulation was determined at various time intervals. The data were consistent with the existence of 2 components of ion uptake, one accounting for ion uptake from solutions below 1 mM and both components contributing to uptake from solutions of concentrations higher than 1 mM.

It is proposed that organic and amino acids play an important role in ion accumulation by providing nondiffusible charges which may bind or retain inorganic ions within the cell. Ions would enter the cell by diffusion or exchange from salt solutions of low concentration and become associated with nondiffusible organic ions, principally organic and amino acids. The electrostatic association between inorganic and organic ions would maintain a gradient and diffusion-exchange would occur until equilibrium between the cell and the external solution was reached. It is proposed that the additional component of ion uptake which becomes important at salt concentrations higher than 1 mM is a result of diffusion of neutral salts according to Donnan phenomena. Ion uptake by this proposed mechanism would not necessarily involve the action of carriers.

The discovery that Michaelis-Menten enzyme kinetics are applicable to kinetics of ion absorption by plant roots has led to the concept of carrier-mediated transport of ions in plant cells. When enzyme kinetics were applied to ion uptake rates over wide ranges of concentration, the existence of multiple binding sites or multiple carriers was suggested (3, 4, 10). The carrier theory of ion uptake has been attractive because of the close agreement of ion uptake kinetics with enzyme kinetics and because this approach provides a rationale for selectivity. Additional evidence for the operation of ion-carrier systems in plant cells is notably lacking. Nevertheless, evidence is convincing that there are 2 components of ion accumulation by plant roots and storage tissue. Epstein *et al.* (3) have reported that the mechanism responsible for the low-salt-concentration component of ion uptake in barley roots (low-concentration mechanism) operates at half-maximal velocity at a K^+ or Rb^+ concentration of about 0.018 mM and operates near maximum velocity at concentrations exceeding 0.2 mM. The high-concentration mechanism contributes negligibly to ion uptake from solutions of less than 1 mM but at salt concentrations exceeding 1 mM, ion uptake is equal to the sum of the absorption by both mechanisms. Ion uptake by the low-concentration mechanism appears to be indifferent to the nature of the counterion, while uptake by the high-concentration mechanism is markedly influenced by the counterion (3, 6, 21).

Evidence has been presented that electrostatic binding of cations by organic anions is involved in ion accumulation by certain unicellular organisms. Leggett *et al.* (14) reported that Rb^+ and Na^+ distribution in baker's yeast cells at equilibrium closely followed simple exchange equations used with cation exchange resins. Schaedle and Jacobson (17) proposed that K^+ accumulation by *Chlorella* was limited by the ability of the cells to create organic acid anions. Both of these organisms accumulated cations but not anions. When roots of higher plants absorb cations without concurrent anion absorption the cations exchange for H ions from the roots and equivalent quantities of organic acids are synthesized (6, 11). It has been proposed that organic acid levels in the cells are regulated by H ion concentration and that the synthesis of organic anions might serve as a driving force of cation accumulation in the absence of anion accumulation (7, 8, 9).

Plant root cells contain relatively large quantities of organic cations and anions that do not diffuse through cell membranes. These organic ions could act as nondiffusible ions and may bind inorganic ions by electrostatic attraction. Six-day old barley roots contain 25 to 30 μeq each of organic and amino acids per gram. In addition, there are other

[1] Contribution (Article No. 68-3-5) of the Department of Agronomy, Kentucky Agricultural Experiment Station, Lexington, and published with the approval of the Director.

ionic charges associated with protein and other organic molecules. The organic and amino acids alone supply a total of 50 to 60 μeq of negative charges and 25 to 30 μeq of positive charges per g fresh weight. Thus they can potentially bind 50 to 60 μeq of cations and 25 to 30 μeq of anions per gram. These quantities are of the same order of magnitude as the total K^+ and Cl^- accumulation from dilute KCl solutions.

Previous studies in this laboratory have indicated a close correlation between organic ions and retention of inorganic ions by barley roots. Hiatt and Lowe (9) have shown that the maintenance of organic and amino acids in cells of barley roots depends on respiratory metabolism. When organic acids and amino acids were lost from high-KCl roots as a result of anaerobiosis, an equal quantity of inorganic ions of opposite charge was lost from the roots. It was suggested that organic acids exist in the cells as K^+ salts and that amino acids exist as KCl salts. It is conceivable that electrostatic attraction of inorganic ions by nondiffusible organic ions could maintain a gradient for the accumulation of inorganic ions.

The data in this report are not sufficient to refute the hypothesis that salts are accumulated in cells by the action of carriers. However, the evidence is sufficient to support an alternative mechanism to explain salt accumulation. Basically the proposed mechanism involves diffusion-exchange and Donnan phenomena. It is proposed that roots accumulate ions from dilute salt solutions (low-concentration component) in the following manner. When roots are placed in dilute salt solutions (less than 10^{-3} N), the ions diffuse or exchange into the cell along a concentration gradient. Once inside the cell the ions become associated with organic ions of opposite charge. This maintains a concentration gradient and diffusion-exchange occurs until equilibrium is achieved. For such a mechanism to operate the cell must be able to synthesize new organic ions or otherwise possess a reservoir of organic ions. Accumulation of cations without concurrent anion accumulation can be explained on the basis of synthesis of organic acid anions (7, 8). However, when roots absorb cations and anions in equivalent amounts, neither organic nor amino acids change in concentration. It appears likely that in low-salt roots the positive and negative charges of amino acids are intramolecularly neutralized or the amino acids may exist as dimers. They might therefore serve as a reservoir of positive and negative charges. Accumulation would continue until a new equilibrium with the substrate salt solution was attained; however, accumulation of ions from dilute salt solutions would not exceed the concentration of available organic ions within the cell.

