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Adaptation of Barley Roots to Low Oxygen Supply and its Relation to Potassium and Sodium Uptake

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Abstract. The uptake of Na^+ and K^+ by barley seedlings grown on aerated or non-aerated solutions was studied. Plants growing in culture solution took up K^+ with high selectivity whether the solution was aerated or not. Roots of plants grown on aerated $CaSO_4$ and transferred to a solution of KCl and NaCl had a lower preference for K^+ than roots of plants grown on non-aerated $CaSO_4$. Both kinds of low-salt roots were much less able to discriminate between K^+ and Na^+ than high-salt roots grown on a culture solution. The different levels of K^+ selectivity are suggested to be related to H^+ release from the tissue.

Roots of barley seedlings can become adapted to low oxygen in their immediate environment by development of an extensive air path that facilitates gas exchange with the air *via* the intercellular spaces of the shoot. Consequently, the seedlings are able to grow in solutions whether aerated or not (1, 23, 24).

Physiological differences have been observed between plants adapted to different levels of oxygen supply. Van der Heide *et al.* (23) found that the sugar level in barley roots growing on one-fourth Hoagland solution was higher when nitrogen was bubbled through the solution than when air was used. In addition, the rate of K^+ and Cl^- uptake (relative to dry weight) was about 20 % higher in the 'nitrogen' plants.

A distinction needs to be made between kinds of barley root preparations used to study salt uptake, that is, between low- and high-salt roots. Preparations commonly used to study rates of uptake are grown from germination on dilute $CaSO_4$ and are low-salt roots. In this case K^+ is restricted to that originally in the seed and so is at a low level in the plant. Roots of seedlings grown on a culture solution are useful for studies of tracer exchange as there is little or no net salt uptake and the cells are in flux equilibrium with the solution. In general high salt roots show more preference for K^+ than do low salt roots (13, 18). Low-salt roots can reach high-salt status as a result of salt uptake and then also show a higher preference for K^+ *vs.* Na^+, as measured by tracer uptake. Measurement of the level of K^+ and Na^+ in such roots when they reach high salt status need not show much discrimination. These levels will have been determined mainly by the initial uptake which was of low selectivity. For this reason the history of the roots needs to be taken into account when judging the selectivity for K^+ *vs.* Na^+.

The purpose of this paper is to examine the effects that adaptations to aeration, or lack of it, have on K^+ and Na^+ uptake into roots of barley seedlings. The majority of workers in this field have used roots grown on aerated solutions; in my own work I have used roots of plants grown on non-aerated solutions. The comparisons given here provide a basis for relating results for these different kinds of roots.

It is shown that uptake into plants growing in a nutrient or salt solution is strongly preferential for K^+ whether aerated or not. However, low-salt roots grown on aerated $CaSO_4$ solution are much less selective for K^+ *vs.* Na^+ than roots grown on non-aerated $CaSO_4$. The differences in K^+ selectivity are suggested to be related to H^+ release induced by salt uptake.

Materials

Seeds of *Hordeum vulgare* cv. Compana, were soaked in aerated water for about 8 hr then germinated on cheesecloth over stainless steel gauze above an aerated solution of 0.5 mM $CaSO_4$. When the roots were about 1 cm long, and reached into the solution from the gauze, the pots of seedlings were divided into 2 sets; one was aerated and in the other aeration was discontinued. Roots from these 2 sets will be referred to as AER and N-AER, respectively.

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The seedlings were used when 5 days old. At this stage, the AER roots were about 11 cm long and 450 μ diameter; N-AER roots were about 6 cm long and 600 μ in diameter. Microscopic examination showed that this difference in root diameter was due to greater cell width and not to an increase in cell number. The cell length in AER roots was about 20 % greater than in N-AER roots. As a result of differences in cell size, the surface area of cortical cells per gram of roots was greater in AER roots (1400 cm^2/g) than in N-AER roots (1000 cm^2/g) and the volume of intercellular spaces was less. The N-AER roots floated on solution and were intensely white due to internal scattering of light; the AER roots were slightly denser than water and translucent. Some properties of AER and N-AER roots are listed in table I.

The basal (upper) 3 to 4 cm of AER roots and 2 to 3 cm of N-AER roots were not used in experiments. It was found that uptake by various parts of the rest of the root was about the same.

For analysis of salt content, roots were ashed at 450° and dissolved in a mixture containing 0.1 N HNO_3 + 10 % v/v acetic acid. Concentrations of K^+, Na^+, Ca^{2+}, and Mg^{2+} were determined by atomic absorption spectroscopy; Cl^- was measured by coulombic titration.

