Equilibrium and Ion Exchange Characteristics of Potassium and Sodium Accumulation by Barley Roots

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ABSTRACT Potassium ion and Na^+ influx and efflux rates into and from excised barley roots are compared with the maximum capacity of accumulation. Potassium ion and Na^+ influx and efflux involve a cation exchange that is independent of simultaneous exchange of the accompanying anion. These exchange fluxes depend on the concentration and cation composition of the solutions from which they originate. Selective differences between K^+ and Na^+ fluxes are sufficient to account for a cationic distribution within the roots that differs markedly from that of the external solution and that persists for extended time periods. The accumulation maximum is a cation exchange equilibrium with the cation influx and efflux rates approaching equality. The equilibrium level is independent of the individual cation fluxes and the external solution concentration. It is a finite quantity which appears to be determined by the internal anion concentration including accumulated as well as endogenous anions.

INTRODUCTION

Potassium and sodium ion concentrations in barley roots may depend on both the initial constant rates of cation accumulation and a maximum capacity of accumulation. It has been generally observed that barley roots can accumulate K^+ or Na^+ from salt solutions at constant rates for several hours under carefully controlled conditions. Ultimately, the accumulation rates decrease with continued incubation in such a way that the cation content of the roots appears to approach a maximum value. The cation accumulation has been considered to be largely irreversible (4); however, the eventual decrease in the initial accumulation rate could be the result of an increasingly significant efflux of accumulated cations. The apparent irreversibility may be a reflection of the degree of departure from equilibrium.

The present work concerns the characterization of the maximum capacity of accumulation in comparison with K^+ and Na^+ influx and efflux rates into and from excised barley roots. These studies demonstrate the degree to which each factor may regulate the relative cation contents of the roots. Cation flux experiments reveal that the maximum capacity is an equilibrium level with the cation influx and efflux rates approaching equality. The equilibrium level was found to be independent of the individual cation fluxes and dependent upon anion accumulation. The cation fluxes involve cation exchange that is independent of simultaneous anion exchange flux.

MATERIALS AND METHODS

The excised barley roots were from 6 day old etiolated seedlings of barley (*Hordeum vulgare* var. *Compana* or var. *Trebi*). The two varieties were both qualitatively and quantitatively similar in their responses. The seedlings had been grown in continuously aerated 2×10^{-4} M $CaSO_4$ solution at 25°C, essentially as described by Epstein and Hagen (2). The roots were excised, rinsed three times with approximately 50 times the roots' volume of water, and then suspended in continuously aerated water in the same proportion no longer than 15 minutes prior to use in the experiment. Demineralized distilled water was used throughout the experimental procedures.

Potassium ion fluxes were determined by using K^{42} or Rb^{86} as an isotopic tracer for K^+. Validity of the use of Rb^{86} was substantiated previously (8) and is consistent with experiments of others (5).

For the accumulation experiments, 4 to 10 gm of roots were placed in 4 liters of rapidly aerated salt solution and successive root samples were removed periodically, rinsed 4 times with 50 volumes of water, blotted gently, and weighed. One-half gm samples were assayed for radioactivity and total K^+, Na^+, and Cl^- content. Efflux was determined by incubating the roots first in a radioactive salt solution for a requisite period of time, usually 3 hours. They were then removed, drained, placed in a non-radioactive solution, and sampled periodically. The influx which occurred during the efflux period was determined by first incubating roots in a non-radioactive salt solution and then in a radioactive salt solution from which root samples were withdrawn periodically as described before. Sampling times were usually at 0, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360 minutes, and at 24 hours. The 4 liter volumes of salt solution sufficed to prevent more than a 10 per cent decrease in salt content during the entire incubation time.

The pH of the salt solutions was adjusted and maintained during the first 6 hours within 0.05 unit and the temperature was 25°C. The pH change during the 6 to 24 hour period usually did not exceed 0.2 unit. The pH was adjusted by measuring the pH of small aliquots of the solutions which were discarded to avoid contamination of the incubating solutions with salt from the pH meter electrode. Base or acid used for pH control was appropriate to the incubation solution and the amounts required were not sufficient to change the salt ion concentrations measurably. K^+ and Na^+ from the roots also did not alter the solution concentration measurably. K^+ and Na^+ solutions contained less than 0.1 mole per cent Na^+ or K^+, respectively, at all times.

Potassium and sodium contents of the roots and incubation solutions were determined by flame photometric analyses. Dried root samples were twice extracted for 30 minutes with 5 ml of 0.1 N HNO_3 or by boiling with 10 ml H_2O and diluted to 25 ml for flame analyses. Root samples which were analyzed for Cl^- as well as K^+ and Na^+ were dried in the presence of excess $Ca(OH)_2$ and twice extracted by boiling 15 minutes with 10 ml of water. Cl^- content of the diluted extracts was measured with an Aminco-Cotlove automatic chloride titrator (1). Ion accumulations and fluxes over a time period are expressed as micromoles per gram fresh weight of roots. Rates of flux or accumulation are calculated from linear time course data by the method of least squares and are expressed as millimicromoles per minute-gram fresh weight of roots. Fluxes are expressed on a weight basis since this was the basis of sampling. A gram of roots (about 60, 10 to 12 plants) has a surface area of about 100 cm^2.