Simple diffusive equilibrium of a salt solution with root cells should not contribute significantly to total uptake at salt concentrations less than 1 mM since diffusive equilibration of roots with a solution of this concentration will result in the absorption of no more than 1 μeq salt per g of roots (fr wt). Since the high-concentration mechanism of ion uptake is functional only at salt concentrations higher than 1 mM, the possibility exists that this component of ion uptake is diffusive. Furthermore, diffusive uptake requires that cation and anion uptake be stoichiometric. Data, presented in this report, indicate that the high-concentration mechanism does not accumulate salts against a gradient and that this mechanism results in the absorption of equal quantities of cations and anions. It is proposed that the high-concentration component of ion uptake is a result of diffusion of neutral salts according to Donnan phenomena.

Materials and Methods

Barley seedlings (*Hordeum vulgare*, var. Campana) were dark-grown in continuously aerated 0.2 mM $CaSO_4$, essentially as described by Epstein and Hagen (2). Excised roots from 6-day old plants were rinsed several times in 0.2 mM $CaSO_4$ and were suspended in approximately 30 times the root volume of aerated $CaSO_4$ for 30 minutes before use.

In most experiments 1.2 g of excised barley roots were placed in 4 or 8 liters of the aerated substrate solution. At the end of the experiment, the roots were rinsed for 2 minutes with distilled water, blotted dry and duplicate 0.5 g samples were weighed for analysis. Each solution, in addition to the experimental salt under consideration, contained $CaSO_4$ at 0.2 mM. Calcium was added to maintain membrane integrity during the experiments (16). Calcium and SO_4^{2-} are absorbed in negligible quantities by barley roots. Depletion of the solutions by absorption did not lower the concentration of the substrate ion more than 10% during the course of the experiments. All experiments were repeated 2 or 3 times.

Potassium was determined by flame photometric analysis, sodium by atomic absorption and chloride by means of a Buchler-Cotlove automatic chloride titrator. Organic acids were determined by the procedure described by Hiatt and Hendricks (8). All analyses are expressed as μeq/g fresh weight.

Results and Discussion

Chloride Absorption vs Concentration of KCl and $CaCl_2$. The effect of concentration of KCl and $CaCl_2$ on Cl^- and K^+ uptake during a 4-hour experiment was determined (fig 1). At low salt concentrations, Cl^- uptake from KCl was slightly higher than Cl^- uptake from $CaCl_2$ but at concentrations greater than 10^{-4} N, Cl^- uptake from $CaCl_2$ was markedly depressed compared with uptake from KCl. Compared with Cl^- absorption, Ca^{2+} absorption was

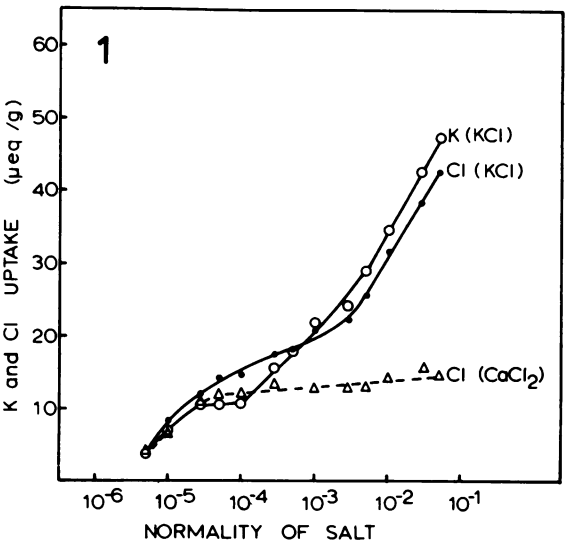
negligible over the range of concentrations used in this experiment. Whereas Cl^- absorption from low salt solutions is practically unaffected by the identity of the counterion, Cl^- absorption from solutions of high concentrations is markedly influenced by the rate of absorption of the counterion. At KCl concentrations greater than 10^{-3} N, K^+ and Cl^- uptake were parallel.

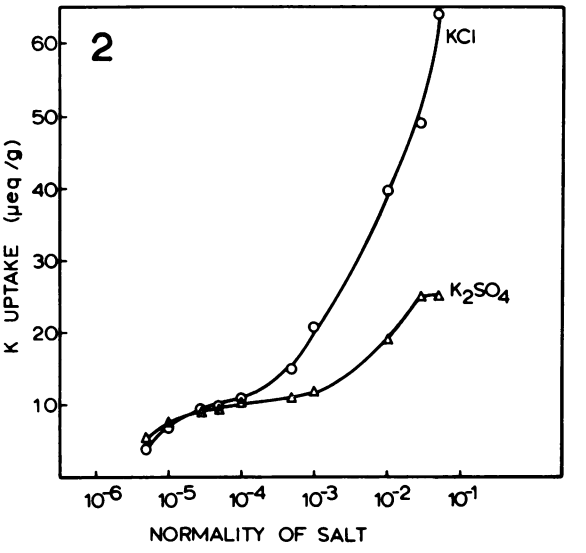
Potassium Absorption vs Concentration of KCl and K_2SO_4. Figure 2 shows the effect of concentration of KCl and K_2SO_4 on absorption of K^+ during a 4-hour experiment. At K^+ concentrations of less than 10^{-4} N, absorption from KCl and K_2SO_4 were approximately equal. At K^+ concentrations greater than 10^{-4} N, K^+ uptake from K_2SO_4 was depressed compared to K^+ uptake from KCl. As with Cl^- uptake (fig 1), K^+ uptake was influenced by the counterion at high salt concentrations only. In a similar experiment of 6-hour duration SO_4^{2-} absorption from 10^{-3}, 10^{-2}, and 5×10^{-2} N K_2SO_4 solutions was less than 1, 2.1, and 6.4 μeq/g, respectively (6). These results substantiate the results of Epstein *et al.* (3) who compared the uptake of Rb^+ from the Cl^- and SO_4^{2-} salts during a 10-minute absorption period. Absorption of K^+ in excess of SO_4^{2-} coincides with the exchange of H ions to the substrate solution (6, 10) and a mechanism has been proposed whereby continued uptake of K^+ from K_2SO_4 depends on malic acid synthesis (7, 8).

