Cation Effects on Chloride Fluxes and Accumulation Levels in Barley Roots

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ABSTRACT Accumulation of Cl^- by excised barley roots, as of K^+, approaches a maximum level at which the ion influx and efflux rates become equal. The rate of Cl^- influx at this equilibrium is close to the initial rate while the efflux rate increases with time from zero to equality with influx. The Cl^- fluxes are independent of simultaneous exchange flux of the cations, but depend on the nature and concentration of the salt solutions from which they originate. The Cl^- content at equilibrium, however, is largely independent of the external concentrations. The approach to equilibrium reflects the presence of the cation. Cl^- flux equilibrium is attained more rapidly in KCl than in CsCl or $CaCl_2$. This is presumably an effect of much slower distribution of Cs^+ and Ca^{++} than of K^+ within the roots. Accumulated Cs^+ appears to form a barrier to ion movement primarily within the outermost cells, thereby reducing influx and ultimately efflux rates of both Cl^- and cations. Slow internal mixing and considerable self-exchange of the incoming ions suggest internal transport over a series of steps which can become rate-limiting to the accumulation of ions in roots.

INTRODUCTION

Rates of K^+ and Na^+ absorption by barley roots have been shown to be independent of the nature, concentration, and absorption rates of the anions of the external solution (8). H^+ release during excess cation absorption and base release during excess anion absorption substantiate a process of independent ion exchange for the absorption of cations and anions. Amounts of K^+ and Na^+ accumulated, however, ultimately approach a maximum level at which the cation influx and efflux rates become equal (9). As equilibrium is approached, the cation content reflects the internal anion concentration, including accumulated as well as endogenous anions. This and differences in cation selectivity indicate distinction between the flux rates and maximum accumulation levels in the uptake of K^+ and Na^+ by barley roots.

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Chloride accumulated by barley roots in recent experiments appeared to be largely nonexchangeable (22). In fact, exchangeability of "metabolically" accumulated ions in plant cells frequently has been considered negligible (20). The eventual decrease in accumulation rates that is generally observed is attributed to an inhibitory effect of a high endogenous salt content on the uptake mechanism rather than an approach to equal influx and efflux (19). However, experimental conditions in these studies do not preclude the possibility that Cl^- accumulated in barley roots, like K^+ and Na^+, is exchangeable, with the apparent irreversibility being a feature of the departure from flux equilibrium. To wit, Briggs has considered the possible contribution of an increasing rate of efflux to a decreasing net flux in plant cell vacuoles (2) and Pitman's isotope exchange studies in beet root tissue reveal that differences between Br^- influx and efflux across cell and vacuolar membranes are comparable to the net accumulation (15, 16).

Although uptake of Br^- and Cl^- by barley roots has been observed to vary with the salt cation (6), Cl^- accumulation does not always reflect the amount of cation uptake (4). Differences between the cation effects on anion influx rates and on maximum accumulation levels were not assayed, however, and under the experimental conditions of long uptake periods (hours) at salt concentrations of 10^{-3} eq/liter or greater, either could be determinant.

Accordingly, the work reported here considers the relative effects of a rapidly absorbed cation, K^+, and slowly absorbed cations, Cs^+ (1) and Ca^{++} (11), on Cl^- fluxes and maximum accumulation levels in excised barley roots. Cl^-, like the cations, approaches flux equilibrium with the Cl^- fluxes independent of simultaneous cation exchange flux. The Cl^- accumulation at flux equilibrium and the flux rates are influenced differentially by the presence of the cation, emphasizing the distinction between the rates and accumulation levels.

MATERIALS AND METHODS

The excised roots were from 6 day old etiolated seedlings of barley (*Hordeum vulgare*, var. Trebi). The plants had been grown in the dark in 2×10^{-4} M $CaSO_4$ at pH 5.6 and the roots were excised and rinsed just prior to experimentation as described previously (8). Demineralized distilled water and HNO_3-washed glassware were used throughout all procedures.

In the usual experiment, 4 to 10 g of roots were placed in 4 liters of rapidly aerated salt solution. The proportion of roots to solution did not exceed 1 g/400 ml, which sufficed to prevent a significant change in solution content due to depletion or salt exudation by the roots. Successive root samples were then withdrawn periodically, rinsed 4 times with 50 volumes of water, blotted gently, and weighed. For the influx experiments, roots were first maintained in a nonradioactive salt solution after which they were removed, drained, and transferred to a radioactive solution of the same salt composition. Root samples were then withdrawn periodically. Efflux was de-

termined from the difference between tracer influx and the total ion accumulation. Sampling times usually were at 0, 15, 30, 60, 90, 120 min, every hour from 2 to 6 or 9 hr, and at 24 hr. One-half g root samples from these procedures were dried in tin cups in the presence of excess $Ca(OH)_2$ and assayed for radioactivity. Cl^-, K^+, and Na^+ were extracted from the dried root samples after counting by twice boiling the roots for 15 min in 10 ml of water. The extracts were diluted to 25 ml and analyzed. The absorption solutions were also analyzed, both before and after each experiment. Cl^- content was measured with an Aminco-Cotlove automatic titrator, and K^+ and Na^+ contents were determined by flame photometry. ^{36}Cl was used to measure Cl^- fluxes and ^{134}Cs was used for Cs^+. K^+ was labeled with ^{42}K or ^{86}Rb, the valid use of which has been substantiated (8).

