Cation-Anion Balance during Potassium and Sodium Absorption by Barley Roots

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ABSTRACT   Steady-state rates of potassium ion and sodium ion absorption by excised barley roots accompanied by various anions were compared with the rates of anion absorption and the concomitant H^+ and base release by the roots. The cation absorption rates were found to be independent of the identities, concentrations, and rates of absorption of the anions of the external solution, including bicarbonate. Absorption of the anion of the salt plus bicarbonate could not account for the cation absorption. H^+ is released during cation absorption and base during anion absorption. The magnitude by which one or the other predominates depends on the relative rates of anion and cation absorption under various conditions of pH, cation and anion concentration, and inhibitor concentrations. The conclusion is that potassium and sodium ions are absorbed independently of the anions of the absorption solution in exchange for H^+, while anions are exchanged for a base. The H^+ release reflects a specificity between K^+ and Na^+ absorption such that it appears to be H^+ exchanged in the specific rate-limiting reactions of the cation absorption.

INTRODUCTION

Maintenance of a constant charge balance in the roots during salt absorption could require that cation absorption rates be equal to absorption rates of the accompanying anions. Absorption of anions and cations by barley roots is so closely related, according to Steward and Sutcliffe (16), that an effect on one results in an effect on the other. For example, stimulation of K^+ absorption by beet root disks at high pH is attributed to an increased uptake of the associated anions, bicarbonate and chloride (7). They agree with Lundegardh (12) that anion accumulation is limiting for cation absorption. Ulrich (18) also considers that cations absorbed in excess of the anion of the salt are absorbed in association with bicarbonate, resulting in the observed increase in organic acids.

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On the other hand, Jacobson, Overstreet, and coworkers hold that cations and anions are absorbed independently in exchange for H^+ and OH^-, respectively (11). The absorption of potassium ion and bromide by barley roots is unequal (10) and bicarbonate was not absorbed rapidly enough to support potassium ion absorption (14). Many workers have observed pH changes in the external solution during salt absorption, but direct observations of the relationship between rates of ion absorption and rates of H^+ and base exchange in short times have not been reported.

In the work presented here absorption rates of K^+ and Na^+ accompanied by various anions are compared with rates of anion absorption and the concomitant H^+ and base release by the roots. These studies lead to the conclusion that cations are absorbed independently of the anion in the absorption solution in exchange for H^+, while anions are absorbed in exchange for a base.

MATERIALS AND METHODS

Excised roots from 6-day-old seedlings of barley (*Hordeum vulgare*, var. *compana*) were the experimental plant material. The seedlings had been dark-grown in continuously aerated 2×10^{-4} M $CaSO_4$, essentially as described by Epstein and Hagen (3). Roots were excised about 15 minutes prior to the experiment, rinsed 3 times with approximately 50 times the root volume of water, and then suspended in continuously aerated water in the same proportion. Distilled demineralized water was used throughout the root preparation and absorption procedures.

The absorption solutions were salt solutions containing a radioactive tracer for the ion under study. Salts of potassium, rubidium, sodium, bromide, chloride, sulfate, phosphate, and succinate were labeled with Rb^{86}, Na^{24}, Br^{82}, Cl^{36}, S^{35}, P^{32}, and C^{14}, respectively. Sufficiently large volumes of the absorption solutions were used to maintain an approximately constant concentration of the salt under study during absorption. The pH was adjusted and maintained within 0.05 unit and the temperature was 25°C.

Potassium ion absorption rates were determined by using Rb^{86} as an isotopic tracer for K^+. Validity of this is suggested by experiments of Epstein and Hagen (3) and Fried and Noggle (5). It is also shown directly by comparisons of absorption rates from Rb^{86}-KCl solutions with absorption from Rb^{86}-RbCl and K^{42}-KCl solutions (Table I). Effects of pH, oxygen, 2,4-dinitrophenol (DNP), and temperature are closely the same with Rb^{86}-KCl as with Rb^{86}-RbCl.

Potassium absorption solutions contained Na^+ in sufficiently high concentration (usually 10^{-3} M) so that Na^+ concentration was not a variable in the adjustment and maintenance of pH. This also insured against the anion concentration being a variable during most experiments. Sodium salt concentrations as high as 10^{-3} M had no significant effect on K^+ absorption at any concentration from 10^{-6} to 3×10^{-2} M (Table I).

The amount of electrolyte leaking from the calomel electrode during pH measurements was also considered. The electrode may leak enough salt to increase the

salt concentration of 10 ml of water to 10^{-3} M in 1 minute. In most cases, pH was adjusted without placing the electrode in the solutions used for absorption, by measuring the pH of small aliquots which were discarded. Where the electrode was placed in the absorption solution, a salt which had no effect on the absorption of the ion under study was used as the electrolyte. NaCl was used for potassium ion and succinate studies. Solutions for Na^+ absorption contained 10^{-3} M LiCl and were titrated with LiOH, using LiCl in the electrode. Na_2SO_4 or K_2SO_4 was used for bromide and chloride experiments and KCl for sulfate and phosphate experiments. The pH meter was calibrated for the small differences in the pH 3.5 to 7.5 range that occurred with some of the electrolytes.