Table I. *Comparison of Properties of Barley Roots Grown on Aerated or Non-Aerated $CaSO_4$ Solution*

The difference in root diameter is due to differences in cell width, not cell number. Of the amino acids present, the main difference was in alanine level. Total nitrogen levels, and respiration rates were the same for AER and N-AER roots. N-AER roots contained much less Ca^{2+} than AER roots. All quantities per g are relative to 1 g fresh weight of root.

Plants grown on $CaSO_4$	AER	N-AER
Length (cm)	11.0	6.0
Diameter (μ)	450	600
No. of cortical cells/section	250 ± 20	220 ± 20
Surface area of cortical cells (cm^2/g)	1400	1000
Dry weight (mg/g)	50 - 70	50 - 70
K^+ (μeq/g)	18	18
Na^+ "	2.0	2.0
Ca^{2+} "	7.0	3.1
Mg^{2+} "	5.2	3.4
Total N (μmoles/g)[1]	180	190
Soluble N " [2]	31	34
Alanine " [2]	0.5	6.0
Total sugar "	90	130
Glucose "	30	48
Respiration (μmoles O_2/g•hr)	14.0	13.5
Salt respiration (10 mM) (μmoles O_2/g•hr)	3.5	3.5
Potential difference (mV)[3]	—110	—80

[1] Assayed by J. E. Leggett
[2] Amino acid separation by Miss M. E. Engelhaupt.
[3] In collaboration with Prof. N. Higinbotham.

Tracer ^{86}Rb and ^{22}Na uptake was measured by counting γ-radiation. Samples were rinsed for 60 sec in ice-water after removal from the labeled solution, blotted and weighed.

Selectivity of K^+ is defined as

$$S_{K,Na} = \frac{\text{uptake of } K^+}{K^+ \text{ in solution}} \Big/ \frac{\text{uptake of } Na^+}{Na^+ \text{ in solution}}$$

Uptake may be net uptake or tracer uptake. When $S_{K,Na}$ is greater than 1.0, there is a preference for potassium, and when less than 1.0, a preference for sodium.

Results

Uptake by High-Salt Roots. High $S_{K,Na}$ seems to be a general property of high-salt barley roots grown on a culture solution. Fig. 1 shows that uptake by roots of non-aerated plants in the range 10 to 100 mM (K^+ + Na^+) has a uniformly high preference for K^+, with $S_{K,Na}$ about 8.5 (14). Table II compares the content of seedlings grown on aerated and non-aerated culture solutions containing 10 mM (K^+ + Na^+). For both kinds of root, $S_{K,Na}$ was relatively high but there was a slightly higher preference for K^+ in aerated roots, possibly due to the more efficient stirring. Uptake to the shoot was the same in each case, and it appears that aeration, or lack of it, had little effect on salt uptake.

Uptake Into Low Salt Roots. When roots of plants grown in dilute $CaSO_4$ (low-salt roots) are put into salt solution, $S_{K,Na}$ calculated from rates of uptake decreases with increasing concentration (Fig. 1). Thus selectivity of uptake by low-salt roots

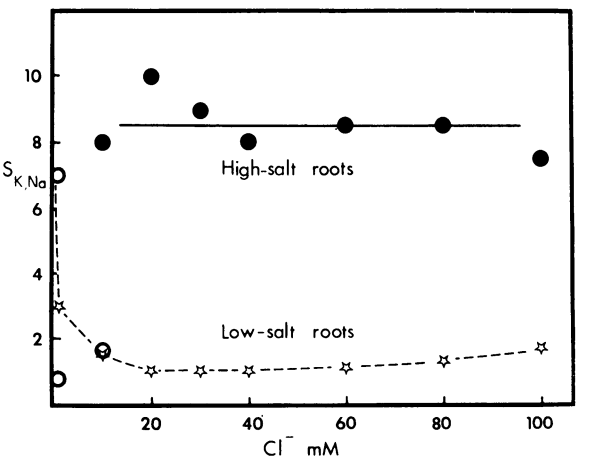

FIG. 1. Comparison of $S_{K,Na}$ in high- and low-salt roots. Uptake to high-salt roots (●) calculated from previously published data (14) was at a uniformly high selectivity. Uptake to low-salt roots, (○, open star) became much less selective at higher concentrations. (Open star), calculated from data of Rains and Epstein (19) and (20). (○), from table III.