The pH of the absorption solutions was adjusted and maintained at pH 5 to within 0.05 unit over the first 9 hr. Base or acid used for pH control was appropriate to the salt solution and the amounts used did not alter the salt content measurably. Contamination by insertion of electrodes into the experimental solution was avoided by measuring the pH of small aliquots of solution which were discarded. The temperature was usually 25°C, maintained to within ±0.5°C.

Root respiration was determined by measuring the decrease in O_2 content of solutions in which roots were incubating with a Clark O_2 electrode (18) in a closed system. Five electrodes operated simultaneously with a switching arrangement such that the current of each was recorded every minute. O_2 uptake rates were measured over periods of 10 to 30 min.

Ion accumulations and fluxes over a given period are expressed as microequivalents per gram fresh weight of roots. The surface area of a gram of roots is about 100 cm². Rates of ion flux and accumulation, and of O_2 uptake are calculated from linear time course data by the least squares method and are expressed as millimicroequivalents per minute-gram. The rates are determined from five or more measurements at various periods, usually from 5 min to an hour or longer.

RESULTS AND DISCUSSION

Accumulation at Various Salt Concentrations

Initial Cl^- content of the roots in these experiments averages 5 $\mu eq/g$ fresh weight of roots. When placed in KCl or NaCl solutions at pH 5, the roots usually accumulate Cl^- at constant rates for 2 to 3 hr in 10^{-2} eq/liter salt, 6 to 10 hr in 10^{-3} eq/liter, and 20 to 24 hr in 10^{-4} eq/liter salt (Fig. 1). The rates appear to decrease when a finite amount of Cl^- is accumulated irrespective of the external solution concentration. The initial constant rates differ greatly, reflecting the large differences in Cl^- concentration of the solution, as in Table I. This is in contrast to the levels approached within 24 hr, which vary less than 30% in roots maintained in KCl. As a result, the amount of Cl^- accumulated between 6 and 24 hr in these roots varies inversely with the external solution concentration.

The effect of salt concentration on K^+ accumulation is similar as has been shown previously (9), although K^+ initial accumulation rates generally
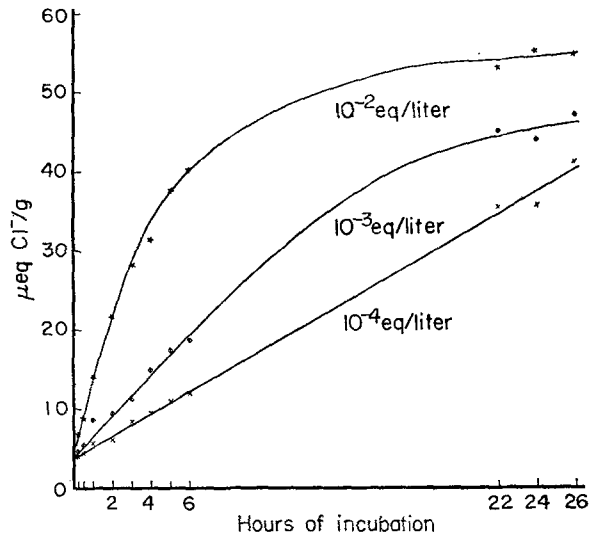
FIGURE 1.   The time course of Cl⁻ accumulation from KCl at pH 5. Temperature = 24°C.

exceed those of Cl⁻. This is reflected by a constant release of H⁺ by the roots over the first 6 hr. Between 6 and 24 hr, Cl⁻ accumulation from KCl exceeds K⁺ accumulation and this is accompanied by an equivalent increase in alkalinity of the external solution.

TABLE I

CHLORIDE AND CATION ACCUMULATION BY ROOTS
IN VARIOUS SALT CONCENTRATIONS

[Salt]	Initial rate	Cl⁻ accumulation			Total Cl⁻ content at 24 hr	Initial rate	Cation accumulation			Total cation content at 24 hr
		0-3 hr	3-6 hr	6-24 hr			0-3 hr	3-6 hr	6-24 hr	
$eq/liter$	$m\mu eq/ min\text{-}g$		$\mu eq/g$			$m\mu eq/ min\text{-}g$		$\mu eq/g$		
KCl						K⁺				
10^{-4}	27	+5.5	+4.4	+21.7	35.3	55	+10.8	+9.0	+27.0	62.8
10^{-3}	48	+8.6	+8.1	+18.5	38.9	90	+17.6	+13.6	+25.4	72.6
10^{-2}	182	+27.2	+16.4	+3.1	50.3	211	+38.6	+18.6	+0.4	73.6
CsCl						Cs⁺				
10^{-4}	28	+5.0	+4.8	+13.3	27.5	58	+9.1	+2.8	+6.8	18.7
10^{-3}	48	+7.9	+5.8	+17.4	35.5	87	+10.6	+3.9	+10.2	24.7
10^{-2}	185	+19.1	+9.1	+29.1	61.7	140	+16.8	+5.8	+17.6	40.2

The roots initially contained 3.7 μeq/g Cl⁻ and 16.0 μeq/g K⁺ in the KCl experiment and 4.4 and 17.7 μeq/g Cl⁻ and K⁺, respectively, in the CsCl experiment. Accumulations were calculated from differences in total content from one period to the next. Initial rates were calculated from linear time course data obtained over periods from 15 min to several hours (>5 points). Losses of K⁺ were less than 2 μeq in CsCl solutions.