TABLE I

COMPARISON OF RUBIDIUM AND POTASSIUM
ISOTOPES AS A MEASURE OF POTASSIUM ION
ABSORPTION, AT PH 5, 25°C

Cation	Absorption rate		
	Rb^{86}-KCl	Rb^{86}-RbCl	K^{42}-KCl
moles/liter	*mμmoles/min. gm*	*mμmoles/min. gm*	*mμmoles/min. gm*
10^{-5}	29	31	—
10^{-3}	124	154	—
10^{-5} + 10^{-3} M NaCl	28	31	27
10^{-3} " "	135	141	156
$V_{max\,1}$	80 mμmoles/min. gm	—	100 mμmoles/min. gm
" 2	325 " / " "	—	350 " / " "
$K_{m\,1}$	2×10^{-5} M	—	3×10^{-5} M
" 2	4×10^{-3} M	—	4×10^{-3} M

V_{max} and K_m values are calculated from data of absorption rates over a range of the cation concentrations assuming two Michaelis-Menten reactions (Fig. 2). $V_{max\,1}$ and $K_{m\,1}$ are the kinetic constants of the reaction predominating at low cation concentrations. $V_{max\,2}$ and $K_{m\,2}$ are the constants of the reaction predominating at high concentrations. NaCl at a concentration of 10^{-3} M was present in the experiments from which these calculations were made.

For the absorption experiments, 3 or 4 gm of roots were weighed, rinsed 4 times, and then placed in 500 ml of continuously aerated absorption solution. Successive portions of the roots were removed at each of 5 sampling times, usually every 2 or 3 minutes, rinsed 4 times with 50 times the root volume of water, blotted gently, and weighed. ½ gm samples were assayed for radioactivity. Absorption rates were calculated from the regression of the absorption-time function by the method of least squares.

H^+ and base released by the roots were determined by incubating 1 gm samples of roots in 100 ml of rapidly aerating solution for various periods, usually up to 1 hour. After incubation, the roots were removed and the solutions were titrated back to the initial pH with standardized NaOH or H_2SO_4. Rates of H^+ and base release were calculated from 6 sampling periods in the same manner as the absorption rates.

Chloride concentrations which were measured for some of the experiments were determined by means of an Aminco-Cotlove automatic chloride titrator, which is a commercial adaptation of an instrument developed by Cotlove *et al.* (1). Total potassium and sodium content of the roots was determined by flame photometric
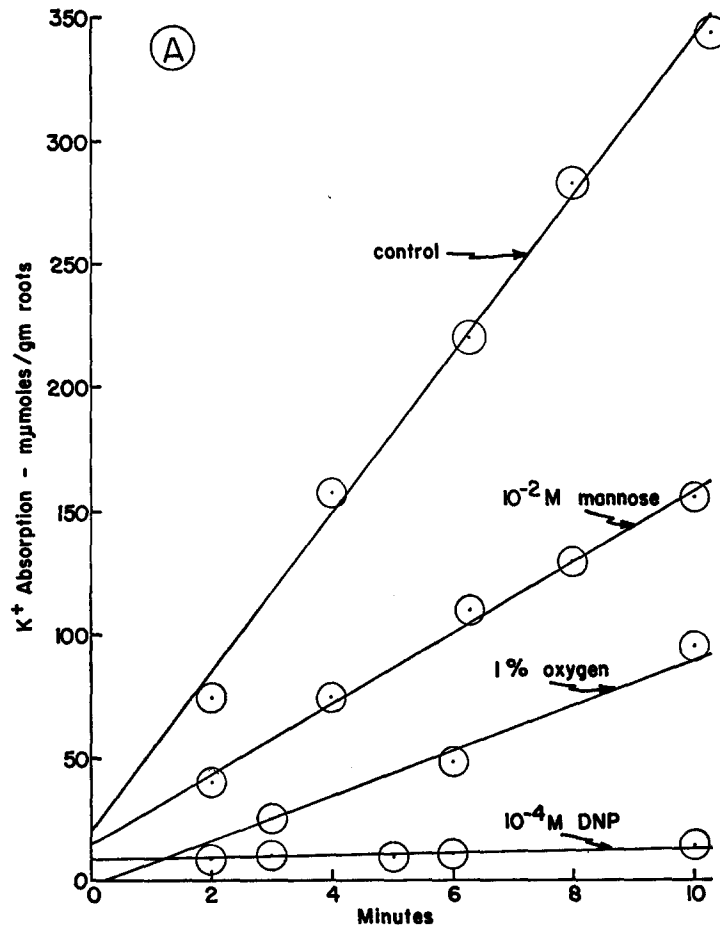

FIG. 1 A

FIGURE 1. The effects of various agents on K^+ and Na^+ absorption as a function of time. Fig. 1 A. K^+ absorption at pH 5 from 10^{-5} M KCl with 10^{-3} M NaCl. Fig. 1 B. Na^+ absorption at pH 7 from 10^{-3} M NaCl with 10^{-3} M LiCl.

analysis. For the photometric analysis, root samples were rinsed, blotted, weighed, and then ashed at 600°C. The ashed residues were dissolved in hot HCl and then diluted.