RESULTS AND DISCUSSION

Roots in these experiments initially contain 14 to 20 μmoles of K^+ and 1 to 5 μmoles of Na^+ per gram. This is analogous to 2×10^{-2} molal K^+ on a basis of uniform distribution. Total K^+ content of the roots may increase by a factor of 4 or 5 during accumulation.

Potassium ion accumulation rates from KCl solutions are constant with time until accumulation of 20 to 30 μmoles/gm roots (Fig. 1A). The rate at 10^{-2} M KCl is constant for approximately 120 minutes with a net accumulation of about 27 μmoles. Thereafter, the rate decreases with each time increment. At 10^{-3} M, the rate is constant for a longer period of 300 minutes, but it also decreases upon the accumulation of about 27 μmoles. Since less than 20 μmoles are accumulated in the 6 hour experimental period at 10^{-4} M, the rate is constant throughout the time. Roots in 10^{-4}, 10^{-3}, and 10^{-2} M KCl (Table I) all eventually accumulate about equal amounts of K^+. The 3 hour accumulations, which are determined predominantly by the initial steady-state accumulation rates, differ considerably, reflecting the large differences in external solution concentration. Potassium ion accumulations during the 3 to 6 hour period differ less since the accumulation rates decrease sooner and to a greater extent as the concentration is increased. In fact, the amount accumulated between 6 and 22 hours decreases with an increase in solution concentration. A similar pattern is obtained with roots incubated in different concentrations of K_2SO_4; however, the rate decreases appear earlier and at a lower K^+ content (Fig. 1B). The total amount of K^+ accumulated in 22 hours is again very closely the same in spite of a tenfold solution concentration difference and the differences in the initial rates (Table I).

Steady-state isotopic K^+ influx rates and K^+ initial accumulation rates are identical as illustrated in Table II by roots incubated in 10^{-2} M KCl labeled with Rb^{86} or K^{42}. A high salt concentration is used in these experiments in order to approach the accumulation maximum within relatively short times. Total K^+ and isotopic K^+ accumulations over 6 hours closely agree in *Compana* roots since little efflux of endogenous K^+ occurred during incubation. When the roots are first placed in unlabeled KCl for 3 hours and then in labeled KCl, K^+ influx measured by the isotope is undiminished over the second 3 hours. However, the 8.4 μmole outward flux of previously accumulated K^+ during the second 3 hours is quantitatively sufficient to decrease the K^+ accumulation to 10.5 μmoles during this period.

Fluxes are more rapid in *Trebi* barley roots and endogenous K^+ contributes significantly to the K^+ efflux during the second 3 hours. Apparently little endogenous K^+ efflux occurs in the first 3 hours with *Trebi* roots since isotopic influx and total K^+ increases closely agree (Table II). However, net isotopic influx during the second 3 hours exceeds the total K^+ accumulation (*e.g.*, 33.6 − 10.9 > 17.3). This is accounted for by a 5 to 6 μmole efflux of endogenous K^+ (out of 19 μmoles present initially).

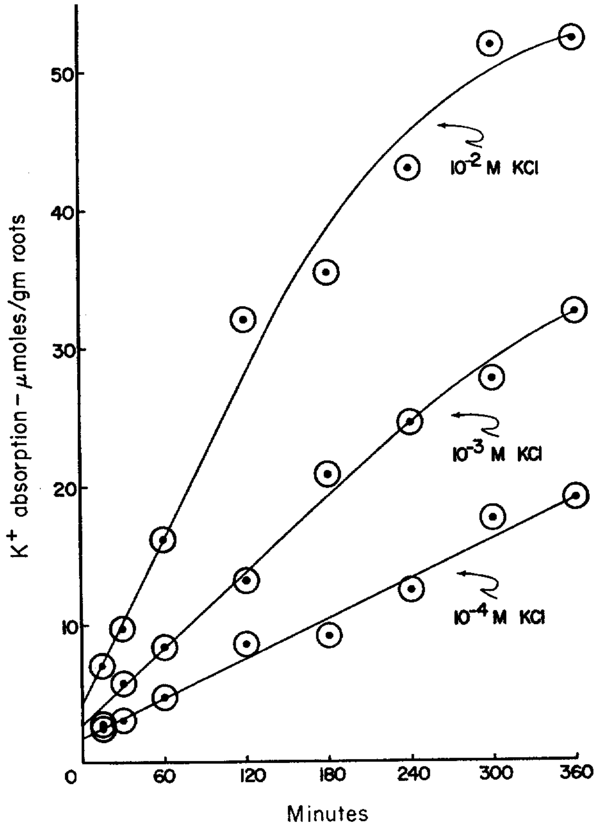

FIGURE 1A

FIGURE 1. The time course of K^+ absorption by *Trebi* barley roots at pH 5 from: Fig. 1A, KCl solutions; Fig. 1B, K_2SO_4 solutions. The absorption values are the increases in total K^+ content determined by flame photometry.