The retardation of K^+ or Cl^- uptake when these ions are supplied in high concentrations with slowly absorbed ions indicate that the high-concentration component of uptake requires coincidental uptake of both cations and anions. This property is consistent with diffusive uptake.

Na^+ and Cl^- Absorption vs Concentration of NaCl. The effect of NaCl concentrations on Na^+ and Cl^- uptake during a 4-hour experiment is shown in figure 3. At concentrations greater than 10^{-3} N, Na^+, and Cl^- uptake were parallel.

Potassium and Cl^- Uptake vs Time at Various Concentrations of KCl. To determine equilibrium levels of K^+ and Cl^- in roots at various substrate concentrations, roots were incubated for time intervals up to 24 hours. The results are shown in figure 4. Roots in KCl concentrations of 10^{-2} M or greater equilibrated with the substrate solution after 8 to 15 hours. Roots in 10^{-4} and 10^{-3} M KCl





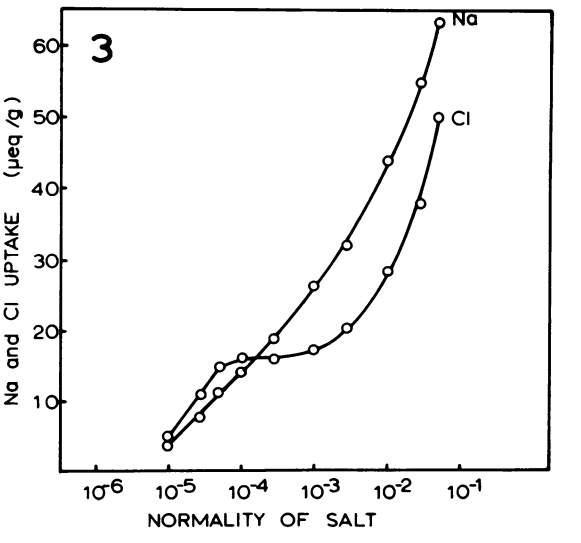
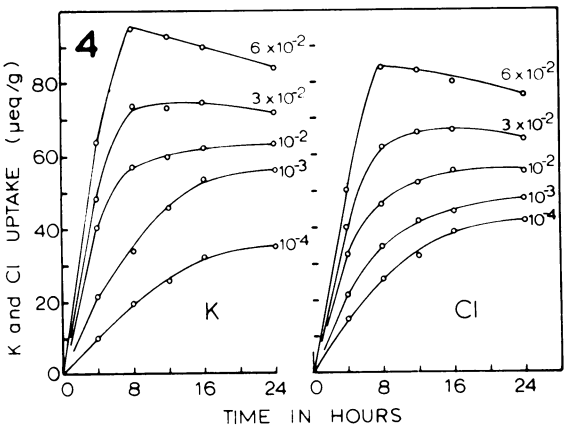


FIG. 1. Effect of KCl and $CaCl_2$ concentration on K^+ and Cl^- uptake by 6-day old barley roots. The absorption period was 4 hours.

FIG. 2. Effect of KCl and K_2SO_4 concentration on K^+ uptake by 5-day old barley roots. The absorption period was 4 hours.

FIG. 3. Effect of NaCl concentration on Na^+ and Cl^- uptake by 6-day old barley roots. The absorption period was 4 hours.



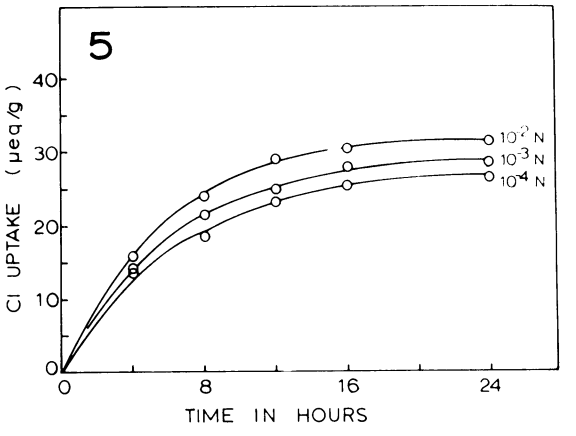


FIG. 4. Effect of time and KCl concentration (molarity) on K^+ and Cl^- uptake by 6-day old barley roots.
FIG. 5. Effect of time and $CaCl_2$ concentration on Cl^- uptake by 6-day old barley roots.

appeared to approach equilibrium with the solutions after 24 hours.

Roots in 10^{-4} M KCl accumulated more Cl^- than K^+. At higher concentrations K^+ accumulation exceeded Cl^- accumulation. If it is considered that uptake from 10^{-3} M KCl is due primarily to the low-concentration mechanism, substraction of this component from uptake at higher concentration should give an approximation of the contribution to uptake by the high-concentration mechanism. Computed in this manner, additional K^+ and Cl^- absorption resulting from increasing KCl concentration levels greater than 10^{-3} M are approximately equal. Increasing KCl concentration from 10^{-3} M (1 μmole/ml) to concentrations of 10, 30, and 60 μmoles/ml resulted in additional absorption of 7, 16, and 28 μmoles/g of both K^+ and Cl^- at 24 hours. Thus, accumulation ratios for the high-concentration mechanism are less than 1.