Table II. *Content of Seedlings Grown for 6 Days After Germination on a Full Nutrient Containing 2.5 mM K^+ and 7.5 mM Na^+*

Composition of nutrient solution: Ca^{2+} = 6, Mg^{2+} = 4, K^+ = 2.5, Na^+ = 7.5, NH^+_4 = 0.4, NO^-_3 = 16, SO_4^{2-} = 4, $H_2PO_4^-$ = 0.4 (all mN); Fe^{3+} as Fe-EDTA, trace elements.

	Fresh wt	K^+	Na^+	Ca^{2+}	Mg^{2+}	$S_{K,Na}$
	mg/plant	*μeq/g fresh wt*				
Roots						
Aerated	125	90	28	2.2	8.2	9.6
Non-aerated	121	74	36	2.4	7.7	6.2
Shoots						
Aerated	249	134	15	9.5	15 7	27
Non-Aerated	228	139	15.5	6.1	12.4	27

from a 10 mM (K^+ + Na^+) solution can be very much less than that of roots grown from germination in a 10 mM (K^+ + Na^+) solution, and hence at high-salt status (16, 18).

In general $S_{K,Na}$ was higher for N-AER than for AER roots, as seen in table III and Fig. 2.

Table III. *Comparison of Selectivity of Uptake of K^+ and Na^+ by AER and N-AER Roots From Solutions of Different Concentrations*

Uptake was measured over the first 4 hr in salt solution. N^+ and Na^+ concentrations were equal and pH was maintained at 5.3.

Solution	Uptake of (K^+ + Na^+)		$S_{K,Na}$	
	AER	N-AER	AER	N-AER
Cl^-	*μeq/g*			
10 mM	64	45	0.4	1.6
1 mM	31	23.5	0.3	0.75
0.2 mM	22	24	1.75	7.0
SO_4^{2-}				
10 mN	35.5	29	0.07	0.70

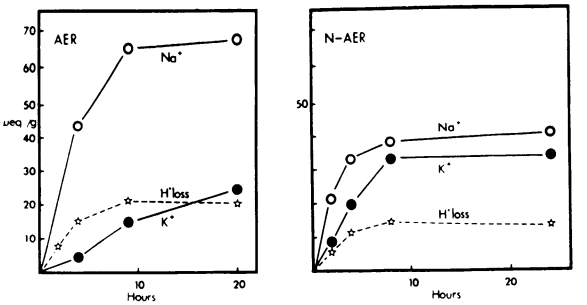

FIG. 2. Time course of net K^+ and Na^+ uptake and H^+ loss from roots in [2.5 mM KCl + 7.5 mM NaCl + 0.5 mM $CaSO_4$] at 23°. $S_{K,Na}$ in AER roots during the first 4 hr (0.3) was much less than that in N-AER roots (18). H^+ release was less rapid from N-AER roots than AER roots and stopped after about 8 hr.

Table III shows that this difference between N-AER and AER roots was found at concentrations where Mechanism I should be dominant (0.2 mM Cl^-) and also when Mechanism II was well developed (10 mM Cl^-). Other experiments have shown that this effect would be found up to at least 50 mM Cl^-. Table III also shows that the difference occurs in 10 mN SO_4^{2-} solutions though $S_{K,Na}$ was lower than in a similar Cl^- solution. The pH of the solution was maintained at 5.3 ± 0.2 by titration with a mixture of KOH and NaOH of the same K^+ and Na^+ concentrations as the uptake solutions.

Uptakes determined in a number of experiments are combined in table IV. The solution contained the usual level of 2.5 mM KCl + 7.5 mM NaCl + 0.5 mM $CaSO_4$. As in table II, these results show that more Cl^- and more cation (K^+ and Na^+) were taken up by AER than by N-AER roots. However, the rates of uptake into AER and N-AER roots expressed on the basis of cell surface are the same. It appears that rates of transport across the cell surface in each kind of root are the same, and that

Table IV. *Uptake Into AER and N-AER Roots on Basis of Root Fresh Weight and Surface Area of Cells*

The greater uptake into AER roots appears to be due to the larger surface area of cells in these roots.