Very little accumulated K^+ is lost to water in contrast to K^+ efflux in salt solution, demonstrating that K^+ efflux occurs by means of an exchange rather than by leaking or free diffusion of salt. Significant reabsorption of K^+ released from the roots is unlikely in these experiments since the solutions are well stirred and the dilution factor is a minimum of 1/500.

Initial rates of K^+ accumulation and isotopic influx from K_2SO_4 agree well with each other (*ca.* 140 mμmoles/min.-gm for *Compana* roots and 190 for *Trebi* roots) and with the rates in KCl (Tables II and III) whether the isotopic influx is measured at the beginning of the first or second 3 hours. Most of the sulfate experiments were at pH 7 because the accumulation rates, although essentially the same as at pH 5, were constant for a longer time period at pH 7 and could be measured more precisely. Total K^+ accumulation from K_2SO_4 in the first 3 hour period is generally less than the isotopic influx during this period. This is attributed to endogenous K^+ efflux since labeled endogenous K^+ loss from *Compana* roots in unlabeled K_2SO_4 is sufficient to decrease the total K^+ accumulation over the first 3 hours. Potassium ion influx into *Compana* roots from sulfate, as from chloride, is not significantly diminished during the second 3 hours, but the efflux of 10.0 μmoles is sufficient to account for the accumulation of only 7.2 μmoles during this period.

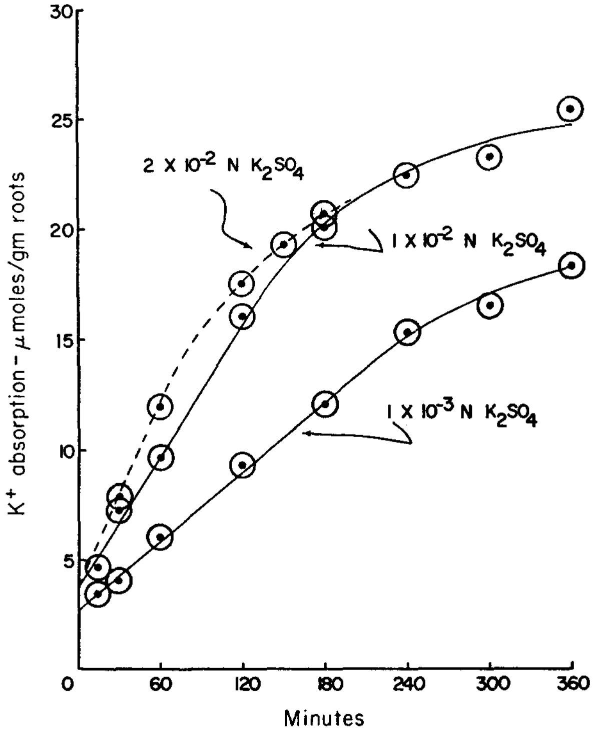

FIGURE 1B

Trebi roots in $K_2^{42}SO_4$ lose 5 to 7 μmoles of endogenous K^+ during the first

3 hour period, as shown by the difference between the K^{42} influx and the increase in total K^+. This is a marked increase in K^+ efflux over the 0 to 2 μmole endogenous K^+ efflux in the first 3 hours when these roots are incubated in KCl (Tables II and VII). During the second 3 hours, *Trebi* roots apparently lose approximately 8 μmoles additional endogenous K^+ since the difference between the isotopic uptake and total K^+ increase cannot be accounted for by K^{42} efflux alone. Thus, total K^+ efflux in K_2SO_4 during the second 3 hours must be about 18 μmoles in order to account for the difference between K^{42} influx and the total K^+ increase. It is quite likely also that the somewhat lower values of K^{42} influx obtained during the second 3 hours compared to the first 3 hour values are the result of K^{42} exchange with previously accumulated K^{42}. Differences between the isotopic uptake and total K^+ increase over the entire 6 hour period demonstrate a total endogenous K^+ efflux of 15 μmoles—80 to 90 per cent of the initial K^+ content of the roots. Endogenous K^+ efflux from *Trebi* roots in chloride is only 6 μmoles during this period. Total K^+ efflux from these roots over the 6 hours is approximately 25 μmoles in sulfate compared to 16 μmoles in chloride. Thus, the lower level of K^+ accumulated by roots in K_2SO_4 rather than in KCl can be accounted for by greater efflux of both endogenous and accumulated K^+ from the roots in sulfate. The pH difference between the chloride and sulfate experiments minimizes rather than accentuates the difference between levels with the two salts since the levels appear higher at the lower H^+ concentration.