Chloride Uptake vs Time at Various Concentrations of $CaCl_2$. Figure 5 shows Cl^- uptake from substrate solutions of various $CaCl_2$ concentrations for time intervals of 4 to 24 hours. Equilibrium with the substrate solution was reached after approximately 16 hours. Increasing $CaCl_2$ concentration from 10^{-4} to 10^{-2} N resulted in little increased Cl^- uptake. Although the initial (4-hr) rates of Cl uptake from 10^{-4} N $CaCl_2$ were similar to initial rates of uptake from 10^{-4} M KCl (fig 4) total accumulation of Cl from $CaCl_2$ after 24 hours was markedly lower than accumulation from KCl.

Potassium Uptake vs Time at Various Concentrations of K_2SO_4. Figure 6 shows K^+ uptake from substrate solutions of various K_2SO_4 concentrations for time intervals of 4 to 24 hours. Except at 10^{-4} N K_2SO_4, equilibration between roots and solutions occurred much more slowly with K_2SO_4 than with KCl. With K_2SO_4 concentrations of 10^{-2} N to 6×10^{-2} N the accumulation of K^+ after 4 hours was approximately half the accumulation from KCl; however, at equilibrium the differences in total accumulation were much smaller.

Evidence that Organic and Amino Acids are Involved in Ion Accumulation. The data reported in the previous figures are consistent with the idea that ions may enter excised roots by 2 separate mechanisms. Ion uptake by the low concentration mechanism approaches maximum rates at salt concentrations of 10^{-4} to 10^{-3} N. The initial rate of absorption of cations or anions is not dependent upon the concurrent absorption of the counterion and ions are accumulated in the roots to concentrations greatly exceeding the salt concentration of the substrate solution by this mechanism.

It is theorized that ion accumulation by this process is due to ionic binding of inorganic ions by organic ions within the cell. Such inorganic ions may enter the cell by diffusion of neutral salts such

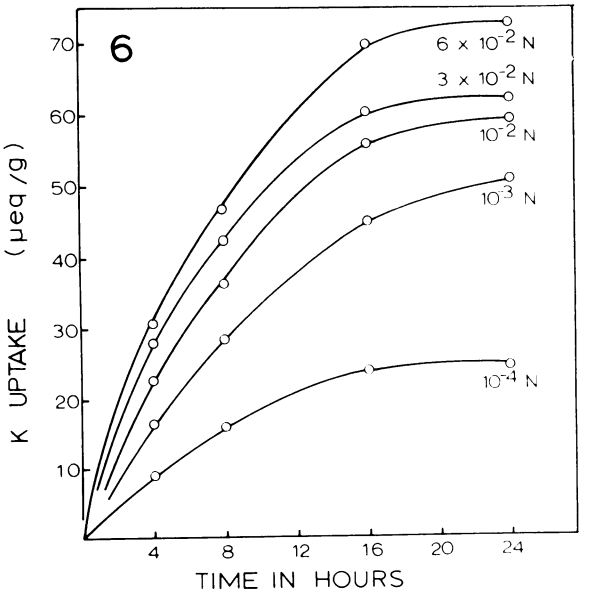


FIG. 6. Effect of time and K_2SO_4 concentration on K^+ uptake by 6-day old barley roots.

Table I. *K, Cl, Organic Acid, and Amino Acid Content of Roots after 24 Hours in 10^{-3} M KCl*

Substance assayed	Conc
	μequivalents per gram
K	69
Cl	52
Organic acids	25
Amino acids	30

as KCl or by the exchange of an inorganic ion for an ion of like charge from the root. It has been demonstrated that when K^+ is absorbed unaccompanied by an anion, H^+ is exchanged from the root (6, 7, 8). Anions entering the root unaccompanied by an alkali metal ion apparently enter the root in combination with H^+ or exchange for OH ions.

Since ion uptake by the high-concentration mechanism is negligible at salt concentrations of 10^{-3} N or below, roots in 10^{-3} N salt should absorb ions by the low concentration mechanism only. Furthermore, the upper limit of ion accumulation at this concentration should be approximately equivalent to the available organic ions of opposite charge within the plant. It has been shown that when roots absorb K^+ without an accompanying anion there is a stoichiometric increase in organic acid content of the tissue (6, 11). Table I shows the K^+, Cl^-, organic, and amino acid content of roots after 24 hours in 10^{-3} N KCl. These organic and amino acids at the pH of cell sap could bind approximately 50 μeq of K^+ and 30 μeq of Cl. The excess K^+ and Cl^- could logically be bound by other charged sites in the tissue such as cell walls, protein, etc. For example, approximately 5 μeq/g of K^+ is adsorbed onto the root surface and is rapidly removed by other cations.

Organic acid content of the tissue may change during ion uptake if either cations or anions are absorbed in excess of the counterion (6). The amino acid content of the tissue, however, remains relatively constant. This raises the question of what changes occur in amino acids to allow them to bind ions. Roots initially contain approximately 25 μeq inorganic cations (primarily K, Na, Mg, and Ca), 4 to 6 μeq of anions (primarily Cl and NO_3), 25 μeq of organic acids, and 30 μeq of amino acids per g fresh weight. At cell sap pH the organic acids are 80 to 85 % dissociated. They can, therefore, bind approximately 21 μeq of cations. The remaining cations and the anions may be bound by the amino acids which have both a positive and negative charge or by other charges in the protoplasm. It is apparent then that initially there are not enough inorganic ions to neutralize the charges associated with the amino acids. The amino acids could possibly (1) be intramolecularly neutralized, (2) exist as dimers, or (3) be associated with other charged sites in the protoplasm. The pattern of $^{14}CO_2$ incorporation into organic and amino acids after pretreatment in KCl and K_2SO_4 appears pertinent. Roots were incubated for 16 hours in 10^{-3} N K_2SO_4, KCl, or $CaSO_4$ and then were incubated for 30 minutes in a solution of 2×10^{-4} M $CaSO_4$ (pH 6.0) containing 0.5 μc/ml $^{14}CO_2$. The organic and amino acids were separated and ^{14}C activity in the organic and amino acids was determined. The results are shown in table II. Roots in K_2SO_4 take up K^+ but absorb essentially no SO_4^{2-}. The absorbed K^+ is balanced by an equal increase in organic acids. On the other hand, there is negligible change of organic acid content of roots in KCl or $CaSO_4$.