Initial rates of Cl⁻ accumulation from CsCl and KCl are closely similar, but the rates decrease sooner in CsCl resulting in lower levels of Cl⁻ accumulation over the first 6 hr (Table I). In general, the initial rates are maintained for only 4 to 6 hr in 10^{-4} eq/liter CsCl, 2 to 3 hr in 10^{-3} eq/liter, and 1 hr or less in 10^{-2} eq/liter CsCl, as illustrated in Fig. 2. Although the initial rates of K⁺ and Cs⁺ accumulation are rather similar at 10^{-4} and 10^{-3} eq/liter, K⁺ accumulation is usually much greater than Cs⁺ accumulation. At 10^{-2} eq/liter, K⁺ accumulation also exceeds Cs⁺ by an amount which cannot be attributed entirely to the relative initial influx rates.
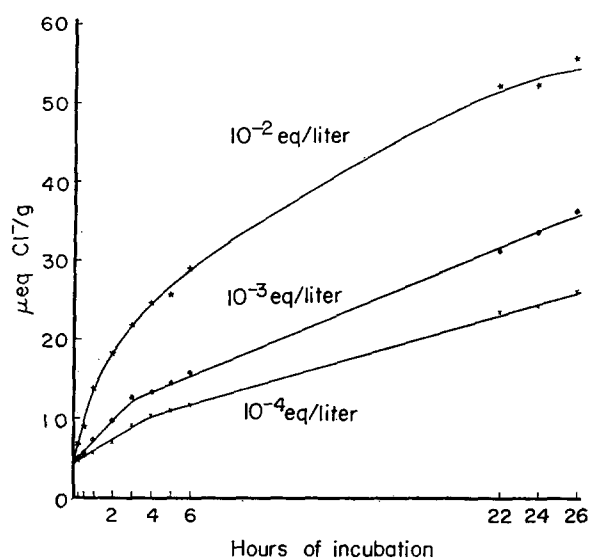
Figure 2. The time course of Cl⁻ accumulation from CsCl at pH 5. Temperature = 24°C.

Influx and Efflux

Although the rate of Cl⁻ accumulation from 10^{-2} eq/liter KCl is constant for only 2 hr (Fig. 1), the rate of Cl⁻ influx, measured by ³⁶Cl after various periods of Cl⁻ accumulation, is relatively constant for at least 24 hr (Table II). Consequently, the decreasing rate of Cl⁻ accumulation from 2 to 24 hr is explained by an increasing rate of Cl⁻ efflux. This is substantiated by measured ³⁶Cl influx and efflux rates which give a net flux of 140 mμeq/min g (184–44), compared to the Cl⁻ accumulation rate of 138 in roots in 10^{-2} eq/liter KCl for 3 hr. No significant efflux occurs over the first 2 hr since rates of accumulation and influx closely agree. Thereafter, efflux increases rapidly until a flux equilibrium is attained. Influx and efflux are apparently equal at 24 hr since no Cl⁻ accumulation could be measured.

Chloride accumulation and influx from CsCl are essentially the same as

from KCl initially, but the rates from 10^{-2} eq/liter CsCl are constant for only 30 min (Table II). The ^{36}Cl influx rates rapidly decrease over the subsequent 90 min to approximately 60% of the initial rate and remain unchanged thereafter. Nevertheless, the decrease in influx rate is not sufficient to account for the slowly diminishing Cl$^-$ accumulation rates. This discrepancy can be attributed only to a slowly increasing efflux rate. Thus, Cl$^-$

TABLE II

CHLORIDE AND CATION FLUXES OF ROOTS
IN KCl AND CsCl SOLUTIONS

Incubation time	Total content	KCl			Total content	CsCl		
		Accumulation rate	Influx rate	Efflux rate (calc.)		Accumulation rate	Influx rate	Efflux rate (calc.)
hr	$\mu eq/g$		$m\mu eq/min$-g		μeq-g		$m\mu eq/min$-g	
			Cl^-				Cl^-	
0	6.3	175	175	0	5.6	181	181	0
1	16.3	175	172	0	14.8	117	139	−22
2	26.9	175	179	0	18.7	68	107	−39
3	36.9	152	181	−29	23.5	59	109	−50
4	42.9	100	182	−82	25.5	49	107	−58
6	53.4	79	174	−95	30.8	37	106	−69
24	60.3	0	178	−178	62.1	11	114	−103
			K^+				Cs^+	
0	20.1	206		0	0	163	163	0
1	31.1	206		0	9.8	163	169	0
2	45.9	206		0	14.6	81	154	−73
3	57.6	206	206	0	18.7	45	154	−109
4	63.4	124	205	−81	20.6	37	170	−133
6	76.8	52	193	−141	24.6	31	123	−92
24	78.6	0	–	$\sim$−200	52.4	4	88	−84

Roots were first in 10^{-2} eq/liter Cl$^-$ solutions for the requisite period and then in ^{36}Cl or ^{134}Cs-labeled salt solutions at the same concentration. Roots in the tracer solutions were sampled every 1 to 5 min for periods from 2 over 10 to 40 min. Influx rates were calculated from linear time course data obtained over these periods (usually >5 points). Accumulation rates were determined from linear time course data (initial periods) or graphically from tangents to the drawn curve. Efflux rates were calculated from the difference between influx and accumulation.

efflux in CsCl exceeds Cl$^-$ efflux in KCl over the first 3 hr. Subsequently Cl$^-$ efflux in CsCl is less than that in KCl, indicating that Cs$^+$ eventually reduces Cl$^-$ efflux as well as influx.