RESULTS

General Features of Absorption   Excised barley roots were found to accumulate potassium and sodium at steady rates for several hours in concen-

trations of K^+ or Na^+ from 10^{-6} M to 3×10^{-2} M. A typical time curve for 10^{-5} M KCl is shown in Fig. 1 A, and for 10^{-3} M NaCl in Fig. 1 B. All the experimental results given are the steady-state rates determined from the slopes of time curves and are expressed as mμmoles/minute gram (fresh
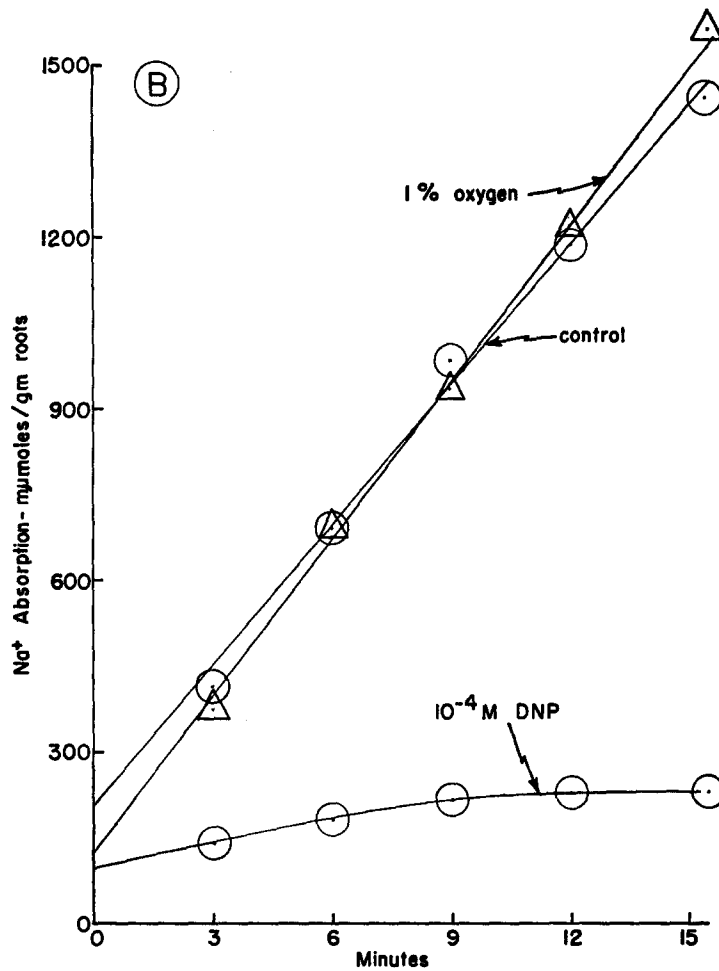

FIG. 1 B

weight) of roots. None of the rates includes the fraction of ions taken up or released which equilibrates within the first few minutes of incubation and is represented by the intercept of the time curve. Salt in the apparent free space was removed by washing.

The roots contain the order of 10^{-5} moles of potassium and 10^{-6} moles of sodium per gram, determined by flame photometric analyses. No observable efflux of absorbed or endogenous potassium or sodium occurs in water, RbCl, KCl, NaN$_3$, or DNP solutions within the relatively short times

measured provided the desorption solutions are well aerated and not more acid than pH 4, as shown in Table II. This was measured both by flame photometric analyses and by the more sensitive isotope exchange studies. Thus, neither K^+ nor Na^+ absorption is accompanied by exchange for Na^+ or K^+.

Steady-state rates of K^+ absorption increase with K^+ concentration in a manner consistent with the observations that absorption can be analyzed as arising from 2 rate-limiting reactions (5) (Fig. 2 A). Sodium ion absorption also is described by 2 rate-limiting reactions (Fig. 2 B). The increase in rate from 10^{-6} M to 10^{-3} M is due to an increase in the cation concentration, since the anion concentration was constant over this range.

TABLE II

RETENTION OF ABSORBED POTASSIUM AND SODIUM
BY ROOTS IN VARIOUS SOLUTIONS

The roots were grown for 5 days in a potassium-sodium nutrient solution. Desorption solutions were at pH 5.

	Desorption time, min.							
	0	10	20	30	0	10	20	30
Desorption solution	K, μmoles/ gm				Na, μmoles/gm			
None	70				16			
Water		65	77	73		12	17	14
10^{-3} M RbCl		70	78	77		16	13	13
10^{-3} M NaN$_3$		70	79	73		16	12	14
10^{-3} M DNP		70	75	88		15	13	15

Absorption rates of both cations are inhibited by hydrogen ions as is evident when the cation concentration is less than or equal to the H^+ concentration. For example, in Table III, K^+ absorption from 10^{-5} M K^+ is less at pH 4 than at pH 5. When the cation concentration is much greater than the H^+ concentration, change in pH has little effect as shown by K^+ absorption in Table IV. This is in agreement with the competitive effects of pH described by Fried and Noggle (5), but differs from the deductions of Steward and Sutcliffe (16).

The Effect of Anions and Anion Concentration Rates of K^+, Rb^+, and Na^+ absorption from solutions of various salts were measured with the results shown in Table III. The anion concentration in the experiments was 5 × 10^{-4} M to 2 × 10^{-2} M, whatever concentration was sufficient to balance the cation concentration. Chloride concentrations in the solutions with other anions were less than 5 × 10^{-6} M, a concentration contributed by the isotope used. Potassium ion absorption rates from KCl solutions were the same as

from K_2SO_4 solutions within the accuracy of measurement over a tested range of K^+ concentrations from 3×10^{-6} M to 3×10^{-2} M (Fig. 2 A). V_{max} and K_m values were the same for both rate-limiting reactions in sulfate or in chloride solution. With succinate, absorption from 10^{-5} M K^+ was 98 per cent at pH 4 and 102 per cent at pH 5 of the absorption rate with chloride. Sodium absorption with succinate was 100 per cent from 10^{-5} M Na^+ and 109 per cent from 10^{-3} M Na^+. With ATP, K^+ was absorbed from 10^{-5} M K^+ at 97 per cent and from 10^{-3} M K^+ at 94 per cent of the rate with chloride. Potassium ion absorption from 10^{-5} M and 10^{-3} M K^+ with nitrate, thiosulfate, and phosphate also shows no significant difference from chloride. The absorption is unaffected by a 100-fold change in total salt concentration, as shown in Table I, where the rate of absorption from 10^{-5} M K^+ is the same with no added chloride as with 10^{-3} M NaCl. Sodium ion absorption rates are similarly independent of the anion of the salt. Rubidium absorption is also the same from sulfate solutions as from chloride and not affected by a 100-fold change in total chloride concentration. Thus, absorption of K^+ and Na^+ is independent of the concentration and identity of the anion of the salt. Potassium ion absorption is also independent of the concentration of bicarbonate (Table IV). Bicarbonate concentration as high as 3.6×10^{-3} M has no effect on K^+ absorption.

