Ion Uptake by Soybean Root Tissue Depleted of Calcium by Ethylenediaminetetraacetic Acid [1, 2]

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Calcium has a fundamental role in the cellular processes of ion absorption and retention. The ion is generally credited with having beneficial effects on the semipermeability of membranes and structure of protoplasm (9, 12). The electron microscope shows Ca deficiency to result in disorganization of membrane structure (19). In addition, the presence of calcium in solution was found by Viets (25) to increase the uptake of other ions, a phenomenon widely confirmed, but with variations in extent and conditions of response.

Calcium is important in cation selectivity. The K/Na (or Rb/Na) absorption ratio increases in the presence of calcium (14, 26), and the inhibitory effect of Na on K uptake is practically eliminated (7). The importance of calcium is sufficiently great in salt uptake studies that the root-to-solution ratio can affect K/Na absorption due to variable dissociation of calcium from the root tissue (15). In the presence of adequate calcium there is no initial exchange adsorption of the rubidium ion (8).

Epstein (7, 8) notes that Ca in the solution around root tissue represents the normal physiological condition, and the ion is essential for the integrity of the selective ion transport mechanism. The selectivity is thought to reside in specific ion carriers. Jacobson et al. (13), on the other hand, conclude that the stimulation of K absorption by Ca results from blocking an interfering cation such as H or Li. Waisel (26) maintains that Ca increases the diffusion of K across the outer cell membrane, which he considers to be the rate-limiting step of metabolic accumulation. Tanada (23) has recently reiterated his suggestion that a ribonucleoprotein complex containing free -SH groups is involved in the Ca activation of Rb uptake by mung bean root tips.

In an initial investigation of ribonucleoprotein as a carrier moiety, an EDTA-initiated degradation of RNA in soybean roots resulted in impaired respiration, ion accumulation, and ion retention (10). This result was attributed to removal of Ca and Mg by EDTA.[3] However, treatment with EDTA in the cold, which would limit degradative metabolism, increased Rb^{86} uptake, a result similar to that reported by Tanada for ultraviolet treatment (20), ribonuclease treatment (21), sulfhydryl inhibition (22), and HCl or NaCl treatment (20). Rb^{86} uptake may be increased and $P^{32}O_4$ uptake decreased by EDTA, citric acid, or alkaline phosphate pretreatment (20).

We have now explored in some detail the effect of a short-term pretreatment with EDTA on ion uptake by soybean root tissue. Pretreatment removes much of the root Ca, and increases the subsequent metabolic uptake of Rb^{86}-labeled K while decreasing that of phosphate. These effects are reversed by addition of Ca. Calcium removal appears to affect accumulation of the ions into the cytoplasm.

Materials and Methods

Soybean seeds (*Glycine max* (L.) Merr., var. Hawkeye) were germinated in the dark at 29° in paper towel scrolls saturated with 10^{-4} M $CaCl_2$. Sections of root tissue excised between 0.5 to 1.5 cm from the tip of the primary roots of three-day-old seedlings were used in all experiments except where noted. In the ribonuclease and ultraviolet light experiments the 0.0 to 1.0 cm tips were used. Serial segments were used as indicated in the tables.

The root sections were cut into ice cold deionized water, rinsed, and blotted. About 100 sections were placed in a 50-ml Erlenmeyer flask containing 10 ml of a pretreatment solution consisting of 1 % sucrose and either 2×10^{-3} M KCl or 10^{-3} M EDTA adjusted to pH 6 with KOH; the KCl solution provides a control treatment with similar K concentration. The flasks were placed on a unidirectional shaker at room temperature (usually about 23°) for 30 to 40 minutes. In one set of experiments pretreatment was at ice temperatures.

At the end of this pretreatment period the solution was decanted from the roots. The sections were rinsed from the flask with water, blotted, and divided into 4 lots containing about 25 sections each. Each lot was weighed and placed in a 50-ml Erlenmeyer flask containing 10 ml of absorption solution consisting of 1 % sucrose and 10^{-3} M salt solution. Potassium absorption was followed from KCl labeled with Rb^{86} (16), calcium from $Ca^{45}Cl_2$, chloride from KCl^{36} and phosphate from $KH_2P^{32}O_4$ adjusted to pH 6. The flasks were replaced on the shaker at room temperature for a 2-hour absorption period. The roots were rinsed from the flasks with water and blotted as before. Sections from half of the flasks were placed in planchets, dried over a hot plate, and the radioac-

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[3] Abbreviations: K-EDTA, potassium salt of ethylenediaminetetraacetic acid. DNP, 2,4-dinitrophenol.