TABLE I

K^+ ACCUMULATION BY ROOTS IN VARIOUS SALT CONCENTRATIONS

Incubation solution	Steady rate time	Initial rate	K^+ accumulation 0–3 hrs.	 3–6 hrs.	 6–22 hrs.	 22 hr. total
equiv./liter	*min.*	*mμmoles/ min.–gm*		*μmoles/gm*		
pH 5.3						
10^{-4} KCl	360	53	+ 9.2	+ 9.9	+20.7	+39.8
10^{-3} KCl	300	90	+17.1	+12.9	+ 8.3	+38.3
10^{-2} KCl	150	165	+30.5	+10.0	+ 3.3	+43.8
pH 5.6						
10^{-4} K_2SO_4	270	59	+11.7	+ 5.5		
10^{-3} K_2SO_4	240	82	+14.2	+ 8.5	+24.8	+47.5
10^{-2} K_2SO_4	120	145	+20.6	+ 8.1	+22.8	+51.5

The roots initially contained 16 to 18 μmoles/gm K^+. Total K^+ contents were determined by flame photometric analyses and accumulations are calculated from the differences in total K^+ from one period to the next.

Potassium ion influx into roots after incubation in Na^+ salt is the same as

TABLE II

ACCUMULATION AND EXCHANGE FLUXES BY BARLEY ROOTS IN CHLORIDE SALT SOLUTIONS AT pH 5

Incubation solution		Initial rates		Isotopic K^+ flux			Total K^+ (or Na^+) changes		
0–3 hrs.	3–6 hrs.	ΣK^+ or Na^+ accumulation	Isotopic K^+ influx	0–3 hrs.	3–6 hrs.	6 hrs. total	0–3 hrs.	3–6 hrs.	6 hrs. total
		mμmoles/min-gm			*μmoles/gm*				
Variety Compana roots									
K*Cl	K*Cl	145 K^+	144	+18.8	+10.5	+29.3	+19.2	+10.2	+29.4
KCl	K*Cl	133 K^+	128	—	+19.2		+18.2	+12.6	+30.8
K*Cl	KCl	—	—	+19.4	−8.4		+19.0	+9.8	+28.8
					+10.8 net	+30.2			
NaCl	K*Cl	133 K^+	130	—	+19.2		+19.3 Na^+	+20.4 K^+	
K*Cl	NaCl	—	—	+18.4	−7.1				
					+12.1 net	+30.5			
K*Cl	H_2O			+17.5	−0.7				
Variety Trebi roots									
$K^{42}Cl$	KCl	196 K^+	195	+33.8	−10.9		+33.6	+17.3	+50.9
KCl	$K^{42}Cl$	194 K^+	197	—	+33.6		+33.4	+17.9	+51.3
						+56.5			
$K^{42}Cl$	NaCl	218 Na^+	190	+36.7	−11.6		+35.0 K^+	−17.1 K^+	
							−0.7 Na^+	+34.9 Na^+	
							+34.3 net	+17.8 net	+52.1
NaCl	$K^{42}Cl$	217 Na^+	195	—	+33.7		−6.2 K^+	+25.0 K^+	
						+58.8	+39.6 Na^+	−4.5 Na^+	
							+33.4 net	−20.5 net	+53.9
KCl	H_2O						+33.2 K^+	−1.9 K^+	

The solutions contain 1 × 10^{-2} equivalents/liter salt.

K* designates isotopically labeled K^+. K^+ was labeled with Rb^{86} in the *Compana* root experiments and in the *Trebi* root experiments, with K^{42}. Total K^+ or Na^+ changes are measured by flame photometry.

TABLE III

K^+ ACCUMULATION AND EXCHANGE FLUXES BY BARLEY ROOTS IN SULFATE SOLUTIONS

Incubation solution		Initial rates		Isotopic K^+ flux			Total K^+ (or Na^+) changes		
0–3 hrs.	3–6 hrs.	ΣK^+ or Na^+ accumulation	Isotopic K^+ influx	0–3 hrs.	3–6 hrs.	6 hrs. total	0–3 hrs.	3–6 hrs.	6 hrs. total
		mμmoles/min-gm			*μmoles/gm*				
Variety Compana roots, pH 5									
$K_2{}^*SO_4$	$K_2{}^*SO_4$	141	142	+13.2	+6.0	+19.2	+11.6	+6.6	+18.2
$K_2{}^*SO_4$	K_2SO_4			+15.5	−9.1		+12.1		
K_2SO_4‡		145		−4.2					
				+11.3 net					
Variety Compana roots, pH 7									
$K_2{}^*SO_4$	$K_2{}^*SO_4$	130	138	+18.8	+6.0	+24.8	+17.6	+7.2	+24.8
$K_2{}^*SO_4$	K_2SO_4			+17.3	−10.0				
K_2SO_4	K_2SO_4‡			−3.8	−0.5		+14.9	+7.8	+22.7
Na_2SO_4	$K_2{}^*SO_4$		139		+16.6		−6.6 K^+	+16.1 K^+	
							+24.7 Na^+	−9.5 Na^+	
							+18.1 net	+6.6 net	+24.7
$K_2{}^*SO_4$	Na_2SO_4			+18.1	−10.4		−18.1 K^+	−18.5 K^+	
							±0.0 Na^+	+24.5 Na^+	
							+18.1 net	+6.0 net	+24.1
Variety Trebi roots, pH 7									
$K_2{}^{42}SO_4$	K_2SO_4			+32.8	−9.7		+27.8	+9.9	+37.7
K_2SO_4	$K_2{}^{42}SO_4$	193			+27.2		+25.9	+9.1	+35.0
						+50.3			
$K_2{}^{42}SO_4$	Na_2SO_4		217 Na^+	+31.9	−13.0		+25.1 K^+	−16.6 K^+	
							−0.4 Na^+	+31.2 Na^+	+39.3
							+24.7 net	+14.6 net	
Na_2SO_4	$K_2{}^{42}SO_4$	181			+29.3		−7.3 K^+	+21.0	
							+31.5 Na^+	−6.0 Na^+	
						+48.2	+24.2 net	+15.0 net	+39.2

Salt concentrations are 1 × 10^{-3} equivalents/liter.