That Cl$^-$ efflux is an ion exchange flux is demonstrated by the negligible efflux of Cl$^-$ in water (Table III) and the independence of the Cl$^-$ and cation flux rates (Tables I, II, and IV; also reference 8). In contrast to Cl$^-$, K$^+$ efflux over the first 3 hr in 10^{-2} eq/liter KCl is negligible since the accumulation rate is generally constant and equal to the influx rate. K$^+$ efflux increases rapidly thereafter, resulting in nearly equal net fluxes (accumulation) of K$^+$

and Cl⁻ over the 3 to 6 hr period, and a greater net flux of Cl⁻ over the 6 to 24 hr period. Thus, K⁺ appears to arrive at a flux equilibrium level sooner than Cl⁻.

Influx of Cs⁺, measured with ¹³⁴Cs, is constant for 4 to 6 hr at 70 to 80% of the K⁺ influx rate (Table II). Onset of Cs⁺ efflux is between 1 and 2 hr, with the accumulation of about 10 μeq/g. This is an hour or two earlier than the onset of K⁺ efflux, with the result that Cs⁺ efflux rates initially exceed those of K⁺. After 3 hr, Cs⁺ efflux rates increase more slowly, and eventually, the rates of both influx and efflux are reduced. At 24 hr, the roots continue to slowly accumulate both Cs⁺ and Cl⁻, indicating that the attainment of flux equilibrium is delayed.

TABLE III

CHLORIDE AND K⁺ CONTENTS OF ROOTS
INCUBATING IN H_2O AFTER 3 HR IN 10⁻²
EQ/LITER SALT AT pH 5

Time	Cl⁻	K⁺
hr	*μeq/g*	
CsCl 3 hr → H_2O		
0	4.2	17.0
3	21.3	16.1
6	20.7	16.1
24	20.9	16.3
KCl 3 hr → H_2O		
0	4.2	17.0
3	30.3	50.2
6	28.4	48.7
24	27.1	

Effects of CsCl and KCl Pretreatment

When roots which have been in 10⁻² eq/liter CsCl for 3 hr are transferred to 10⁻² eq/liter K³⁶Cl, Cl⁻ and K⁺ influx rates are the same as initially in KCl (Table IV). The rate of Cl⁻ influx during the 3 hr in CsCl had decreased 35%. Recovery of the initial rate occurred within the 20 sec of transfer from CsCl to K³⁶Cl. The 3 hr K⁺ accumulation after 3 hr in CsCl is likewise the same as initially in KCl. Much of the accumulated Cs⁺ exchanged for K⁺. However, net K⁺ and Cl⁻ accumulation at 24 hr exceeds accumulation from KCl alone. This suggests that while influx rates are undiminished, efflux is reduced by the 9 to 11 μeq of previously accumulated Cs⁺ remaining in the roots, thereby delaying attainment of flux equilibrium. Greater Cl⁻ accumulation from 6 to 24 hr and continued slow accumulation at 24 hr by roots of the other CsCl treatments suggest that the Cl⁻ level in these roots would ultimately exceed accumulation from KCl alone, also.

Relatively little nontracer Cl⁻ initially in the roots exchanges with ^{36}Cl over 6 to 24 hr (Δ^{36}Cl $-\Delta\Sigma$Cl⁻, Table IV) when roots have been in CsCl (e.g., 53.2 $-$ 50.9 $=$ 2.3) compared to roots continuously in KCl (31.9 $-$

TABLE IV
CHLORIDE AND CATION ACCUMULATION
IN VARIOUS SALT SOLUTIONS

Treatment	Influx rate	Accumulation			Total content at 24 hr
		0–3 hr	3–6 hr	6–24 hr	
	$m\mu eq/min\text{-}g$		$\mu eq/g$		
KCl 3 hr → K^{36}Cl					
Cl⁻	170	+28.6	+15.3	+18.2	66.3
^{36}Cl	176		+23.3	+31.9	55.2
K⁺	204	+36.0	+19.8	+15.6	88.4
CsCl 3 hr → Cs^{36}Cl					
Cl⁻	179	+19.8	+9.1	+26.4	59.5
^{36}Cl	113		+14.8	+30.2	45.0
Cs⁺	152 (at 3 hr)	+19.1	+7.9	+20.8	47.8
K⁺		−1.3	+0.7	−1.3	15.1
CsCl 3 ½ hr → K^{36}Cl					
Cl⁻	173	+24.1	+21.0	+50.9	100.2
^{36}Cl	180		+21.4	+53.2	74.6
Cs⁺	164	+24.8	−13.8	−1.7	9.3
K⁺	214	−0.2	+37.3	+59.0	113.1
KCl 3 hr → Cs^{36}Cl					
Cl⁻	177	+32.0	+8.2	+30.8	75.2
^{36}Cl	168		+18.0	+39.1	57.1
K⁺	214	+38.6	−7.0	−0.9	47.7
Cs⁺	160		+15.2	+23.3	38.5
CaCl₂ 3 hr → Ca^{36}Cl₂					
Cl⁻	177	+13.7	+8.6	+19.0	45.5
^{36}Cl	141		+14.6	+21.8	36.4
K⁺		−1.3	−0.2	−0.2	15.3
KCl 3 hr → Ca^{36}Cl₂					
Cl⁻		+31.5	+11.9	+11.8	59.4
^{36}Cl	168		+16.4	+19.9	36.3
K⁺		+32.2	−8.1	±0.0	41.1

The salt concentrations are 10^{-2} eq/liter at pH 5. The ^{36}Cl influx rates given were measured at 3 hr.