Table I

Effect of Temperature and Duration of K-EDTA Pretreatment on Subsequent Potassium Absorption

Pretreatment	conditions	Potassium absorbed		
		Total	Accumulated	Exchangeable
min	temp		μmoles/g fr wt	
0	...	4.15	2.44	1.71
30	25°	8.92	5.32	3.60
	3°	6.70	4.05	2.65
120	25°	8.48	2.04	6.44
	3°	8.88	5.07	3.81

tivity measured with a gas-flow counter. Total absorption was calculated from these data.

The root sections from each remaining flask were placed in a 50-ml flask containing 10 ml of exchange solution, consisting of ice cold 1 % sucrose and either 10^{-2} M $CaCl_2$ for exchanging K, Ca, and Cl, or 10^{-2} M potassium phosphate buffer at pH 6 for exchanging phosphate. The exchange period was 30 minutes except for obtaining data of figure 3, where various time periods were used. The roots were rinsed from the flasks and the radioactivity measured as before. The accumulated ion was calculated from these data. Exchangeable ion was taken to be the total minus the accumulated ion.

In some experiments a second pretreatment period of 30 minutes was used after the first pretreatment period. When a second pretreatment period was used, the root sections were rinsed, blotted, divided into lots of 25 and weighed before being put into a 50-ml Erlenmeyer flask containing 1 % sucrose plus indicated additives.

In the experiment designed to study Ca^{45} loss from root sections 2-day-old soybean seedlings were transferred from the scrolls to a wire screen fitted over a Pyrex utility dish containing 1500 ml of 10^{-4} M $CaCl_2$ labeled with Ca^{45}. The dish was kept in darkness on a shaker for 20 hours. The radioactive solution was changed once during this period. Next, the radioactive solution was replaced with nonradioactive 10^{-4} M $CaCl_2$ to remove readily exchangeable ions. Four hours later the root segments were excised, divided into lots of 25, weighed, and placed in 50-ml flasks containing 10 ml of 1 % sucrose and either 2×10^{-3} M KCl or 10^{-3} M EDTA for various periods of time. The sections were rinsed, blotted, and the radioactivity determined as before.

In the determination of cations in the root by emission spectography, 4 cm of the root tip were used. Fifteen grams were placed in 1.5 liters of 10^{-3} M K-EDTA plus 1 % sucrose and in 2×10^{-3} M KCl plus 1 % sucrose. At intervals samples were removed, weighed, ashed, and taken up in spectographic buffer containing 7.5 % HCl, 2.5 % HNO_3, 5 % $LiNO_3$ and 0.12 % $CdCl_2$ as internal standard. Ion content was determined by Dr. M. S. Wang on a Hilger emission spectrograph.

The determination of relative activity of root sections in respiration and Rb^{86} accumulation was made in a Warburg respirometer at 30°. Triplicate determinations were made with 25 sections in 2.5 ml of 10^{-3} M potassium phosphate (pH 6.0) labeled with Rb^{86}. Ion accumulation lasted 90 minutes, followed by 30 minute exchange as above. Protein determinations were made by the method of Lowry et al. (17) on 10 % trichloroacetic acid precipitates of aqueous homogenates of 100 comparable sections. Homogenization was in a ground glass conical homogenizer with a power-driven pestle.

Anaerobic conditions were obtained by bubbling commercial nitrogen gas into the solutions for 20 minutes prior to the addition of the root sections as well as during the treatment period.

Bovine pancreatic ribonuclease (Sigma Chemical Company) was used at the concentration of 1 mg/ml

Table II

Effect of K-EDTA Pretreatment on Potassium Absorption along the Soybean Root Axis

Pretreatment	Root section	Potassium absorbed		
		Total	Accumulated	Exchangeable
	cm from tip		μmoles/g ft wt	
None	0.0–0.5	7.95	4.78	3.17
	0.5–1.5	4.15	2.44	1.71
	1.5–2.5	3.62	1.79	1.83
	2.5–3.5	2.23	1.14	1.09
10^{-3} M K-EDTA (30 min)	0.0–0.5	23.15	16.85	6.30
	0.5–1.5	7.51	5.08	2.43
	1.5–2.5	6.81	3.98	2.83
	2.5–3.5	6.08	3.49	2.59

Table III
Respiration and Rb^{86} Accumulation by Segments of Soybean Root Tissue

Root section	Protein	Uptake			
		O_2		Rb^{86}	
cm from tip	mg/g fr wt	μl/hr g fr wt	μl/hr mg prot	cpm/g fr wt	cpm/mg prot
0.0–0.5	42.6	1640	38	2880	68
0.5–1.5	11.2	698	62	1975	176
0.5–2.5	8.1	477	59	745	92

in 1 % sucrose. The root tips were pretreated for 40 minutes at room temperature.