K^* designates isotopically labeled K^+. K^+ was labeled with Rb^{86} in the *Compana* root experiments and in the *Trebi* root experiments, with K^{42}. Total K^+ or Na^+ changes were measured by flame photometry.

‡ Endogenous K^+ efflux during incubation in unlabeled K_2SO_4. The endogenous K^+ was labeled by growing the roots for 2 days in $CaSO_4$ solution containing a trace amount of Rb^{86}.

after incubation in K^+ salt or with no pretreatment whether the salt anion is chloride or sulfate (Tables II and III). The time course of the K^+ influxes is also closely the same; for example, as in Fig 2. K^+ efflux accompanies Na^+ influx. In fact, K^+ influx and efflux are exactly balanced by compensating Na^+ fluxes with the result that net cation fluxes are identical.

As expected from the fluxes, total cation, *i.e.* Σ total ($K^+ + Na^+$), accumulation by the roots at any one pH is the same whether predominantly K^+ or Na^+ (Table IV). Roots with somewhat lower cation contents in Table IV initially accumulate slightly more cations so that the totals are closely the same at a given accumulation time with potassium or sodium salt. As illustrated in Table IV, the summation of K^+ and Na^+ content is the same at each sampling period, regardless of the K^+ and Na^+ ratio in the external solution. Thus, the accumulation maximum is independent of the nature of the cation with respect to K^+ and Na^+, as well as the rates of individual cation influx or efflux and the concentration of the external solution.

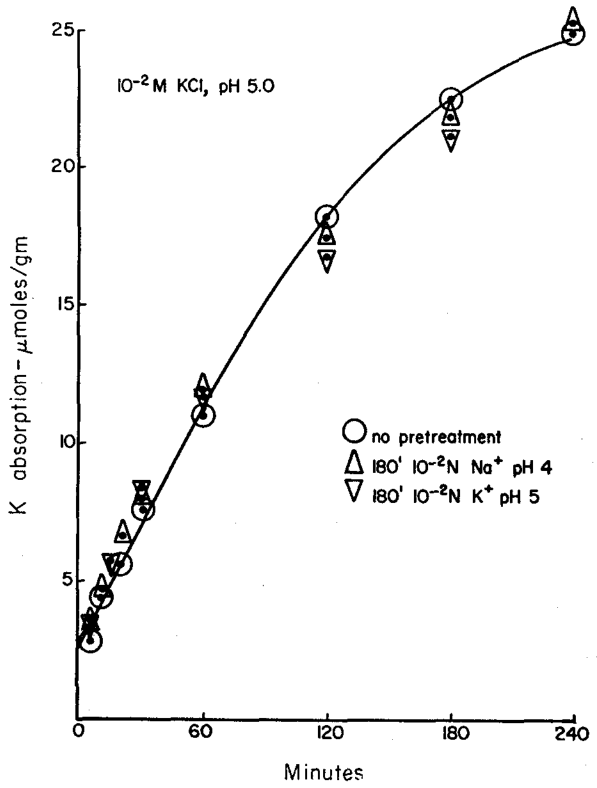

FIGURE 2. Isotopic K^+ absorption by *Compana* barley at pH 5. Initial absorption compared to absorption after 3 hours' incubation in 10^{-2} M NaCl and KCl solutions.

TABLE IV

RELATIVE K^+ AND $\Sigma(K^+ + Na^+)$ CONTENTS OF BARLEY ROOTS INCUBATED IN VARIOUS SALT SOLUTIONS

Incubation solution		Σ(total K^+ + total Na^+)			Relative K^+ content		
0–3 hrs.	3–6 hrs.	Initial	3 hrs.	6 hrs.	Initial	3 hrs.	6 hrs.
			µmoles/gm			*mole fraction* × 100	
Variety Compana roots, pH 7							
K_2SO_4	K_2SO_4	25	43	50	80	86	89
Na_2SO_4	K_2SO_4	20	42	50	80	33	60
K_2SO_4	Na_2SO_4		42	48		90	43
Variety Trebi roots, pH 5							
NaCl	KCl	20	51	70	89	24	52
KCl	NaCl		53	70		98	51
KCl	KCl	19	53	70	89	98	99
$9/1$	$\frac{KCl}{NaCl}$	15	60	79	97	77	77
$5/5$	$\frac{KCl}{NaCl}$	17	62	78	97	40	43
$1/9$	$\frac{KCl}{NaCl}$	16	60	80	97	22	17

The incubation solutions contained 1×10^{-3} equivalents/liter salt.