18.2 $=$ 13.7). In fact, exchange efflux of nontracer Cl⁻ from CsCl-treated roots is much less than expected from the ratio of ^{36}Cl to nontracer Cl⁻ in the roots. As a result, the total Cl⁻ level at 24 hr in roots which have incubated in CsCl exceeds the ^{36}Cl uptake by 14.5 to 25.6 μeq. The difference is only 11.1 μeq in roots continuously in KCl. This indicates that Cs⁺ markedly reduces internal equilibration of Cl⁻.

Features of Cl^- accumulation from $CaCl_2$ (Table IV) are similar to those for accumulation from CsCl. The accumulation rates rapidly decrease within the first hour from an initial rate close to that in KCl to a slowly diminishing rate of approximately 15% of the initial rate. This can be attributed likewise to a decrease in influx rate and early onset of efflux. Efflux in $CaCl_2$ appears to be greater initially than in KCl, as suggested by comparison of the ^{36}Cl influx rate (177 to 141 mμeq/min-g) with the total Cl^- increase over the first 3 hr in Table IV. From 6 to 24 hr in $CaCl_2$, as in CsCl, exchange efflux of nontracer Cl^- (21.8 − 19.0 = 2.8) is less than in KCl and less than expected from the ^{36}Cl/nontracer Cl^- in the roots. Thus, internal equilibration and attainment of flux equilibrium appear to be delayed in roots in $CaCl_2$ also.

Equilibration of Accumulated K^+ and Cs^+ with Endogenous K^+

Efflux of K^+ from the roots in CsCl is also grossly out of proportion to the ratio of Cs^+/K^+ content in the roots (Table IV). Cs^+ efflux is greater than expected even with consideration of interaction between the relative cation concentrations and selectivity of the cation rates of uptake. For example, K^+ influx from 10^{-2} M KCl is uninhibited (94% of control) by the presence of 10^{-2} M CsCl. This suggestion that Cs^+ equilibrates internally much more slowly than K^+ was tested by a "mixing" experiment in which equilibration of accumulated Cs^+ and of accumulated K^+ with endogenous K^+ were compared. Roots were in 10^{-2} eq/liter $^{134}CsCl$ or ^{86}Rb-KCl at pH 7 for 5 min. They were then removed, rinsed, and maintained in an atmosphere of 100% relative humidity for various periods, after which they were placed in 10^{-1} eq/liter Na_2SO_4 at pH 7 (1 g roots/25 ml) for 10 min to exchange out accumulated and endogenous cations, presumably from the outermost cells. The incubation solutions were at pH 7 to minimize differences between the amount of ^{86}Rb-K^+ and ^{134}Cs accumulated and exchanged. Both the roots and the solutions were analyzed for tracer and total K^+ content. The premise is that the radioactivities of K^+ in the roots and the K^+ exchanged into the Na^+ solution are the same when complete mixing occurs. The roots accumulated 0.99 μeq/g of ^{86}Rb-K^+ and 0.76 μeq/g of ^{134}Cs. An average of 20 and 16%, respectively, of the root cations exchanged out into the Na_2SO_4 solution.

The rate of mixing of accumulated Cs^+ with endogenous K^+ is much slower than mixing of accumulated K^+ (Fig. 3). K^+ is completely mixed by 6 hr with a 50% mixing time of about 1 hr. By contrast, Cs^+ is little more than half mixed at the end of 6 hr, thereby demonstrating that Cs^+ distributes through the roots much more slowly than K^+. At 24 hr, the ^{86}Rb-K^+ mixing ratio is 103%, essentially the value of complete mixing. However, the ^{134}Cs mixing ratio at 24 hr is 150%, which exceeds the complete mixing value consider-

ably. This suggests that Cs^+ is preferentially held at binding sites, which could account for the slow equilibration rate of Cs^+.

Consideration has been given to the possibility of selective difference between Cs^+ and K^+ exchange with Na^+. Since Cs^+ flux rates are slower than those of K^+, Cs^+ would be expected to exchange out of the roots more slowly than K^+. This would result in an erroneously low $^{134}Cs/K^+$ ratio in the Na^+ exchange solution, which would in turn result in overestimation of the Cs^+ mixing ratio. Thus, Cs^+ mixing is at least as slow as indicated experimentally.
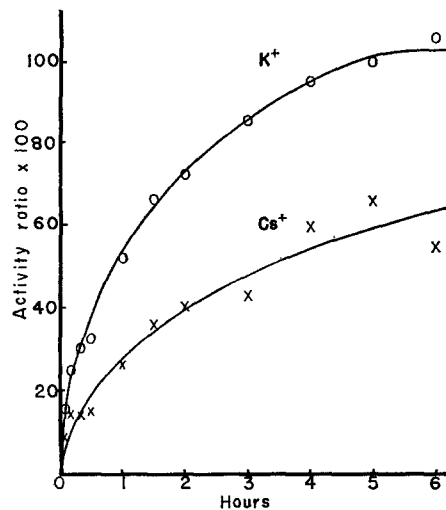

FIGURE 3. The time course of accumulated ^{86}Rb-K^+ and ^{134}Cs mixing with endogenous K^+. Activity ratio (mixing)

$$= \frac{\text{radioactivity}/K^+ \text{ in roots}}{\text{radioactivity}/K^+ \text{ in exchange solution}}.$$