	K^+		Na^+		Cl^-		(K^+ + Na^+)		$S_{K,Na}$
AER roots (7)					*μeq/g*				
4 hr	6	±2	49	±2	40	±1.5	55	±3	0.37
7 hr	11	±1	67	±3	62	±2	78	±3	0.50
N-AER roots (5)									
4 hr	18.5	±1	20.5	±3	26.5	±0.5	39	±3	2.7
7 hr	29	±4	27	±4	44	±3	56	±5	3.2
							μeq/1000 cm² cell surface		
AER roots									
4 hr	4.3		35		28.5		39		
7 hr	8		48		44		56		
N-AER roots									
4 hr	18.5		20.5		26.5		39		
7 hr	29		27		44		56		

larger uptake to AER roots is simply due to the smaller cells in these roots. It is suggested therefore that the difference in $S_{K,Na}$ between the 2 kinds of roots is not due to an effect on rate of total salt transport across the cell membranes, but to a separate action on K^+ and Na^+ transport. As high-salt roots show high $S_{K,Na}$ whether grown on aerated solutions or not, the difference between N-AER and AER roots can be thought of conveniently as a greater reduction of $S_{K,Na}$ in AER roots than in N-AER roots.

H^+ Release and Selectivity Changes. Fig. 2 showed there was a rapid loss of H^+ when low-salt roots were put into 10 mM salt solution. This H^+ loss continued at a nearly constant rate for about 4 hr but after 6 hr in solution the rate of loss decreased. This change in rate of H^+ release takes place at about the same time as an increase in $S_{K,Na}$. For example in 1 experiment with AER roots uptakes of K^+ and Na^+ were 6.9 and 23 μeq/g between 2 and 4 hr, and 5.9 and 6.5 μeq/g between 4 and 8 hr, giving $S_{K,Na}$ of 0.9 and 2.7 respectively. Similar changes in $S_{K,Na}$ were reported previously for non-aerated roots (16, 18).

Tracer ^{86}Rb Uptake. The initial rates of ^{86}Rb uptake by AER and N-AER roots were very nearly the same and about 8.0 μeq per g per hr (Fig. 3). About 20 min after the start of uptake there was a reduction in rate of uptake, which was more marked for AER than for N-AER roots. Between 20 min and 1 hr the rates of uptake were 3.4 μeq per g per hr into AER roots and 6.7 μeq per g per hr into N-AER roots. Consequently over longer periods there was greater ^{86}Rb uptake into N-AER roots.

The shoulder at 20 to 30 min is thought to represent a change in K^+ fluxes and not to be the result of isotopic equilibration of the cytoplasm. The content of K^+ in the cytoplasm of these roots cannot be estimated with much certainty, but tracer efflux measurements show it is not more than 1 μeq/g of root. The values of fluxes estimated here give a maximum time for half-exchange of tracer in the cytoplasm of about 5 min and the real value is likely to be very much less. The time for half-exchange of the free space is about 2 min (15). Within 10 min, then, the cytoplasm specific activity will have risen sufficiently for tracer uptake to be no longer $\phi_{oc} \cdot s_o$, but

$$\phi_{oc} \cdot s_o - \phi_{co} \cdot s_c, \text{ or,}$$

$$\frac{\phi_{oc} \cdot \phi_{cv} \cdot s_o - \phi_{co} \cdot \phi_{vc} \cdot s_v}{\phi_{cs} + \phi_{cv}}$$

where the subscripts o, c, and v show direction of fluxes (ϕ) or location of specific activity (s) [*cf.* Cram (2)].

This view is supported by comparison of uptake of ^{86}Rb into AER roots (a) put from $CaSO_4$ to labeled salt solution and (b) put from $CaSO_4$ to unlabeled salt solution for 1 hr, then transferred to labeled solution. In the period 0 to 30 min uptake into (a) was 3.3 μeq/g and into (b) 1.9 μeq/g. From 30 to 60 min, uptake into (a) was 1.8 and into (b) 1.9 μeq/g.

Tracer ^{22}Na Uptake by Low-salt Roots. Net uptake of sodium by low-salt roots is very nearly the same as tracer uptake, for the roots initially

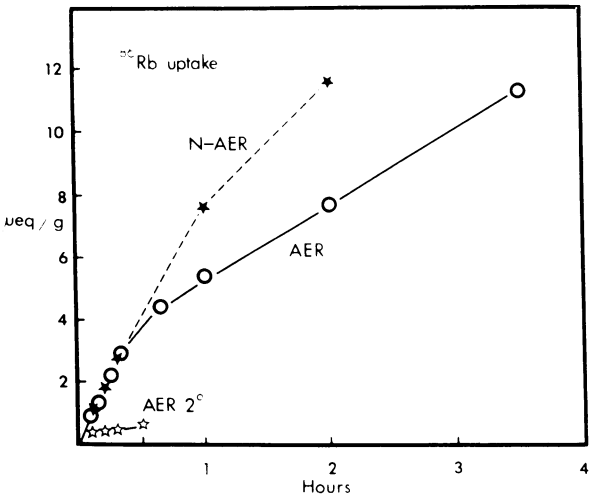

FIG. 3. Uptake of ^{86}Rb by AER (○) and N-AER (★) roots from 2.5 mM KCl + 7.5 mM NaCl + 0.5 mM $CaSO_4$. The initial uptake to both sets of roots at 23° was the same (8.0 μeq/g•hr). The free space uptake at 2° (open star) was 0.3 μeq/g.