TABLE V

THE EFFECT OF pH ON K^+ ACCUMULATION BY *TREBI* ROOTS FROM 10^{-2} M KCl

Solution pH	Initial rate	K^+ accumulation 0–3 hrs.	ΣK^+ 24 hrs.
	mµmoles/min.-gm	*µmoles/gm*	
4	233	+30.2	76.8
5	252	+37.1	90.0
6	235	+42.3	97.2
7	230	+37.7	99.2

Greater K^+ accumulation at pH 6 and 7 than at greater hydrogen ion concentrations (Table V) suggests a need to include hydrogen ion content of the roots in the cation summation. The pH has little effect on the K^+ influx rates, again demonstrating the distinction between the flux rates and accumulation maxima.

Although net fluxes in K^+-Na^+ exchange experiments are the same whether of K^+ or Na^+ in Tables II and III, K^+ efflux exceeds Na^+ efflux and Na^+ influx exceeds K^+ influx. This is a general observation in this type of experiment and suggests differential rates of K^+ and Na^+ influx with differential effects of K^+ and Na^+ concentration on the flux rates. For example, Na^+ efflux may be inhibited by the increasingly greater K^+ concentration in the roots (from 13 to 35 μmoles/gm) resulting in more K^+ exchange with K^+ than with Na^+. On the other hand, K^+ efflux presumably is less inhibited and therefore greater, since the root Na^+ concentration is relatively lower (from 2 to 30 μmoles/gm). From this, the flux rates of individual cations would be expected to influence the relative accumulations of different cations but not the summation of cation accumulations. In fact, differential flux rates among the cations could determine a distribution ratio of the cations in the roots that differs from that of the external solution for a considerable length of time even though total efflux rates approach those of influx. This is indeed the case, as illustrated in Table VI. Roots in various ratios of K^+ to Na^+ at 10^{-2} equivalents/liter total salt concentration contain essentially the same total summation of K^+ and Na^+ at the various sample periods; for example, as shown by the 24 hour total $K^+ + Na^+$ contents. No further increase in the summation cation contents occurred after 8 hours.

TABLE VI
RATIOS OF K^+/Na^+ CONTENT OF ROOTS DURING INCUBATION IN SOLUTIONS OF DIFFERENT K^+/Na^+ RATIOS

Solution	K^+/Na^+	Initial rate	Total content										Total $K^+ + Na^+$ 24 hrs.
			Initial	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	9 hrs.	24 hrs.	
	mole fraction ratio × 100						*mole fraction ratio K^+/Na^+ × 100*						*μmoles/gm*
I	91/9	77/23	96/4	84/16	78/22	76/24	78/22	77/23	79/21	80/20	78/22	84/16	74
II	52/48	36/64	97/3	58/42	43/57	40/60	41/59	41/59	41/59	43/57	44/56	50/50	78
III	9/91	18/82	97/3	40/60	26/74	22/78	18/82	16/84	17/83	18/82	17/83	16/84	78
IV	90/10	64/36	96/4	81/19	74/26	73/27	65/35	68/32	69/31			73/27	79

Solutions II, III, and IV contain 1 × 10^{-2} equivalents/liter chloride salt and are at pH 5.
Solution I contains 1 × 10^{-2} equivalents/liter sulfate salt at pH 7. *Trebi* roots were used in solution IV and *Compana* roots in the other treatments.

Only with roots in the solution of nearly equal K^+ and Na^+ concentration does the K^+/Na^+ ratio in the roots closely approach that of the external solution within 24 hours. The root ratios at first approach the expected value but then overshoot slightly in favor of Na^+ before arriving at the 50/50 value. Root K^+/Na^+ ratios in the 9/91 solution gradually approach, but do not quite attain, the theoretical ratio within 24 hours. The initial rate ratio, which shows a twofold preference for K^+, is maintained by the roots from 4 hours for as long as sampled. This relative preference for K^+ at 10^{-3} M is expected from differential concentration functions of K^+ and Na^+ on the individual absorption rates (2, 5, 8).

Na^+ influx (Table VI) is proportionately faster than K^+ influx although K^+ influx is uninhibited in the solutions of 91/9 and 90/10 ratios. Thus, the initial rate ratios show a preference for Na^+ which is reflected by a root ratio of Na^+ which increases during the first 3 or 4 hours to 2 to 3.5 times the solution ratio. Indeed, the 4 hour K^+/Na^+ ratio of the roots in solution IV is almost exactly the ratio of the influx rates. Subsequently, the relative Na^+ content decreases as if to approach the expected 9/1 ratio, although this is not quite attained even within 24 hours.