Table II. *$^{14}CO_2$ Incorporation into Organic and Amino Acids after Preincubation of Roots in KCl, K_2SO_4, or $CaSO_4$*

Six-day old barley roots were incubated for 16 hours in 10^{-3} N KCl + 2×10^{-4} M $CaSO_4$, 10^{-3} N K_2SO_4 + 2×10^{-4} M $CaSO_4$, or 2×10^{-4} M $CaSO_4$. Following preincubation, roots were rinsed for 1 hour in aerated 2×10^{-4} M $CaSO_4$ and placed for 30 minutes in 2×10^{-4} M $CaSO_4$ containing 0.5 μc of $KH^{14}CO_3$, pH 6. Organic and amino acids were separated and ^{14}C activity in each fraction was determined.

Salt treatment	^{14}C activity	% of activity in OA	AA
	cpm/g		
K_2SO_4	218,800	83.2	16.7
KCl	90,300	65.1	34.9
$CaSO_4$	161,400	75.5	24.5

Amino acid content of the tissue does not change, indicating that ^{14}C incorporation into amino acids is by an exchange reaction. The pathway of ^{14}C incorporation into organic and amino acids is illustrated in figure 7. Incorporation of ^{14}C into amino acids may occur by exchange mediated by the transamination reaction between oxalacetate (OAA), glutamic acid, and aspartic acid without net amino acid synthesis. If KCl forms salts with the amino acids, then the amino acids might be more reactive than the dimers or zwitterions and enter more readily into the transamination exchange reaction. This could explain the greater proportion of ^{14}C in amino acids when roots contain high levels of KCl. Joshi *et al.* (13) determined dark fixation of $^{14}CO_2$ by spinach homogenates and found the ratio of ^{14}C in organic acids and amino acids to be 42:53 (OA:AA) by spinach homogenate alone and 3:94

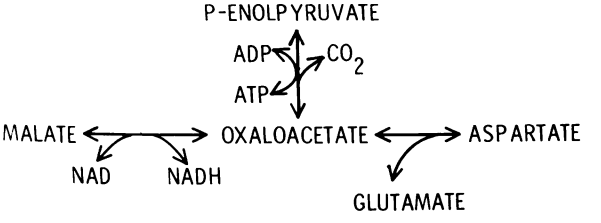


FIG. 7. Reactions by which $^{14}CO_2$ is incorporated into malic acid and aspartic acid.

by spinach homogenate with 0.22 M NaCl added. These results are consistent with the hypothesis that when roots absorb both cations and anions, salts are formed with amino acids; however, these results must be considered very preliminary and further experimental verification is needed. Since ^{14}C incorporation into amino acids is primarily into aspartic acid (13), the low net ^{14}C incorporation in KCl treated roots might be due to re-exchange of ^{14}C through a reverse of the reactions after the small pool of aspartic acid (approximately 1 μmole/g in these roots) has equilibrated.

Potassium absorption from K_2SO_4 occurs without concurrent absorption of the associated anion and organic acids are synthesized in quantities equivalent to the K^+ absorbed. On the other hand, when roots absorb KCl, both K^+ and Cl^- are absorbed and there is little change in organic acids; and it is postulated that the absorbed KCl is primarily associated with amino acids. During K^+ absorption from single salt solutions of K_2SO_4 and KCl, K becomes associated with organic acids and amino acids, respectively. Therefore, if both binding systems can be brought into play, the roots should be able to accumulate a larger amount of K^+. To test this hypothesis, roots were incubated 24 hours in K_2SO_4 or KCl and for 12 hours in KCl followed by 12 hours in K_2SO_4. The results are shown in table III. During the initial 12-hour absorption period, a greater quantity of K^+ was absorbed from KCl than K_2SO_4. During the 12 to 24 hour interval, K^+ uptake from K_2SO_4 exceeded K^+ uptake from KCl. These data are consistent with the results reported in figures 4 and 6 which also illustrate that the roots have equilibrated with the substrate solution after 24 hours. When roots were transferred from KCl to K_2SO_4 during the second 12-hour interval, K^+ uptake during this interval was increased and total K^+ accumulation was increased. These data are consistent with the hypothesis that K^+ accumulation may be increased by utilizing both organic acids and other anions (presumably amino acids) to bind K^+ ionically.