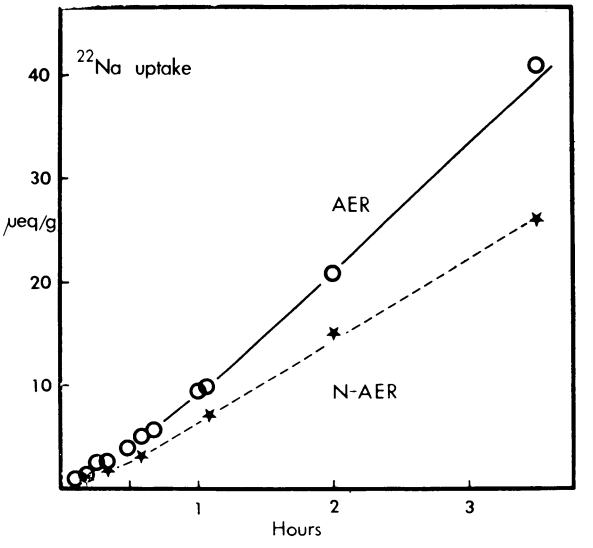

FIG. 4. Uptake of ^{22}Na by AER (○) and N-AER (★) roots transferred from $CaSO_4$ to (2.5 mM KCl + 7.5 mM NaCl + 0.5 mM $CaSO_4$) 4 hr after cutting. The initial rates of uptake in this example were more nearly alike than expected from net uptake differences shown in table III. The initial rate of uptake was lower than the uptake after 30 min. Free space content after rinsing, estimated from uptake at 0°, was 0.6 μeq/g for AER roots.

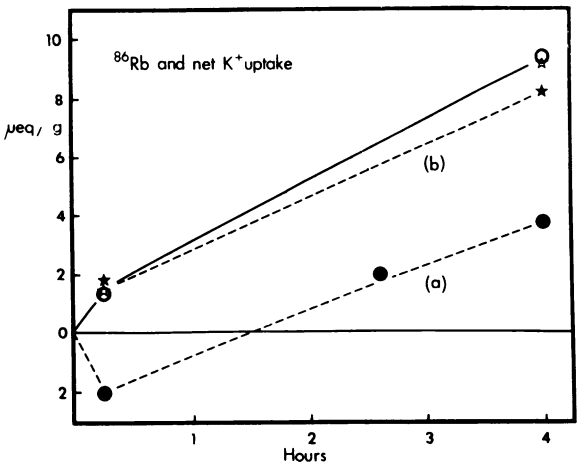

FIG. 5. Comparison of tracer (open symbols) and net K^+ uptake (closed symbols) to AER roots which had been kept in 0.5 mM $CaSO_4$ (a) for 15 min ○, ●, (b) for 4 hr (open star), ★ after cutting. Freshly cut roots show initial loss of K^+ followed by uptake at the same rate as the tracer. Tracer uptake was the same for both sets of roots and in good agreement with that in Fig. 3. Sodium uptake was (a) 48 μeq/g; (b) 42.5 μeq/g, and chloride uptake (a) 38 8 μeq/g, (b) 38.9 μeq/g. The pH was maintained at 53.3.

contain only 2 μeq/g of sodium. Initial rates of uptake of sodium into AER roots are greater than into N-AER roots, as would be expected from the results in table III (Fig. 4). The rate of uptake into the roots increased by about 1.0 to 1.5 μeq per g per hr at about the same time as there was a decrease in ^{86}Rb uptake (Fig. 4).

Pre-treatment of Excised Roots and K^+ Uptake. Comparisons of K^+ and Na^+ uptake were complicated by an effect of pre-treatment, particularly when using AER roots. Roots kept in 0.5 mM $CaSO_4$ for 2 to 4 hr after cutting took up more K^+ than either roots of whole plants or excised roots used after a short rinse in $CaSO_4$ solution. For example uptakes of K^+, Na^+ and Cl^- in 4 hr from the standard solution by roots of whole plants were 4.7, 48.5, and 42 μeq/g. Uptakes by roots kept in 0.5 mM $CaSO_4$ for 4 hr after cutting were 10.6, 41, and 43 μeq/g respectively. Total salt uptake was the same but different proportions of K^+ and Na^+ were accumulated. Fig. 5 shows that the difference in net K^+ uptake was largely due to efflux of K^+ from the roots. Tracer uptake was the same in both cases.