When ultraviolet radiation was used, 100 root tips were placed in a 3.2 × 3.2 × 0.6 cm plastic container with 3 ml of 1 % sucrose. The containers were placed 2 cm from the filter of a Mineralight short wave ultraviolet lamp (Model SL 2537, Ultraviolet Products, Inc.) for 20 minutes. The major energy of this lamp is at 2537 A.

Results

Potassium Uptake as a Function of Calcium Removal. The influence of the length of pretreatment in K-EDTA on Rb^{86} labeled K absorption is shown in figure 1. Pretreatment for 30 minutes increased both exchangeable and accumulated ion. Longer pretreatments resulted in declining accumulation, with the exchangeable fraction continuing to increase. The effect of temperature and duration of pretreatment in K-EDTA on K absorption is shown in table I. At ice temperatures 120 minutes are required to produce the same response occurring in 30 minutes at 25°. The impairment of K accumulation found at 120 min at 25° does not occur in the cold. The effect of K-EDTA is evidently limited by temperature as had previously been noted (10) suggesting that some metabolic act is responsible for the impairment of ion accumulation. The K-EDTA promotion of K accumulation is greatest in the tip 0.5 cm of the root (table II). This region of dividing and expanding cells has the highest protein N content (table III). However, the subjacent 0.5 to 1.5 cm section is more active in ion accumulation and respiration per unit protein and shows a smaller proportionate increase in exchangeable ion (table II). We used the 0.5 to 1.5 cm section for nearly all subsequent experiments in order to have tissue with maximum ion accumulation on a protein basis.

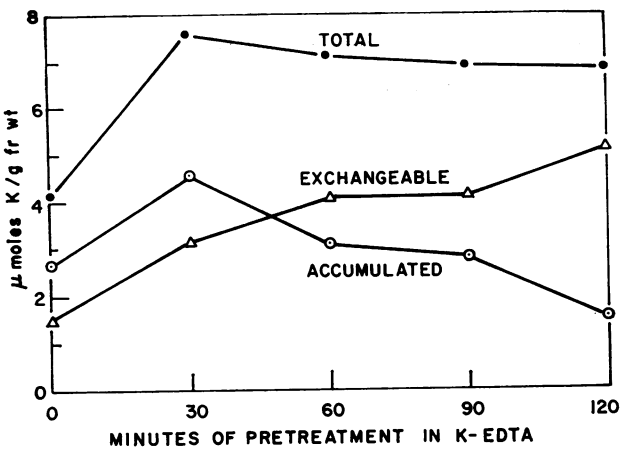
The loss of Ca, Mg, and Fe to K-EDTA as detected by the emission spectrograph is shown in table IV. Calcium is lost at a rapid rate, Mg less so, and there was no significant loss of iron. The KCl effected a much smaller loss of Ca and Mg. The rapid Ca removal from the root tissue was confirmed by growing soybean seedlings in Ca^{45} and following the loss of the tracer from the root tissue in KCl and K-EDTA. In 30 minutes about 65 % of the Ca^{45} was removed from the 0.5 to 1.5 cm section (fig 2). Removal of Ca from the tip section was even more rapid and complete. It may be important that the more mature tissue, which is more effective per unit protein in ion accumulation (table III) can withhold more of its Ca from K-EDTA. Some of the Ca binding of such tissues may be by cytoplasmic chelate entities with high affinities for the ion. Alternatively, the K-EDTA resistant fraction may be located in the vacuoles and only slowly lost.

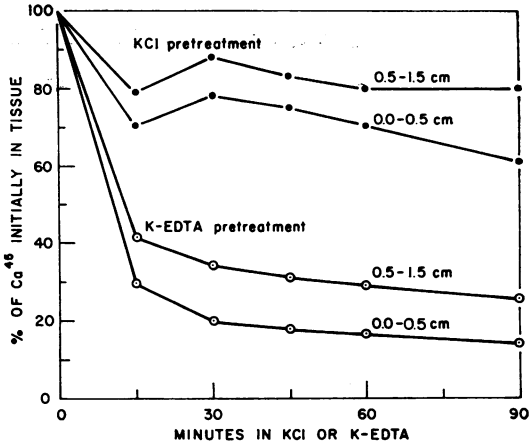
It was confirmed that removal of Ca from the tissue by K-EDTA pretreatment leads to rapid leakage of materials absorbing ultraviolet light between 260 to 290 mμ, presumably nucleotides (10). The loss of nucleotides could potentially affect respiration rate, but in the current experimentation we found no significant difference in the respiration rates of the root tissue when pretreated for 40 minutes in KCl or K-EDTA in the presence of 10^{-3} M phosphate buffer. Treatments with K-EDTA in excess of one hour inhibited respiration (10).