Chloride absorption from $CaCl_2$ is unaccompanied by Ca^{2+} and presumably enters the cell in association with hydrogen ions (6). This results in raising the pH of the substrate solution and lowering the pH of cell sap. The lowering of cell sap pH shifts equilibria of glycolytic reactions such that organic acids are decarboxylated through reversal of the EMP pathway (7). The H^+ ions accompanying Cl^- may be utilized in this reverse pathway. The K^+ initially associated with the organic acids being decarboxylated are not lost from the roots. They may associate with the incoming Cl^- and subsequently, along with Cl^-, become associated with amino acids. Chloride uptake from $CaCl_2$ is initially as rapid as Cl^- uptake from KCl but the rate decreased rapidly (fig 4 and 5) as the endogenous supply of organic acids is depleted (6). This suggests that Cl^- uptake unaccompanied by a cation is limited by the supply of K^+ which becomes available when organic acids are decarboxylated. Therefore, if organic acids and K^+ are increased by pretreatment of roots in K_2SO_4, there should be a subsequent increase in the ability of the roots to accumulate Cl^- from $CaCl_2$. An experiment was conducted to test this hypothesis. The results are shown in table IV. Chloride accumulation from $CaCl_2$ was increasing by preincubation of the roots in K_2SO_4. The data support the hypothesis that Cl^- accumulation from $CaCl_2$ may be increased by increasing the endogenous level of organic acids and K^+ in the tissue.

Table III. *K Accumulation by 6-Day Old Barley Roots from K_2SO_4, from KCl, or from KCl Followed by K_2SO_4*

After incubation for 12 hrs in 10^{-3} N K_2SO_4 or KCl, roots were transferred to new solutions as indicated for an additional 12 hours.

Treatment 0–12 hrs	Treatment 12–24 hrs	K uptake 0–12	K uptake 12–24	Total K uptake
		μequivalents per g		
K_2SO_4	K_2SO_4	35	16	51
KCl	KCl	54	13	67
KCl	K_2SO_4	54	21	75

Table IV. *Effect of Preincubation of Roots in K_2SO_4 on Subsequent Cl Accumulation from $CaCl_2$*

Pretreated roots were placed for 6 hours in the indicated solutions and then rinsed for 10 minutes before being placed in the absorption solutions. All solutions contained 2×10^{-4} M $CaSO_4$.

Pretreatment	Absorption treatment	Cl uptake
		μeq/g
Expt I		
$CaSO_4$ only	24 hr in 10^{-4} N $CaCl_2$	26.9
10^{-4} N K_2SO_4 + $CaSO_4$	"	34.8
Expt II		
None	20 hr in 10^{-3} N $CaCl_2$	28.8
10^{-3} N K_2SO_4 + $CaSO_4$	"	34.3
None	24 hr in 10^{-3} N KCl	52.7

Total Accumulation of K and Na in Combination. According to the proposed hypothesis, the upper limit of cation accumulation is determined by the availability of nondiffusible negative sites within the tissue. Therefore, total cation accumulation at equilibrium should be nearly the same regardless of the identity of the cation, providing the roots are permeable to the cation and providing that organic acid content does not change appreciably. A replacement series experiment was conducted varying NaCl concentration from 10^{-3} N to 0 and KCl concentration from 0 to 10^{-3} N. Total salt concentration was maintained at 10^{-3} N. The results are shown

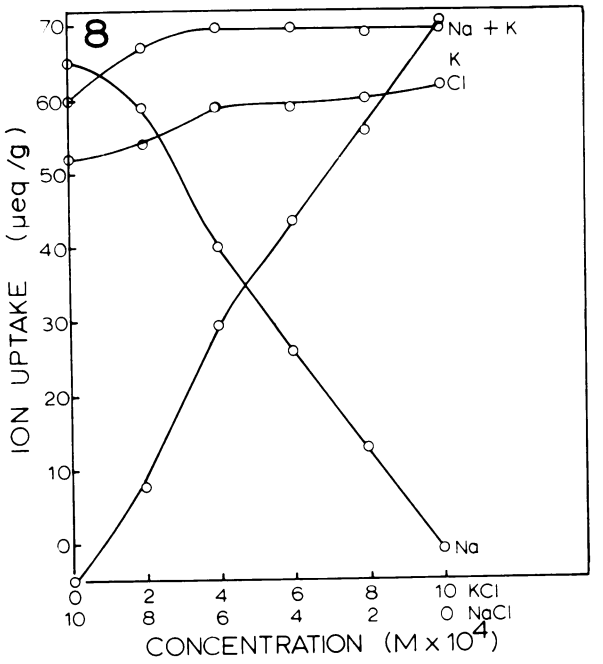


FIG. 8. Na^+, K^+, and Cl^- uptake in a replacement series of NaCl and KCl. Absorption time was 24 hours. Total salt concentration maintained at 10^{-3} M.

in figure 8. Except in the solution containing no KCl and 10^{-3} N NaCl, total cation accumulation was approximately constant at about 70 μeq/g. Deviation from this value in the absence of K is accounted for primarily by loss of K from the roots. When K^+ is not present in the substrate solution, metabolism may be adversely affected since Na^+ cannot replace K^+ in several enzyme catalyzed reactions. The data indicate that K^+ and Na^+ accumulation have a common limiting factor which is postulated to be negatively charged sites. Organic acid content of these roots was not determined, but, based on previous experiments, the organic acid content is estimated to increase by an amount equal to the difference between Cl^- and cation uptake. This value was approximately equal in all treatments.

The results in the foregoing tables and figures support the contention that ion accumulation from salt solutions of less than 10^{-3} N is due to electrostatic association in which the nondiffusible charges are provided primarily by organic and amino acids contained within a semi-permeable membrane. No doubt other charged sites are also involved to a limited degree in the overall association of inorganic ions with organic ions. Organic and amino acids, however, are the only easily measurable organic ions of appreciable quantity in the cell that may act as a readily available source of exchange sites.

Diffusion as the High-Salt Concentration Mechanism of Ion Uptake. Evidence has been presented that plant cells possess a relatively high concentration of organic ions. These ions are within a semipermeable membrane and do not diffuse out of the cell unless membrane integrity is destroyed as by anaerobiosis (9). These organic ions behave as non-diffusible ions within a semipermeable membrane. Such a system must behave as a Donnan system.