Effects of pH on Accumulation and Fluxes of Chloride and Cations

Since Cl^- and K^+ accumulation involves a process of base and H^+ exchange, pH was studied as a variable for comparisons of ion fluxes and accumulations. (Table V). Initial Cl^- influx and accumulation rates from 10^{-2} eq/liter salt are unaffected (<10%) by differences in H^+ concentration of the external solution from pH 7 to 4, whether from $CsCl$ or KCl. When K^+ is the counterion, accumulation from 6 to 24 hr decreases with the pH increase, resulting in an attenuated but similar pattern in the 24 hr levels. K^+ influx rates are likewise unaffected by the pH differences, but accumulation over the first 6 hr increases with the pH. This appears to be due to earlier onset of K^+ efflux at low pH, since the accumulation rates are constant for longer periods at higher pH. Accumulation from 6 to 24 hr decreases with the pH increase, as if flux equilibrium were attained more rapidly at high pH. The 24 hr K^+ levels vary inversely with the Cl^- levels.

Chloride accumulation from $CsCl$ after 3 hr reflects the pattern of the pH effect on Cs^+ from pH 4 to 6, but not at pH 7. The 24 hr Cl^- level at pH 4 is markedly reduced in $CsCl$ over the level in KCl, although accumulation from

CsCl is as great or greater than from KCl at higher pH. Cs^+ influx rates are not only slower than K^+ rates, but are more inhibited by H^+. Accumulation also decreases with the pH, but to a greater degree than can be attributed to the influx rate differences.

Respiration and Cation-Anion Accumulation Balance

Respiration rates of excised roots incubating in water decrease 20 to 30% over 24 hr with little or no change during the first 3 to 6 hr (Table VI, part I). This is a feature of 6 day old roots. Roots of plants which were maintained

TABLE V

CHLORIDE AND CATION INFLUX RATES AND ACCUMULATION FROM KCl AND CsCl SOLUTIONS AT VARIOUS HYDROGEN ION CONCENTRATIONS

pH	Influx rate	Cl⁻ Accumulation				Influx rate	Cation accumulation			
		0-3 hr	3-6 hr	6-24 hr	24 hr total		0-3 hr	3-6 hr	6-24 hr	24 hr total
	$m\mu eq/min\text{-}g$		$\mu eq/g$			$m\mu eq/min\text{-}g$		$\mu eq/g$		
KCl						K^+				
4	185	+29.6	+14.5	+22.1	+66.2	208	+30.4	+12.0	+17.8	+65.2
5	187	+29.2	+16.2	+16.6	+62.0	206	+35.8	+22.3	+14.7	+72.8
6	176	+27.6	+15.6	+13.3	+56.5	218	+41.6	+26.8	+9.6	+78.0
7	177	+28.3	—+30.9—		+59.2	215	+38.2	—+42.2—		+80.4
CsCl						Cs^+				
4	185	+19.2	+0.7	+5.8	+25.7	75	+10.2	+0.1	+1.0	+11.3
5	192	+20.5	+8.0	+37.0	+65.5	158	+18.4	+3.4	+26.8	+48.6
6	180	+18.4	+11.7	+39.6	+69.7	176	+17.6	+8.0	+28.8	+54.4
7	180	+15.6	+10.7	+36.2	+62.5	163	+18.6	+8.9	+35.8	+63.3

Salt concentrations are 10^{-2} eq/liter. Initial Cl⁻ content averaged 4.1 μeq/g and K^+ content averaged 19.5 μeq/g.

intact in the $CaSO_4$ culture solution until just prior to measurement, took up O_2 at similar rates (within 5%). Respiration rates of roots in KCl generally increase about 20% within the first hour or two of incubation, but after 3 hr, the rates decrease gradually to slightly slower rates at 24 hr than of roots in water (about 90% of the water control). Roots in K_2SO_4 respire at essentially the same rates as roots in water despite nearly the same amount of K^+ accumulation over 24 hr as for roots in KCl. Although K^+ accumulation exceeds the anion accumulation in both salt solutions, the excess of K^+ is much greater in K_2SO_4 than in KCl (8). Thus, the rate of respiration is relatively insensitive to high K^+ or Cl⁻ accumulation, per se, and the magnitude of excess K^+ accumulation over the anion.

Respiration rates of roots in CsCl decrease 30% within the first hour and thereafter the rates decrease slowly over 21 hr to less than 60% of the initial

rate. An excess of Cl^- accumulation occurs continuously over 24 hr. Respiration rates of roots in Cs_2SO_4 decrease more slowly, but finally to a level similar to that of roots in CsCl. Cs^+ accumulation from Cs_2SO_4 exceeds the sum of sulfate and the chloride contaminant, demonstrating that an imbalance of cation and anion accumulation is not responsible for the respiratory decrease.