Thus, if K^+, Rb^+, or Na^+ is absorbed in association with the anion of its salt or bicarbonate, the rates of absorption of these anions must all be equal to each other and to the rate of cation absorption. That this is not the case is shown by the rates of anion absorption in Table V compared with K^+ and Na^+ absorption rates in Table III. Rates of K^+ absorption greatly exceed rates of sulfate and phosphate absorption. The K^+ absorption rate at pH 5 from 10^{-3} M K^+ in the presence of phosphate is 126 mμmoles/minute gram. Phosphate absorption is only 52. Under these conditions, $H_2PO_4^-$ is the predominate phosphate ion species absorbed and in the absorption solution (6). Sulfate absorption is not fast enough to support K^+ absorption at any sulfate concentration. Even at V_{max} where the rates are independent of pH, neither sulfate nor phosphate is sufficiently fast to accompany K^+ absorption. Only chloride and bromide absorption rates are fast enough at high concentrations to support the rapid rate of K^+ absorption, but K^+ absorption is independent of the chloride concentration. Bromide absorption rates were not influenced by different accompanying cations, potassium, calcium, and zinc ions, which are absorbed at widely differing rates (4, 15). Phosphate absorption was the same whether in solution as a Na^+ or K^+ salt and a KCl concentration of 10^{-3} M has no effect at any phosphate concentration measured (10^{-6} M to 5×10^{-4} M).

Uptake of bicarbonate and CO_2 has been reported as approximately 2 (17) and 10 (14) per cent of the respiration rate under conditions of greater

bicarbonate concentrations than in the experiments reported here. It is not likely that CO_2 uptake greatly exceeds 10 per cent of the respiration rate since the respiratory CO_2/O_2 quotient is 1.0. The roots in these experiments respire at rates between 200 and 300 mμmoles/minute gram. From this, bicarbonate absorption is estimated to be a maximum of 30 mμmoles/minute
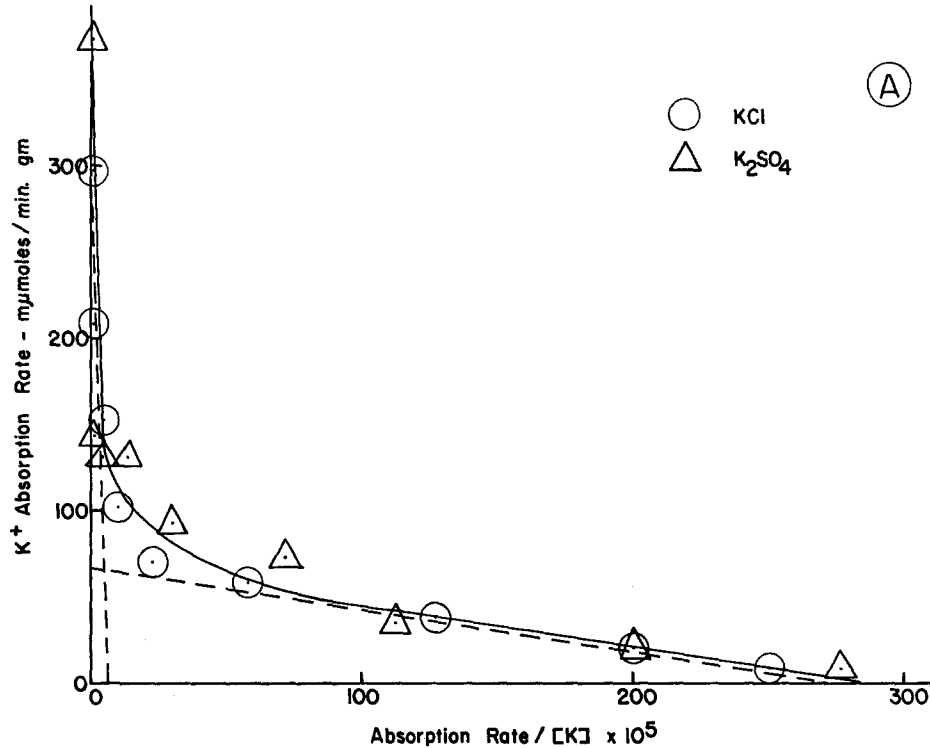

Fig. 2 A

FIGURE 2. K^+ and Na^+ absorption rates as a function of absorption rate/[cation] (Eadie plot). The component reactions of each curve are represented by the dotted lines. Fig. 2 A. K^+ absorption at pH 5 from KCl and K_2SO_4 solutions. K^+ concentrations are from 3×10^{-6} M to 3×10^{-2} M with 2×10^{-3} M Na^+ present at all K^+ concentrations. Fig. 2 B. Na^+ absorption at pH 7 from NaCl solutions with 10^{-3} M LiCl present. Na^+ concentrations are from 10^{-6} M to 10^{-2} M.

gram. This value falls far short of being sufficient to account for the difference between potassium absorption and sulfate or phosphate absorption.