The high-salt mechanism of ion uptake assumes importance above salt concentration of 10^{-3} M. Above 10^{-3} M, total ion uptake consists of ion uptake by the low concentration mechanism, which is operating at near maximum rates, plus the additional contribution from the second, high-concentration mechanism. Ion uptake by the high-concentration mechanism is markedly dependent on the identity of the counterion (fig 1 and 2) and an ion entering the root through this mechanism must be accompanied by an ion of opposite charge. Chloride supplied as $CaCl_2$ does not exhibit this second component of ion uptake because the membrane is relatively impermeable to Ca^{2+} (fig 1). Likewise, K^+ uptake from high concentrations of K_2SO_4 is retarded because of the slow rate of SO_4^{2-} uptake. When concentrations of univalent cation chloride salts are increased above 10^{-3} M, the additional accumulation of cations and anions are approximately equivalent. This relationship is particularly striking at salt concentrations greater than 10^{-2} M. Examples of this consistently observable relationship are shown in table V. A graphical illustration of equivalency of cation and anion uptake at high salt concentrations is observable in figures 1, 3, and 4. Salts enter a Donnan space by diffusion and must, therefore, enter as a neutral salt.

Due to the Donnan phenomenon a diffusible ion of one charge may be maintained at higher concentrations than the diffusible ion of opposite charge. Because of their high organic acid content, barley roots possess a greater quantity of nondiffusible negative charges than positive charges. Thus, they should maintain a higher concentration of K^+ than Cl^- when they are allowed to equilibrate with KCl at high concentrations. Figure 4 illustrates that this is the case. Calculations of the absolute theoretical values for the uptake of salts by Donnan phenomena from a single salt solution are difficult, however, because of the complexity of the system in plant roots. This complexity is due to several factors: (1) the cell contains both nondiffusible cations and nondiffusible anions, (2) the nondiffusible ion content may change during the course of the experiment due to unequal rates of absorption of cations and anions, (3) the cell contains other diffusible cations and anions, (4) the total amount of nondiffusible cations and anions is difficult to determine, and (5) the nondiffusible ion content undoubtedly varies widely in the different types of cells.

Similarity of kinetics of ion uptake to enzyme kinetics, ion selectivity and requirement of respiration for ion uptake have all been explained on the basis of the carrier theory. These observations can be equally well explained on the basis of ion accu-

Table V. *Equivalency of Cation and Anion Uptake from Univalent Cation Salt Solutions Exceeding 10^{-2} M in Concentration*

The values expressed are the differences between uptake from 10^{-2} M solutions and uptake from solutions of higher concentrations.

Expt		Salt conc	Incubation period	Absorption in excess of absorption from 10^{-2} M salt	
		M × 10^2	*hr*	*μeq/g*	
				K	Cl
A	KCl	3	4	8	7
		6	4	23	18
		3	8	17	15
		6	8	38	36
B	KCl	3	4	10	8
		5	4	25	22
		3	12	20	19
		5	12	38	36
C	NaCl	3	4	11	9
		5	4	20	21
		3	8	23	22
		5	8	32	37

mulation by exchange and Donnan phenomena.

It is beyond the scope of this paper to present a detailed treatment of kinetics of ion uptake. When the reciprocal of ion uptake is plotted against the reciprocal of ion concentration a straight line, consistent with Michaelis-Menten enzyme kinetics is obtained. Epstein and Hagen (*2*) hypothesized that the absorption of inorganic ions involves their combination with binding compounds or carriers to form a labile complex, presumably in the cell membrane. This concept is analogous to the combination of substrate with enzyme to form an enzyme-substrate complex which then breaks down to yield the free enzyme and the products of the reaction. In applying the Michaelis-Menten equation, it is assumed that steady-state conditions between the free and combined enzyme is rapidly achieved and, therefore, the concentration of the enzyme-substrate complex does not change. If inorganic ions traverse the cell membrane by being alternately adsorbed and displaced through a series of fixed ion exchange sites, steady-state conditions between the adsorbed ions and the ions in the ambient solution should be rapidly achieved. Such a system would be quite analogous to the carrier system proposed by Epstein and Hagen (*2*) and kinetic studies would not distinguish between mobile carriers and fixed ion exchange sites.

Waisel (*22*) concluded that passive movement of ions across the outer cell membrane frequently constitutes the rate limiting step of cation accumulation and that Ca^{2+} affects the selective permeability of the membrane. Handley *et al.* (*5*) reached a similar conclusion and proposed that the Ca^{2+} effect may be related to membrane pore size. The hydrated radii of Rb^+, K^+, Na^+, and Li^+ have been given as 5.09 A, 5.32 A, 7.90 A, and 10.03 A, respectively (*12*). Estimates of the equivalent pore radius of different animal membranes based upon the osmotic pressure developed across membranes in the presence of diffusible salts ranged from 3.5 to 6.5 A (*18*). The equivalent pore radius of kidney slices from *Necturus* became significantly larger when Ca^{2+} was removed from the medium (*23*). Handley *et al.* (*22*) proposed that Ca^{2+} stabilizes the cell membrane with a consequent decline in permeability. Because of their large hydrated radii, passage of Na and Li should be greatly restricted by the presence of Ca^{2+} while the passage of Rb^+ and K^+ should be restricted to a lesser extent (*22*). Handley *et al.* (*22*) suggested that the elimination of Na^+ interference with K^+ uptake reported by Epstein (*1*) could be explained on this basis. Undoubtedly factors other than membrane pore size and size of the hydrated ion are involved in determining relative rates of ion passage across membranes. Since cell walls and cell membranes possess numerous exchange sites, most ions perhaps move chromatographically through the membrane by a series of exchange reactions. It is well known that any exchange system preferentially adsorbs certain ions. Movement of ions along a series of adsorption sites in combination with the sieve effect of membranes could very well explain selectivity in ion uptake.