TABLE VI

RESPIRATION RATES OF ROOTS INCUBATING
IN VARIOUS SALT SOLUTIONS

Treatment	Initial	Rate of oxygen consumption				Ion accumulations at 24 hr	
		3 hr	6 hr	9 hr	24 hr	Anions	Cations
		$m\mu atoms/min\text{-}g$				$\mu eq/g$	
I H_2O	438	462	396	—	372		
Intact plants	438	—	435	—	344		
KCl	530	552	494	476	324	+60.1	+66.8
K_2SO_4	392	468	428	—	372	+28.8	+60.5
CsCl	482 → 360	338	304	290	278	+57.1	+51.8
Cs_2SO_4	476	402	396	316	250	+24.0	+35.2
$CaCl_2$	476	474	460	406	352	+35.1	+2.6 K^+
$CaSO_4$	422	488	420	480	400	+8.9	−3.0 K^+
II H_2O	462	—	439		339		
KCl	440	426	440		288		
3 hr → CsCl			452		244		
K_2SO_4	392	414	434		—		
3 hr → CsCl			350		190		
CsCl	552 → 442	374	306		200		
3 hr → H_2O			448		396		
→ KCl			440		320		
→ K_2SO_4			380		334		

Salt concentrations are 10^{-2} eq/liter at pH 5. The Cs_2SO_4 solution contained a contaminant Cl^- concentration and $CaSO_4$, a contaminant K^+ concentration, of 10^{-5} eq/liter. The anion accumulation values are $\Sigma(\Delta Cl^- + SO_4^{--})$ for sulfate solutions and the cation values are $\Sigma(Cs^+ + \Delta K^+)$.

The two Ca^{++} salts have little, if any, effect on the respiration rates although Cl^- accumulation from $CaCl_2$ must greatly exceed the cations, including contaminant K^+.

When roots were in CsCl for 3 hr (Table VI, part II), during which time the respiration rate dropped 32%, and then were placed in water, KCl, or K_2SO_4, the rates recovered within 3 hr to the rate of roots continuously in water. Incubation in CsCl after an initial 3 hr in K_2SO_4 results in the same degree of respiratory inhibition at 24 hr as for roots in CsCl continuously. Inhibition is somewhat less in the roots incubated in KCl before CsCl, probably because of the greater K^+ accumulation by roots in KCl.

GENERAL DISCUSSION

Levels of Cl^- accumulated by barley roots, like levels of the balancing cation (9), approach a flux equilibrium. The amounts of each accumulated are a function of the relation of the individual influx and efflux rates. Influx and initial accumulation rates in low salt roots are the same, since Cl^- exchanges primarily for a base and the cation for H^+ under these conditions (8). This accounts for the apparent initial irreversibility of Cl^- and cation accumulation (19, 20, 22), which is a feature of departure from flux equilibrium. With continued incubation, the Cl^- and cation accumulation rates ultimately decrease because of increasingly greater efflux of the previously accumulated ions, with the influx and efflux rates approaching equality. Chloride influx from KCl is the same at flux equilibrium as initially, so that the decrease in the accumulation rate is entirely attributable to an increasing efflux rate.

Chloride accumulation approaches the same level at flux equilibrium from low salt concentrations as from high, much as does K^+ accumulation. The amount appears to be 60 to 90 μeq/g in either KCl or CsCl. This is equivalent to 0.07 to 0.10 eq/liter tissue water which results in a large concentration gradient even in ambient salt solutions of 10^{-2} eq/liter. Prevot and Steward in 1936 observed nearly the same maximum accumulation of Br^- (about 60 μeq/g) from 5×10^{-3} eq/liter KBr by low salt barley roots, which was attained by 47 hr (17). In the present studies, about 9 hr were required to reach this level in 10^{-2} eq/liter KCl, but more than 24 hr were required at 10^{-4} eq/liter.

Both the Cl^- initial accumulation and influx rates and the flux equilibrium level are independent of the accompanying cation fluxes. The cation, however, greatly influences the manner of the approach to equilibrium. Cl^- flux equilibrium is attained more rapidly in KCl than in CsCl or $CaCl_2$. K^+ accumulation approaches flux equilibrium sooner than Cl^-, although onset of Cl^- efflux is earlier. K^+ efflux at pH 5 becomes appreciable with the accumulation of about 35 μeq, while Cl^- efflux begins with a 25 to 30 μeq accumulation. Onset of Cl^- efflux in CsCl also precedes the onset of Cs^+ efflux (by about 30 min), but with the accumulation of only 10 μeq. Onset of Cs^+ efflux, in turn, precedes K^+ efflux, also with the accumulation of about 10 μeq.

K^+ rapidly accumulates and distributes throughout the roots, readily mixing with endogenous and previously accumulated K^+. Cl^- also mixes rapidly with internal Cl^- when K^+ is the counterion, with relatively little "lifo" (last-in-first-out) exchange of the accumulating Cl^-. On the other hand, Cs^+ in the roots mixes much more slowly than K^+, although its rate of influx is 70 to 80% of the K^+ influx rate. Accordingly, Cl^- mixing is much slower in CsCl than in KCl, with lifo exchange a prominent feature of the accumulation.

This, of course, is consistent with the early onset of efflux of both Cl⁻ and Cs⁺. Thus, it appears that internal movement and accumulation of Cl⁻ greatly depend on the cation.

Chloride influx is inhibited by Cs⁺. The inhibition begins within 30 to 60 min and is maximal within 2 hr, with the accumulation of about 10 μeq of Cs⁺. The inhibition of influx is only 30 to 40%, and it does not increase further with the continued accumulation of Cs⁺. Release from inhibition is immediate and complete upon transfer to KCl. Cs⁺ also inhibits the rate of O₂ consumption by the roots. In fact, the sequence of the respiratory decrease quantitatively parallels the Cs⁺ effect on the Cl⁻ influx rates. After 3 hr in 10^{-2} eq/liter CsCl, the roots respire at a constant 70% of the root respiration rate in water for the subsequent 21 hr. The respiratory inhibition was also quickly alleviated upon transfer to KCl or K₂SO₄, presumably by exchange of accumulated Cs⁺ for K⁺. Recovery was also effected by transfer to water, which may have resulted from dilution of Cs⁺ by continued slow equilibration with endogenous K⁺ over the 3 hr.