Acid and Base Release by the Roots   Hydrogen ions are released by roots at steady rates, as shown in Fig. 3, under conditions where the rate of cation absorption is constant and predominates over the anion absorption. At the high salt concentrations of Fig. 3, the absorption rate is independent of the

pH change during the experiments. The rates of H^+ release appear to be net rates, reflecting the difference between anion and cation absorption. For example, the rate of release in KCl is less than in K_2SO_4 at the same potassium concentration. H^+ release in $CaSO_4$ reflects the large initial exchange step that is observed with calcium ion and strontium ion absorption (4). After approximately 20 minutes, the rate is constant with time but relatively slow as expected from the relatively slow rate of calcium ion absorption.
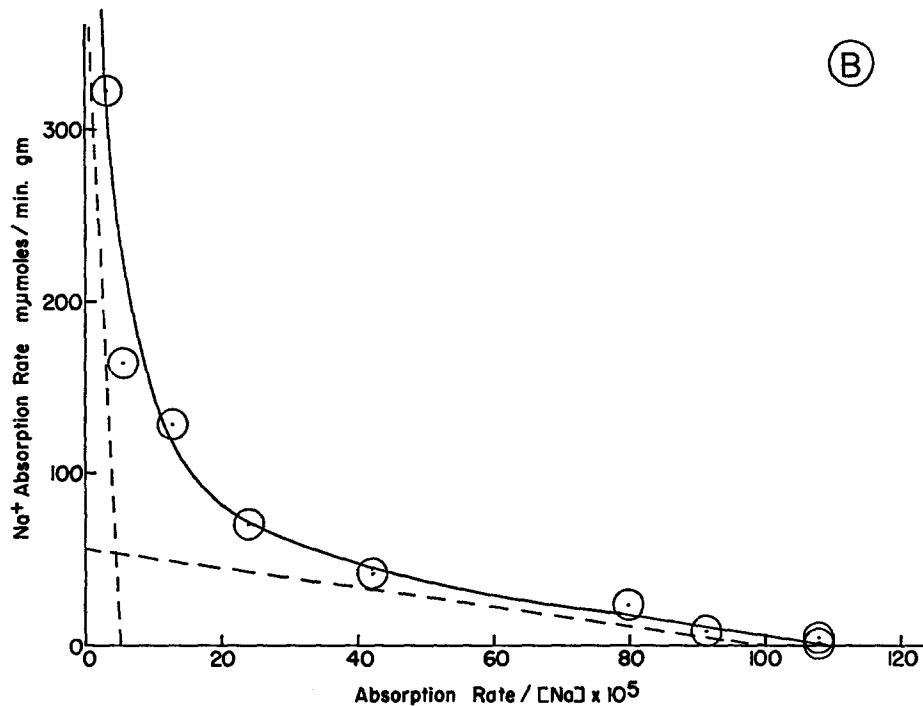

Fig. 2 B

In $CaCl_2$, after the initial exchange H^+ release, a net steady-state release of base is observed which overshadows the slow H^+ release of calcium ion absorption. This is consistent with the relative rates of calcium ion and chloride absorption. Sodium ion absorption is approximately the same as K^+ absorption at 10^{-3} M (Table III) and produces the same rate of H^+ release in the same salt, as in Table VI. The cation induced H^+ release is even faster when acetate is the anion, as expected from the general observation that organic acids enter roots only in the undissociated form.

Rates of H^+ release qualitatively follow the rates of cation absorption. They increase with an increase in K^+ concentration with the same salt, as shown in Table VI. Mannose, 2,4-dinitrophenol(DNP), and low oxygen concentrations, which are inhibitors of potassium absorption (Fig. 1 A), decrease

the rate of H^+ release. On the other hand, Na^+ absorption is not inhibited by oxygen as low as 1 per cent (Fig. 1 B). The H^+ release rate in 5×10^{-5} M NaOH is similarly unaffected. In this case, the rate of H^+ release is approximately equal to the rate of Na^+ absorption.

TABLE III

POTASSIUM ION AND SODIUM ION ABSORPTION RATES WITH VARIOUS ANIONS

Anion concentrations are 10^{-3} M to 2×10^{-2} M for monovalent species and 5×10^{-4} M to 10^{-2} M for divalent species. No significant concentrations of trivalent species under the experimental conditions. 10^{-3} M Na^+ is also in the solutions for K^+ and Rb^+ absorption and 10^{-3} M Li^+ is in the Na^+ absorption solutions.

| | Chloride | Anion | | | |
		Sulfate	Phosphate	Nitrate	Thiosulfate
	Absorption rate	Per cent absorption rate with chloride			
	mμmoles/min. gm				
K^+ absorption + 10^{-3} M Na^+					
10^{-5} M K^+, pH 5	30	110	115	100	94
pH 4	16				
10^{-3} M K^+, pH 5	124	106	102	97	107
pH 4	116				
10^{-2} M K^+, pH 5	214	113	—	—	—
ΣV_{max} pH 5	340	110	—	—	—
Rb^+ absorption + 10^{-3} M Na^+					
10^{-3} M Rb^+, pH 5	154	101	—	—	—
Na^+ absorption + 10^{-3} M Li^+					
10^{-5} M Na^+, pH 7	12.3	101	74	115	—
10^{-3} M Na^+, pH 5	113	91	98	84	—
ΣV_{max} pH 7	310				

ΣV_{max} is the sum of the V_{max} values of the component absorption reactions and is derived from an absorption rate *vs.* absorption rate/[cation] plot extrapolated to infinite cation concentration.