TABLE VII

COMPARISON OF CATION FLUXES WITH ANION FLUXES

Measurement	Treatment	
	0–3 hrs.	3–6 hrs.
	KCl	K_2SO_4
	µmoles/gm	
I		
ΣCl^-	+21.2	−0.8
ΣK^+	+30.0	+1.5
K^*	—	+15.4
K^+ efflux in exchange for K^* ($K^* - \Sigma K^+$)	—	−13.9
K^+ flux without Cl^-	+8.8	−13.1
H^+ efflux	−9.3	—
	$K^{42}Cl$	$Na_2S^{35}O_4$
II		
$S^{35}O_4$	—	+3.6 equivalents
ΣCl^-	+22.7	−0.8
K^{42}	+39.2	−10.9 −5.4 endogenous K^+
ΣK^+	+39.4	−16.3
ΣNa^+	—	+34.5 +18.2 net
Cation flux without Cl^- or $SO_4^=$	+16.6 K^+	−15.5 K^+ +30.9 Na^+

Salt solutions are 1×10^{-2} equivalents/liter at pH 7.0. K^* designates Rb^{86}-labeled K^+.

Comparisons of K^+ fluxes with chloride flux demonstrate that K^+ exchanges independently of the anion (Table VII). The roots initially contain 1 to 5 µmoles of chloride per gram. Hydrogen ion release was determined from the amount of base required to maintain the pH at 7.0. No change in pH was observed in aerated salt solutions without roots or with roots in water. The roots in KCl for the first 3 hours accumulated an excess of K^+ over Cl^- which was balanced by simultaneous release of hydrogen ion to the external solution as demonstrated in the first experiment of Table VII. The roots were then transferred, without rinsing, to a Rb^{86}-K_2SO_4 solution at pH 7 for an additional 3 hours. The isotopic K^+ uptake greatly exceeds the total K^+ accumulation during this period and the difference (13.9 μmoles) between these two values is a measure of the K^+ efflux in exchange for K^+. Apparently some exchange of the isotopic K^+ with previously incorporated isotope occurred in this experiment since the rate of incorporation continuously decreased with time and the 3 hour amount represents only half the influx. The roots released only a very slight amount of chloride while incubating in sulfate. Thus, 9 μmoles of K^+ entered the roots in exchange for H^+ and at least 13 μmoles of K^+ exchanged out of the roots unaccompanied by chloride.

Potassium ion also exchanges with Na^+ without simultaneous exchange of the accompanying salt anions (Experiment II, Table VII). The roots in KCl for 3 hours, as in the previous experiment, accumulated an excess of K^+ over Cl^-. The K^{42} influx and the total K^+ accumulation rates were constant over the entire 3 hour period with no measurable efflux of endogenous K^+. In Na_2SO_4 during the second 3 hours, 11 μmoles of previously accumulated K^{42} and 5 μmoles of endogenous K^+ exchanged for Na^+ without simultaneous efflux of Cl^-. Na^+ influx greatly exceeds sulfate accumulation (determined with S^{35} in a previous experiment under similar conditions) resulting in a 31 μmole influx of Na^+ unaccompanied by the anion, half of which is balanced by K^+ exchange efflux. The other half of the Na^+ influx presumably is balanced by H^+.

TABLE VIII

K^+ ACCUMULATION BY EXCISED ROOTS COMPARED TO ROOTS OF INTACT PLANTS

Treatment	K^+ accumulation			
	Initial rate	0–3 hrs.	3–5 hrs.	5 hr. total
	mμmoles/min.-gm	*μmoles/gm*		
1 × 10^{-2} M KCl, pH 5.0				
Intact	137	28.2	8.9	37.1
Excised	147	31.8	10.3	42.1
1 × 10^{-2} equiv./liter K_2SO_4, pH 5.0				
Intact	139	16.9	4.5	21.4
Excised	145	15.2	6.1	21.3

The accumulation maximum and cation exchange flux may be considered to be a feature of the cut end of the excised roots. For that reason, K^+ accumulation and influx in excised roots and roots of intact plants were studied (Table VIII). Little difference is apparent in either the initial accumulation rates, *i.e.* influx rates, or the total accumulation from one period to the next.

If there is any difference at all, influx and accumulation with excised roots may slightly exceed influx and accumulation with roots of intact plants.

GENERAL DISCUSSION

The maximum K^+ accumulation approached by roots during K^+ salt uptake is a cation exchange equilibrium with the influx and efflux rates approaching equality. The ultimate decrease in K^+ accumulation rates with long times of salt uptake is quantitatively accounted for by the efflux of accumulated and endogenous K^+. Although equilibrium is most apparent at salt concentrations greater than 10^{-3} M, the same level is approached by roots in much lower concentrations, differential concentration effects on the flux rates notwithstanding. Accumulation rates are sufficiently slow that at 10^{-4} M KCl, 24 hours are required to reach equilibrium, while with the slow rates of 30 mμmoles/min-gm at 10^{-5} M K^+, equilibrium would not be expected to be attained until the order of 2 days. This accounts for the apparent irreversibility of K^+ accumulation from solutions of low K^+ concentration and within short incubation times, since K^+ exchanges primarily for H^+ as a partial process under such conditions (8).