This K^+ efflux appeared to be from all the cells of the cortex and not from the cut end of the stele. Measurements were made of K^+ lost from the cut ends and K^+ uptake over the whole root surface. Using freshly cut roots in a solution of 0.1 mM KCl + 10.0 mM NaCl + 0.5 mM $CaSO_4$ there was a loss of 0.4 μeq/g from the cut end and an uptake of 1.1 μeq/g•hr. When roots were kept for 4 hr in $CaSO_4$ after cutting, loss from the cut end was the same but uptake along the root was 4.0 μeq per g per hr.

Table V. *Effect of pH and Ca^{2+} on Uptake of Na^+, K^+, and ^{86}Rb Over a Period of 4 Hr*

The salt solution contained 2 5 mM KCl + 7.5 mM NaCl + 0.5 mM $CaSO_4$ (except when Ca^{2+} was absent). Roots were kept in aerated 0.5 mM $CaSO_4$ after cutting.

pH	Root	Na^+	Uptake K^+	^{86}Rb
pH varied			*μeq per g per 4 hr*	
4.3	AER	46	5.8 ± 0.3	10.4 ± 0.4
5.5		41	10 6 ± 1.0	9.6 ± 0.8
4.3	N-AER	34	9.4 ± 1.0	16.0 ± 0.4
5.5		34	16.0 ± 0.6	15.2 ± 0.4
pH 5.5 Ca^{2+} varied				
Check	AER	52	8.4	
No Ca^{2+}		52	0.8	
Check	N-AER	29	14.0	
No Ca^{2+}		28	13.0	

Effect of pH and Ca^{2+} Level on K^+ Uptake. Measurements of uptake over the first 4 hr in salt solution are convenient for study of the effect of H^+ and Ca^{2+} on K^+ uptake. Roots rinsed in $CaSO_4$ for 4 hr were used.

At low pH, uptake into both AER and N-AER roots was less selective than at high pH. Table V shows that the lower K^+ uptake at low pH was due to greater K^+ efflux. Tracer influx was not affected by pH. Absence of Ca^{2+} from the solution severely reduced K^+ uptake into AER roots, but uptake into N-AER roots was the same as in the control. The actions of pH, low Ca^{2+}, and pretreatment were to some extent additive. Using freshly cut roots in a solution without Ca^{2+} and at pH 4.6, there was a loss of 13.5 μeq/g K^+ from the tissue (Fig. 6).

Total salt uptake into both N-AER and AER roots was about 10 % less at pH 4.3 than at pH 5.5. Lack of Ca^{2+} did not affect uptake of Cl^- into N-AER roots, but reduced Cl^- uptake into AER roots by about 20 %.

Relation to Other Published Data on K^+ and Na^+ Uptake. Table VI compares results for K^+ and Na^+ uptake from several authors. The differences in $S_{K,Na}$ within the same variety can be explained adequately by the differences described in this paper between roots grown on aerated and non-aerated $CaSO_4$ solution, or by differences in the method used to measure uptake. Uptake over the first few hr is less selective than that over 24 hr (Fig. 2) and $S_{K,Na}$ measured by tracer uptake can be larger than that measured by net uptake, particularly if there is efflux of K^+.

In general, differences in K^+ and Na^+ uptake between varieties of barley do not seem to be as large as the changes in selectivity due to pretreatment or method of measuring uptake. Greenway (5) has also found that selectivity of uptake by a number of varieties was very nearly the same despite differences in salt tolerance.

Table VI. *Comparison of Published Results of K^+ and Na^+ Uptake From a Solution of 5 mM KCl + 5 mM NaCl + 0.5 mM $CaSO_4$*

The pH was between 5 and 5.7. Temp 25° except Rains and Epstein's data at 30°.