Rates of base release can be observed under conditions where anion absorption rates exceed cation absorption. Base release rates are constant with time under the experimental conditions of relatively high salt concentration (Fig. 4) when the absorption rate is constant. Thus, there is a net steady base release with 10^{-2} M $CaCl_2$ at pH 4. This was also true for 10^{-3} M KCl at

pH 4. The rate is less at 10^{-2} M KCl (Table VII) as expected from the increase in net H^+ release in 10^{-2} M KCl at pH 7. Base release rates are consistent with the relative rates of anion absorption. They are much greater with $CaCl_2$ at pH 3.5 where chloride absorption is increased and calcium ion absorption would be expected to be more inhibited. The initial H^+ exchange reaction with calcium ion is overshadowed by the large base release so that the rates appear constant from 1 second (Fig. 4). On the other

TABLE IV

THE EFFECT OF BICARBONATE CONCENTRATION
ON K^+ ABSORPTION

K^+ concentration was 3×10^{-3} M with 10^{-3} M Na^+ present. Solutions with HCO_3^- concentrations greater than 5×10^{-6} M were prepared by titrating $KHCO_3$—$NaHCO_3$ solutions with HCl to the desired pH. The HCO_3^- concentration was calculated from the pH and the difference between the cation concentration and the measured Cl^- concentration. Since these solutions were not equilibrated with air, pCO_2 values were from 0.2 per cent at pH 7.7 to 10 per cent at pH 4. Solutions with HCO_3^- concentrations at 5×10^{-6} M and 5×10^{-8} M were prepared with KCl and NaCl and equilibrated with air. The pH was adjusted with HCl.

[HCO$_3^-$]	pH	[Cl$^-$]	Potassium ion absorption rate
moles/liter		*moles/liter*	*mµmoles/min. gm*
5×10^{-8}	4	4×10^{-3}	102
2×10^{-5}	4	4×10^{-3}	98
5×10^{-6}	6	4×10^{-3}	139
8×10^{-4}	6	3×10^{-3}	136
1.2×10^{-3}	7	2.8×10^{-3}	134
3.6×10^{-3}	7.7	4×10^{-4}	137

hand, with $CaSO_4$, the initial exchange of both calcium ion and sulfate (2) is reflected by an initial state of no change in the amount of base released for the first 20 minutes. Thereafter, a relatively slow steady rate ensues. With Na_2SO_4, H^+ release overshadows the base release even at pH 4, owing to the greater rate of cation absorption. DNP reverses this, showing the same base release rate that it shows without the addition of salt. Hydrochloric, sulfuric, phosphoric, and acetic acids all give rise to a base release with the relative rates in qualitative agreement with the relative rates of anion absorption.

The results show that H^+ is released during cation absorption and base during anion absorption. The magnitude by which one of these predominates depends on the relative rates of cation and anion absorption. However, the

actual rate of base release exceeds the rate of anion absorption. For example, base release with 5×10^{-5} M H_2SO_4 is 15 mμmoles/minute gram when the absorption is less than 1 (results in Table V compared to those in Table VII). There are similarly quantitative discrepancies between absorption

TABLE V

ABSORPTION RATES OF VARIOUS ANIONS

The potassium salt was used in all experiments

	Anion			
	Bromide	Chloride	Sulfate	Phosphate
Anion concentration	Absorption rate			
moles/liter	mμmoles/min. gm	mμmoles/min. gm	mμmoles/min. gm	mμmoles/min. gm
10^{-5}				
pH 4	—	16	0.18	3
pH 5	—	10	0.07	—
10^{-4}				
pH 4	12	37	0.27	8
pH 5	—	34	0.13	—
10^{-3}				
pH 4	67	62	1.90	—
pH 5	—	54	1.96	52
pH 7	—	52	1.46	—
10^{-2}				
pH 4	194	218	12.8	162
pH 5	—	160	14.6	—
pH 7	—	126	11.1	—
ΣV_{max}				
pH 4	375	320	≈ 40	200

rate and base release with 10^{-4} M H_3PO_4, although the discrepancy is not as great as with sulfate. This explains why the differences in H^+ release between KCl and K_2SO_4 are not as great as expected from the relative absorption of chloride and sulfate.

DISCUSSION

Potassium ion and sodium ion absorption are independent of the identities, concentrations, and rates of absorption of the anions of the external solution. Independence of cation and anion absorption is also shown by the effect of 10^{-2} M mannose which inhibits potassium absorption but does not affect phosphate absorption (8). Mannose has been shown to alter the distribution