Ion accumulation by diffusion or exchange should not have a direct requirement for respiratory energy; however, numerous investigations have shown that ion accumulation is inhibited by blocking respiration. Respiratory metabolism is apparently required for the maintenance of nondiffusible ions in the cell. Studies in this laboratory have shown that inhibition of respiration results in the loss of organic and amino acids from barley roots by 2 separate processes (*9*). A rapid initial response is attributed to decarboxylation of organic acids resulting from lowered CO_2 levels in the cell. Furthermore, after

1 to 2 hours under anaerobiosis, apparent loss of membrane integrity results in the rapid leakage of organic and amino acids from the cells. Marschner *et al.* (15) noted derangement in the fine structure of meristematic cells of corn roots when treated anaerobically or exposed to solution pH levels below 4.4 for 3 hours.

The theory of ion accumulation proposed here is, in many respects, a return to some old ideas summarized by Stiles (20, see Steward 19). A major objection to the proposal that Donnan phenomena are involved in ion accumulation has been that both cations and anions are accumulated by cells. An ideal Donnan system will accumulate ions having a charge opposite the charge of the nondiffusible ion and will exclude ions of the same charge as the nondiffusible ions. This does not appear to be a valid objection because the cell contains high levels of both nondiffusible cations and nondiffusible anions. Carrier mediation of transport cannot be excluded on the basis of evidence presented here; however, there seems to be no need to resort to the action of membrane-located carriers. The membrane would serve a 2-fold purpose in ion accumulation by this process; the selectivity or preferential exclusion of ions and the retention of organic ions within the cell.

Literature Cited

1. Epstein, E. 1961. Essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437–44.
2. Epstein, E. and C. E. Hagen. 1952. A kinetic study of absorption of alkali cations by barley roots. Plant Physiol. 27: 457–74.
3. Epstein, E., D. W. Rains, and O. E. Elzam. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. Proc. Natl. Acad. Sci. 49: 684–92.
4. Fried, M. and J. C. Noggle. 1958. Multiple site uptake of individual cations by roots as affected by hydrogen ion. Plant Physiol. 33: 139–44.
5. Handley, R., A. Metwally, and R. Overstreet. 1965. Effects of Ca upon metabolic and nonmetabolic uptake of Na and Rb by root segments of *Zea mays*. Plant Physiol. 40: 513–20.
6. Hiatt, A. J. 1967. Relationship of cell sap pH to organic acid change during ion uptake. Plant Physiol. 42: 294–98.
7. Hiatt, A. J. 1967. Reactions *in vitro* of enzymes involved in CO_2 fixation accompanying salt uptake by barley roots. Z. Pflanzenphysiologie 56: 233–45.
8. Hiatt, A. J. and S. B. Hendricks. 1967. The role of CO_2 fixation in accumulation of ions by barley roots. Z. Pflanzenphysiologie 56: 220–32.
9. Hiatt, A. J. and R. H. Lowe. 1967. Loss of organic acids, amino acids, K, and Cl from barley roots treated anaerobically and with metabolic inhibitors. Plant Physiol. 42: 1731–36.
10. Jackson, P. C. and H. R. Adams. 1963. Cation-anion balance during potassium and sodium absorption by plant roots. J. Gen. Physiol. 46: 369–86.
11. Jacobson, L. and L. Ordin. 1954. Organic acid metabolism and ion absorption in roots. Plant Physiol. 29: 70–75.
12. Jenny, H. and R. F. Reitemeier. 1935. Ionic exchange in relation to the stability of colloidal systems. J. Phys. Chem. 39: 593–604.
13. Joshi, G., T. Dolan, R. Gee, and P. Saltman. 1962. Sodium chloride effect on dark fixation of CO_2 by marine and terrestrial plants. Plant Physiol. 37: 446–49.
14. Leggett, J. E., W. R. Heald, and S. B. Hendricks. 1965. Cation binding by baker's yeast and resins. Plant Physiol. 40: 665–71.
15. Marschner, H., R. Handley, and R. Overstreet. 1966. Potassium loss and changes in the fine structure of corn root tips induced by H-ion. Plant Physiol. 41: 1725–35.
16. Rains, D. W., W. E. Schmid, and E. Epstein. 1964. Absorption of cations by roots. Effect of hydrogen ions and essential role of calcium. Plant Physiol. 39: 274–78.
17. Schaedle, M. and L. Jacobson. 1965. Ion absorption and retention by *Chlorella pyrenoidosa*. I. Absorption of potassium. Plant Physiol. 40: 214–20.
18. Solomon, A. K. 1960. Measurements of equivalent pore radius in cell membranes. In: Membrane Transport and Metabolism. A. Kleinzeller and Z. Katyk, eds. Academic Press, New York. p 94–99.
19. Steward, F. C. and J. F. Sutcliffe. 1959. Plants in relation to inorganic salts. In: Plant Physiology, Vol. II. Plants in Relation to Water and Solutes. F. C. Steward, ed. Academic Press, New York. p 255–59.
20. Stiles, W. 1924. Permeability. New Phytologist Reprint. 13: 1–296.
21. Torii, K. and G. Laties. 1966. Dual mechanisms of ion uptake in relation to vacuolation in corn roots. Plant Physiol. 41: 863–70.
22. Waisel, Y. 1962. Effect of calcium upon uptake of monovalent ions by excised barley roots. Physiol. Plantarum 15: 709–24.
23. Whittenbury, G., N. Sugino, and A. K. Solomon. 1960. Effect of antidiuretic hormone and calcium on the equivalent pore radius of kidney slices from *Necturus*. Nature 187: 699–701.