Author	Ref	Barley variety	$S_{K,Na}$	Type of measurement
		Grown on aerated $CaSO_4$		
Jackson and Stieff	8	Trebi	0.2	Net uptake in 3 hr
" "	8	Compana	0.5	Initial net uptake
This paper		Compana	0.4	Net uptake in 4 hr
Hiatt	[1]	Compana	1.1	Net uptake in 24 hr
Rains and Epstein	18	Arivat	1.4	Initial tracer uptake
		Grown on non-aerated $CaSO_4$		
Pitman *et al.*	16	Cape	1.3	Tracer uptake in 2 hr
Pitman (unpublished)		Bolivia	1.1	Tracer uptake in 3 hr
" "		Bolivia	2.2	Net uptake in 24 hr
This paper		Compana	1.6	Net uptake in 4 hr

[1] Personal communication.

Discussion

Growing barley seedlings on $CaSO_4$ solution with aeration produced roots that differed in morphology and physiology from roots grown without aeration. Roots grown without aeration had wider cells, larger air spaces and were shorter than those grown with aeration. There were also differences in sugar level and relative abundance of the different amino acids (table I). The higher level of alanine in non-aerated roots has a parallel in the water fern *Marsilea,* for Gaudet (4) found that the water form of this fern contained very much more alanine (and ammonia and glutamine) than the land form.

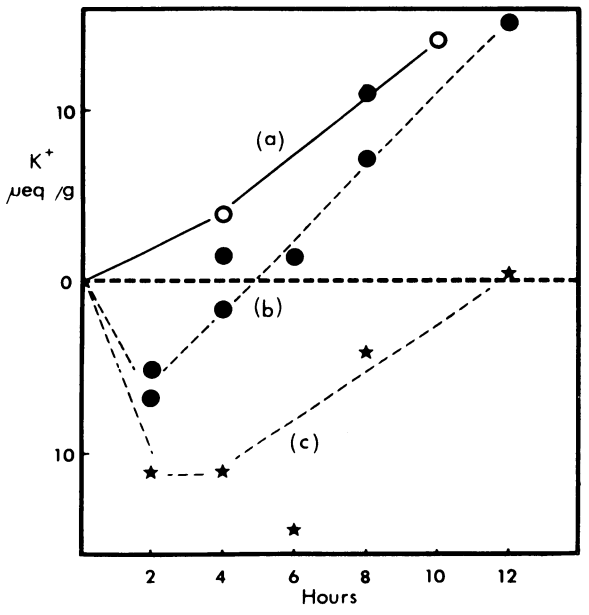

FIG. 6. Changes in K^+ level in AER roots after transfer to 2.5 mM KCl + 7.5 mM NaCl. (a) Solution also containing 0.5 mM $CaSO_4$ and pH adjusted to 5.3, roots in $CaSO_4$ for 30 min after cutting. (b) Solution as (a) but pH not adjusted and roots used within 15 min of cutting. The pH fell to 4.6. (c) No $CaSO_4$ added to the solution, otherwise as (b). Note the initial loss of K^+ and recovery as in Fig. 5.

When these low-salt roots were put into salt solutions containing K^+ and Na^+ they showed a large difference in selectivity for K^+. Roots grown without aeration (N-AER) had much larger $S_{K,Na}$ than roots grown with aeration (AER) (Fig. 2). These differences were found for a wide range of total (K^+ + Na^+) concentrations. Differences between the roots were also found in total salt uptake, but the larger uptake by roots grown with aeration can be explained by the larger surface area of cells per gram of those roots compared with N-AER roots (table IV). The surface area could not account for the difference in $S_{K,Na}$ in the 2 kinds of root.

Tracer measurements showed that there was a change in Na^+ and K^+ influxes into both AER and N-AER roots about 20 to 30 min after putting the roots into a 10 mM salt solution. The difference in $S_{K,Na}$ seen in long term uptake into these roots (Fig. 2) was partly due to larger Na^+ and smaller K^+ influxes into AER roots, but also to a larger K^+ efflux from AER than from N-AER roots. The development of K^+ efflux was also responsible for the low $S_{K,Na}$ for roots in solutions of low pH (*cf.* table V).

Despite the differences in uptake into AER and N-AER roots, both root preparations behaved as low-salt roots, having a large net uptake, and showing less ability to distinguish between K^+ and Na^+ than high-salt roots. Fig. 1 and table II showed that $S_{K,Na}$ for low salt roots was much less than for roots grown in a culture solution. Similar differences between roots of low- and high-salt status have also been found for tracer influx (18).