of P^{32} among the products of phosphate absorption by barley roots (8). Thus, cations must be absorbed as ions or hydroxides. Anions must be absorbed as ions or undissociated acids. Release of hydrogen ions accompanying cation absorption and base release during anion absorption substantiates
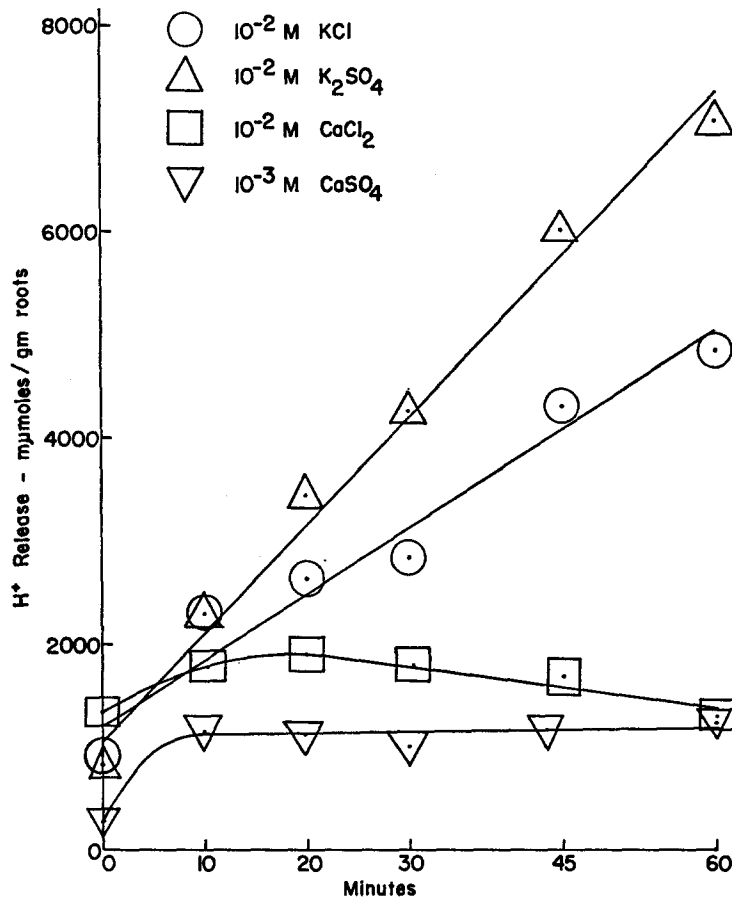

FIGURE 3. Time course of H^+ release by roots, determined by titration back to the initial pH of 7.0. Final pH values were as low as 4.7. The H^+ release was measured for periods from 1 second to 60 minutes.

this. It is also consistent with the competitive effect of hydrogen ion on cation absorption (5) and of hydroxide on phosphate absorption (6). H^+ release cannot be accounted for by bicarbonate absorption since CO_2 uptake is not great enough to account for the excess of cation absorbed over anions. Neither cation is exchanged for the sodium ion or potassium ion in the roots.

Release of hydrogen ion in the external solution does not differentiate between an actual H^+ exchange or hydrogen ion release owing to uptake of

hydroxide. In either case, internal anions must exert some balance for cation absorption if a constant charge balance is to be maintained. Organic acid anions have been shown to increase with excess cation absorption and to decrease with excess anion absorption (9, 18). Lundegardh (12) believes that

TABLE VI

RATES OF H⁺ RELEASE BY BARLEY ROOTS

The amounts of H^+ released were determined by titration back to the initial pH of 7.0. Final pH values were as low as 4.7. The negative rate of $CaCl_2$ indicates a net base release. All solutions contain sufficient base to bring the solution to pH 7 and are equilibrated with air. KOH was used to adjust potassium salt solutions, NaOH for sodium salt solutions and $Ca(OH)_2$ for calcium salt solutions. The amount of base did not increase the cation concentration of the added salt solutions by more than 10 per cent. Solutions without added salt contain only sufficient base to bring the solution to pH 7.

Solution	H^+ release rate
	mμmoles/min. gm
NaOH, 7×10^{-5} M	19
1 per cent oxygen	21
$+ 10^{-2}$ M mannose	4
DNP, 10^{-4} M $+ 3 \times 10^{-4}$ M NaOH	8
KCl, 10^{-3} M	21
10^{-2} M	40
K_2SO_4, 10^{-3} M	47
1 per cent oxygen	23
10^{-2} M	55
1 per cent oxygen	36
$+ 10^{-2}$ M mannose	18
$+ 10^{-4}$ M DNP	2
Na_2SO_4, 10^{-3} M	55
Na acetate, 10^{-3} M	77
$CaSO_4$, 10^{-3} M	2
$CaCl_2$, 10^{-3} M	-27

increase in organic acid anions is accomplished by bicarbonate uptake and is causally related to excess cation absorption. On the other hand, Jacobson (9) observed that CO_2 uptake from the external solution was not sufficient to account for the organic acid increase. The organic acid concentration changes were not always equal to or great enough to account for the excess cation or anion absorbed, particularly in the case of anions. Ulrich (18)

viewed the changes in organic acid content as a respiratory-linked result of unequal absorption of anion and cation. Increased organic acid content associated with an excess of cation absorbed was attributed to concomitant CO_2 uptake. An increase in the respiratory utilization of organic acids was at-
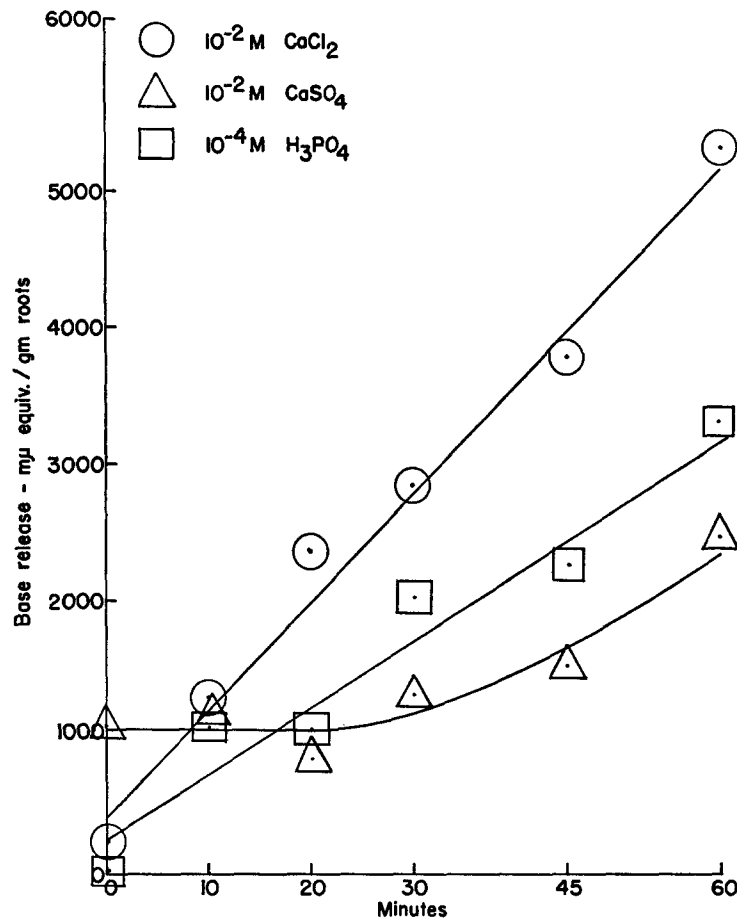