Rates of K^+ or Na^+ influx are constant for at least 6 hours and depend on the cation concentration of the external solution. Influx rates were determined only in the initial stages over time periods during which the measured rate was constant. Efflux rates depend on the internal cation concentrations. They also depend on the external solution in so far as the efflux involves a 1 to 1 exchange with the incoming cation. The equilibrium level, by contrast, is independent of the salt concentration of the external solution and the individual rates of cation influx or efflux. Potassium ions exchanging for H^+ or for Na^+ in the roots approach the same equilibrium level. The equilibrium is the same for K^+ as for Na^+ or any combination of the two cations. By inference, the equilibrium level would be the same for the sum of all the cations accumulated and must include H^+.

Accumulated K^+ efflux accounts for the major portion of the total K^+ efflux in the *Compana* roots, with relatively little endogenous K^+ efflux during the first 3 to 6 hours, particularly in Cl^- salt solutions. For example, despite an endogenous/accumulated K^+ ratio between 1/1 and 2/3, loss of accumulated K^+ accounts for all the efflux between 3 and 6 hours. In K_2SO_4, endogenous K^+ efflux during the second 3 hours was less than a tenth of the accumulated K^+ efflux although endogenous K^+ content was no less than half the accumulated K^+. This infers that the most recently accumulated K^+ would be the first to exchange out of the roots into the external solution. Apparently little mixing of the accumulated K^+ with endogenous K^+ occurred during the course of accumulation initially, although all of the endogenous K^+ appears to be exchangeable eventually. The proportion of endogenous K^+

exchanged over a 24 hour period is generally as great as the proportion of accumulated K^+, demonstrating complete mixing during this time. On the other hand, endogenous/accumulated K^+ efflux in *Trebi* roots in either Cl^- or $SO_4^=$ salt solution corresponds very closely to the root content ratio (*e.g.*, 1/3.6 for efflux in NaCl with the same ratio of content in the roots), demonstrating essentially complete mixing within the first 3 hours in these roots.

The lifo (last-in-first-out) K^+ exchange flux observed with the *Compana* roots has been demonstrated in squid axons (7). Features of such an exchange are also discussed by Harris (6). When transport into a tissue occurs over a series of multiple steps as seems likely in the root uptake, the likelihood of the presence of an ion of the external solution decreases during the course of incorporation as the number of series steps increases. This may account for the low exchange values obtained by Sutcliffe with high salt beet root slices (9).

Differences between the rates of K^+ and Na^+ influx in barley roots suggesting some degree of selectivity have been observed by others and ourselves. These include differential effects of calcium and hydrogen ions on K^+ accumulation with respect to their effects on Na^+ accumulation (5, 10). Hydrogen ions are more inhibitory to Na^+ influx than to K^+ influx. Potassium ion influx rates are immediately and severely inhibited at 1 per cent oxygen, whereas no effect is apparent on Na^+ influx for an hour or more (8). This selectivity of accumulation, however, is inherent in the rate-limiting exchange fluxes but not in the ultimate equilibrium.

While the cation fluxes *per se* do not determine the equilibrium level, they do determine the relative quantities of the cationic species accumulated. They account for ratios of cation species in the roots that differ markedly from those of the external solution for many hours. Indeed, some cationic ratios of the external solution may never be attained in the roots. In this sense, the cation equilibrium attained when the summation $K^+ + Na^+$ influx and efflux rates are equal may be quasi-equilibrium. Persistence of a quasi-equilibrium in roots is not surprising when the slow rates and multiple gradients that must be involved in the cation distribution throughout the roots are considered. A 25 per cent preference for one cation over another need only be compounded 3 times to obtain a twofold preference. Thus, a slight selectivity in influx rates alone can account for a markedly greater accumulation of one cation over another. To wit, the roots reflect a 3/2 ratio of K^+/Na^+ influx rates for as long as 4 hours even in a high salt (9/1 K^+/Na^+) concentration of 10^{-2} M in which quasi-equilibrium is attained within 8 hours. It is apparent that mixing gradients and constantly changing ratios of cations can greatly prolong the attainment of true equilibrium.

Potassium ion influx rates, determined from the initial steady-state rates, are independent of the identity, concentration, and influx rates of the anions of the external solution (8). In the present experiments, K^+ influx rates are the same from chloride or sulfate salt solutions in spite of large differences in the anion accumulation rates. Potassium ion efflux is similarly independent of simultaneous anion efflux. Thus, the cation flux rates, both inward and outward, involve a cation exchange that is independent of simultaneous exchange of the accompanying anion. Potassium or Na^+ ions entering low salt roots from low salt concentrations initially exchange primarily for H^+. In high salt roots, exchange for endogenous K^+ or Na^+ predominates over exchange with H^+ which still occurs. This would explain the lower cation accumulations generally observed with high salt roots.

The equilibrium level, however, does depend on the anion accumulated. Roots in KCl for 6 hours approach equilibrium at a higher level than roots in K_2SO_4. This can be accounted for by earlier appearance and greater rates of K^+ efflux with roots in K_2SO_4 than in KCl solution during this time. Thus, the differences observed between K^+ accumulation in chloride and in sulfate (3) can be explained by K^+ efflux. The difference in equilibrium levels between the two salts is presumably a reflection of the relative quantities of anion accumulated. It may also reflect faster depletion of H^+ in the roots in K_2SO_4 due to the large excess of K^+ accumulation over sulfate accumulation.

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