Comparison of $S_{K,Na}$ and H^+ release shows that low $S_{K,Na}$ occurs when H^+ release is high. Thus

Fig. 2 showed that rate of H^+ release decreased during salt accumulation by AER roots; at the same time $S_{K,Na}$ increased. Again $S_{K,Na}$ was much lower and H^+ loss more rapid from AER than N-AER roots. As another example table III showed that $S_{K,Na}$ in 0.2 mM solution was higher than in 10 mM: H^+ release (not reported) was much less from roots in the low concentration. In 10 mN sulphate solution $S_{K,Na}$ was low. H^+ release into SO_4^{2-} was 8 μeq/g·hr compared with 4 μeq/g·hr into Cl^- solution, possibly due to organic acid formation by roots in the SO_4^{2-} solution (6).

Hydrogen ions released from the cell membrane must diffuse from the site of formation to the external solution. Due to the diffusion resistance, high rates of H^+ release should lead to large local concentrations of H^+ ions. As the cell wall has the properties of a Donnan system of negative charges with pK_a of about 2.8 (15), high H^+ concentrations would reduce local density of fixed changes and so reduce Ca^{2+} concentrations at the cell wall/membrane interface. Both H^+ and Ca^{2+} ions have effects on membrane permeability and/or ion transport.

Low pH and/or low Ca^{2+} lead to increased permeability of the cell membranes. This effect is shown by the increased K^+ efflux at pH 4.3 compared with pH 5.5 (table V) and the K^+ loss from roots in solutions lacking Ca^{2+} (Fig. 6). There are other samples of this action in the literature (*e.g.* 3, 9, 12, 22) and the interaction of Ca^{2+} and H^+ to increase membrane permeability has been discussed by Marschner and Mengel (10, 11).

Active uptake of salt does not seem to be affected by low pH to the same extent as cell permeability. In table V uptake of tracer was the same at pH 4.3 and 5.5, and total salt uptake was only 10 % lower at pH 4.3 than at 5.5. Jackson and Edwards (7) also found that variation in pH in the range 4 to 7 had little effect on K^+ and Cl^- influxes from KCl solutions. Similarly Rains *et al.* (21) studying uptake of K^+ at low concentrations (Mechanism I uptake) found that ^{86}Rb influx at pH 4.1 was only 10 % lower than at pH 5.7.

Welch and Epstein (25) have shown recently that Mechanism I is responsible for almost all the K^+ uptake from solutions containing large proportions of Na^+ (such as the ones used in these experiments). They found that K^+ uptake from 0.1 mM KCl and 10 mM NaCl was very nearly the same as that from 0.1 mM KCl and from 5.0 mM KCl + 10.0 mM NaCl. This Mechanism I process is the same, I think, as the active K^+ influx shown to be present in high salt roots (17), and located at the plasmalemma. Mechanism I appears to have a value of about 4 to 6 μeq/g·hr at 25° while total salt uptake from a 10 mM Cl^- solution is about 10 to 12 μeq per g per hr.

To account for changes in selectivity as low salt roots accumulated salt, I suggested (18) that changes in selectivity reflected changes in cytoplasmic K^+ and Na^+. The combination of active K^+ uptake (Mechanism I) and effect of H^+ ions on cell permeability shows one way that this change in selectivity could be induced.

In high-salt roots a high value of K^+/Na^+ in the cytoplasm can be maintained by the Mechanism I influx of K^+ and possibly by an Na^+ active efflux too (17). The effectiveness of this system depends on the "leak" of Na^+ into the cell past the pumps; if the cell permeability to Na^+ is low then K^+/Na^+ will be high in the cytoplasm. Increasing cell permeability by the action of H^+ ions would increase Na^+ influx and so reduce the ratio of K^+ to Na^+ in the cytoplasm. Selectivity would be further reduced if H^+ loss from the cell is an active secretion which takes place instead of Na^+ efflux in the low-salt condition. Substantiation of this hypothesis requires measurements of permeability of the cell and of Na^+ influx at different stages of uptake. It is doubtful how much reliance could be placed on these kinds of measurements with present techniques. Nonetheless, this hypothesis allows behavior of low- and high-salt roots to be explained on the same model, and has the experimental support of the relationship between H^+ and $S_{K,Na}$ described above.

This comparison of uptake into roots grown on aerated and non-aerated solutions has the advantage that the plant material is genetically the same though modified by environment. Apart from the usefulness of this comparison for salt uptake study, it is a good example of the way plant roots may become adapted morphologically and physiologically to local differences in environment. Plants growing in soil can experience fluctuations in their environment. As synthesis (or activation) of enzymes is a relatively rapid process, adaptational responses of cell metabolism could occur within 12 hr of an environmental change. A study of adaptations of cell metabolism to, for example, low water availability, high salt levels, or flooding should be a necessary part of the study of plant nutrition.

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