FIGURE 4. Time course of base release by roots, determined by titration back to the initial pH of 4.0. Final pH values were as high as 4.5. The base release was measured for periods from 1 second to 60 minutes.

tributed to excess anion absorption with the organic acid decreases not quantitative because of the low levels of organic acid initially in the roots. The time sequence of the organic acid changes with salt absorption was not determined but the qualitative agreement with the relative anion and cation absorption does suggest an internal balance for absorption.

The base release observed with excess anion absorption can be OH^- re-

sulting from H^+ uptake or from HCO_3^- excretion as well as from exchange. HCO_3^- would not be accumulated in the external solution under the experimental conditions of constant equilibration with air. HCO_3^- excretion seems a likely explanation of the relatively large base release compared to

TABLE VII

RATES OF BASE RELEASE BY BARLEY ROOTS

The amounts of base released were determined by titration back to the initial pH of the solution. Final pH values were as high as 4.5. The negative value for Na_2SO_4 indicates a net H^+ release. All solutions contain sufficient acid to bring the solution to pH 4.0 or 3.5. HCl was used for chloride salt solutions and H_2SO_4 for sulfate. The amount of acid did not increase the anion concentrations of the added salt solutions by more than 10 per cent. Solutions without added salt contain only sufficient acid to adjust the pH.

Solution	Initial pH	Base release rate
		$m\mu moles/min.\ gm$
HCl 3×10^{-4} M	3.5	66
10^{-4} M	4.0	40
KCl 10^{-3} M	3.5	354
10^{-3} M	4.0	23
10^{-2} M	4.0	7
CaCl₂ 10^{-3} M	3.5	434
10^{-3} M	4.0	55
10^{-3} M	7.0	27
10^{-2} M	4.0	150
H_2SO_4 5×10^{-6} M	4.0	15
$+ 10^{-4}$ M DNP	4.0	33
Na_2SO_4 10^{-2} M	4.0	-23
$+ 10^{-4}$ M DNP	4.0	26
$CaSO_4$ 10^{-3} M	3.5	71
10^{-3} M	4.0	12
10^{-2} M	4.0	64
H_3PO_4 10^{-4} M	4.0	50
CH_3COOH 4×10^{-3} M	3.5	19

the excess anion absorption since CO_2 is constantly generated in the roots by the respiratory chain. Overstreet *et al.* (14) state that excess absorption of anions is usually accompanied by an increase of HCO_3^- in the external solution. Titration curves do not suggest that sufficient amounts of organic weak-acid anions are excreted.

Potassium ion absorption is inhibited at 1 per cent oxygen but Na^+ ab-

sorption is not. This infers a specificity of the absorption for each cation. The H^+ release rate, in reflecting the absorption rate of each cation, also reflects this specificity. Thus, the H^+ release appears to be production of H^+ in the specific reactions of the cation absorption. It does not appear that a general exchange is rate-limiting to the cation absorption; that is, K^+ or Na^+ absorption is not rate-limited by H^+ exchange from non-specific sources. Further, the cation absorption does not depend on a H^+ gradient in the opposite direction, as is indicated by almost no effect of a change in H^+ concentration from 10^{-4} M to 10^{-7} M on K^+ absorption from 10^{-3} M K^+.

H^+ and base release during cation and anion absorption substantiates a process of H^+ exchange for cations and OH^- exchange for anions, formulated by Jacobson et al. (10). Exchange is consistent with mechanisms of absorption mediated by metabolically produced carriers, with more than one reaction for each ion and with specificity of absorption of individual ions. In addition, it provides an electrical balance for independent absorption of cations and anions.

The rate-limiting steps of cation and anion absorption are only connected by a maintena.·_e of charge. However, independence of the immediate reactions of cation and anion absorption does not preclude the likelihood that absorption of one would have an eventual or indirect effect on the other. For example, an increase in respiration produced by anions which was observed by Lundegardh (13) might be expected to alter the steady-state levels of compounds involved in cation absorption. In turn, the increase in organic acids associated with excess cation absorption may eventually alter the rate of anion absorption. The experiments presented indicate that the rate-limiting step for cation absorption against a salt concentration gradient is neither a gradient produced by simultaneous anion absorption nor an H^+ concentration gradient in the opposite direction. It appears to be the production, utilization, or turnover of compounds in specific reactions.

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