The readily reversible effects of Cs⁺ on Cl⁻ influx and respiration and the slow distribution of Cs⁺ within the roots provide strong evidence that little Cs⁺ passes beyond the outermost cells. Inasmuch as the O₂ consumption determinations are a measure of the respiration from all the cells, a maximum inhibition of 30% would seem to represent inhibition of little more than 30% of the cells. It seems unlikely that a large percentage of the cells are partially inhibited since no further inhibition results with accumulation of an additional 40 μeq of Cs⁺ over the 10 μeq threshold amount. Furthermore, only 14% of the previously accumulated K⁺ exchanged out for Cs⁺, although 80% of the K⁺ exchanged out for Na⁺ in a similar type of experiment over the same time period. Yet K⁺ displaced 63% of the previously accumulated Cs⁺, in spite of indication in the mixing experiment that Cs⁺ is preferentially bound.

The conclusion is implicit in these results that the features of the Cl⁻ influx and its inhibition by Cs⁺ are those of the plasma membranes of the epidermal and cortical cells. That the properties of Br⁻ accumulation by barley roots are principally those of the cortex has been established by Prevot and Steward (17). Evidence that the plasma membrane acts as a premeability barrier to accumulation in the cytoplasm as well as in the vacuole is indicated by values of the electrical potential and measurements of Cl⁻ and cation fluxes across the plasmalemma and tonoplast (3). All the potential difference is across the plasmalemma in oat root cells (5) and ion fluxes across the tonoplast are much faster than across the plasmalemma of *Nitella translucens* (14). Increase in the ability of corn root steles to accumulate Cl⁻ is only attributable to decreased permeability of the plasma membrane which then manifested kinetics similar to those of the epidermal and cortical cells (10). Contraction of the protoplast as a whole, rather than just the vacuole when plants are put into

hypertonic solutions (7), also indicates that the plasmalemma is the permeability barrier.

Although onset of Cl^- efflux is earlier and the efflux is initially greater in CsCl than in KCl, accumulated Cs^+ eventually reduces Cl^- efflux also and its own influx and efflux as well. Inhibition of efflux is not readily reversible as suggested by the greater accumulation of K^+ and Cl^- over 21 hr after 3 hr in CsCl than in KCl alone for 24 hr. The 9 to 11 μeq of Cs^+ remaining in the roots after exchange of 14 μeq for K^+, is apparently adequate to inhibit the Cl^- and cation efflux, but not respiration. It must be sufficiently beyond the outer plasma membranes to inhibit efflux but not influx, and to be inaccessible for exchange with K^+ out into the external solution. Since Cs^+ appears to decrease the permeability of the plasma membrane, it seems likely that it would affect the permeability of the tonoplast and other membranes as well. Thus, reduction of Cl^- and K^+ efflux may arise from decreased permeability of the tonoplast upon transport of Cs^+ to the vacuole. This could explain the relative inaccessibility of a fraction of Cs^+ to exchange with K^+ and the insensitivity of respiration to its presence. In fact, an alternate explanation for the respiratory recovery of CsCl-treated roots after 3 hr in water, is dilution of Cs^+ in the cytoplasm by transport into the vacuoles.

The Cs^+ inhibition of Cl^- and cation fluxes does not appear to be directly linked to the respiratory inhibition. Rather both effects appear to be a result of Cs^+ accumulation. The flux effects of Cs^+ are evidently direct ones on the permeability of the cell membranes. This is further suggested by the effects of Ca^{++} which acts much like Cs^+ in reducing the Cl^- flux rates and the rate of internal equilibration, but does not inhibit respiration. This is consistent with observations that Ca^{++} reduces influx and efflux of Na^+ and K^+ in barley roots (12) and on the effects of Ca^{++} on barley root cell structure (13).

Distinctions between the Cl^- influx rates and accumulation levels are manifest in the comparative effects of various H^+ and salt concentrations of the external solution. Similar distinctions between influx and accumulation are also inherent in the cation absorption. Cation selectivity is characteristic of the exchange fluxes but not of the flux equilibrium level (9). Ratios of K^+/Na^+ in the roots ultimately approach the ratios of the external solution in spite of the selectivities of the influx rates. While the anion and cation accumulation levels partially reflect each other, the Cl^- and cation flux rates are independent. This implies that ion fluxes into the roots are limited by an ion exchange membrane, presumably the plasma membrane, having highly anion-selective constituents or parts and highly cation-selective constituents or parts. The anion and cation selectivities must be independent of each other while the interior contents are more mixed. Such an exchange process is entirely consistent with the intermediate (Michaelis-Menten) type of kinetics which the ion influxes and initial accumulation rates exhibit.

Exchange neither supports nor invalidates the concept of carriers and active

transport. In low salt roots, according to the electrochemical potential defini-
tion of active transport (21), "passive" K^+ influx is balanced by "active" H^+
release and active Cl^- influx is balanced by passive base release. In so far as
the anion and cation influx rates are the same, whether exchanging for base
and H^+ or previously accumulated anions and cations, it would seem that
the influx rates are limited by exchange diffusion through the membrane
while accumulation levels are dependent upon the driving forces, or asym-
metries. This, of course, does not preclude the possibility of dynamic metabolic
control of the membrane characteristics and the driving forces as well.

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