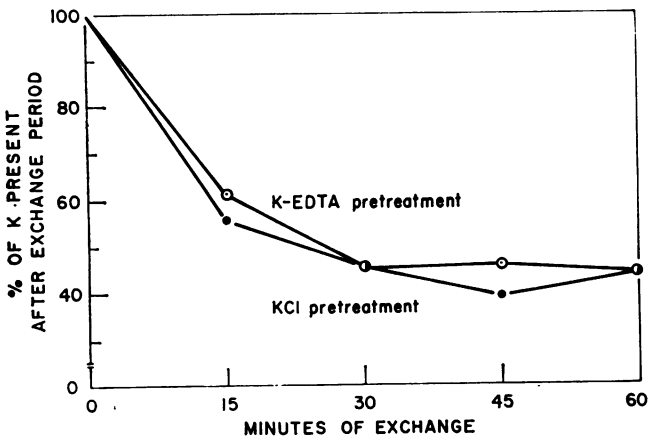
In previous experiments with longer exposure to K-EDTA, a degradation of RNA was initiated in root tissue (10). The addition of K-EDTA to root homogenates accelerated degradation of endogenous RNA by endogenous enzymes. The removal of Ca

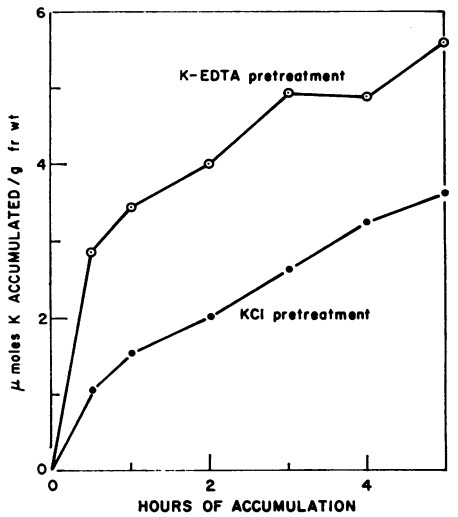
Table IV
Cation Removal From Soybean Root Tissue by K-EDTA as Determined by Emission Spectrography

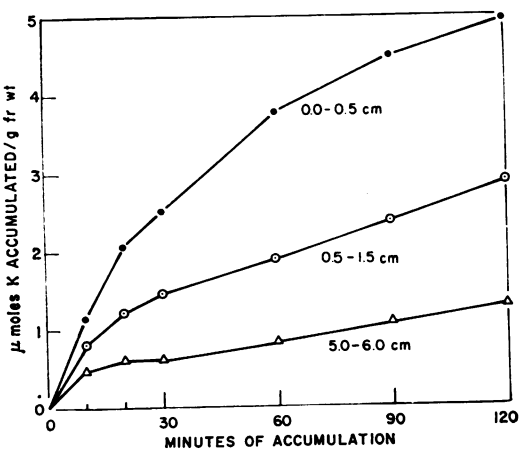
Pretreatment		Cation		
		Ca	Mg	Fe
		μg/g dry wt		
None	...	279	334	34
70 min	KCl	203	282	24
	K-EDTA	26	206	27
135 min	KCl	227	237	33
	K-EDTA	39	139	33











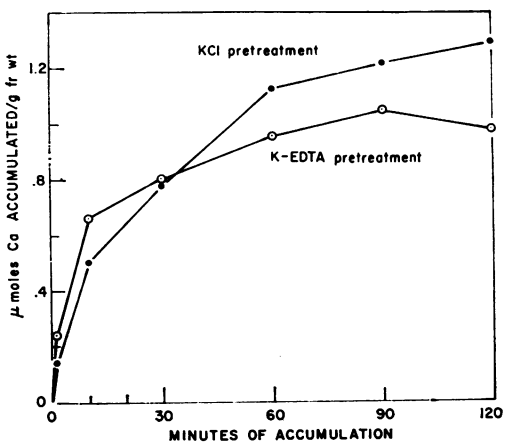


FIG. 1 (*upper left*). The absorption of K labeled with Rb^{86} by soybean root tissue as affected by preincubation in 10^{-3} M EDTA (potassium salt). Two hours absorption peroid.

FIG. 2 (*upper right*). Percent of Ca^{45} accumulated during germination remaining in soybean root tissue after treatment for various time periods in 10^{-3} M K-EDTA or 2×10^{-3}M KCl.

FIG. 3 (*middle left*). Percentage of total initial absorbed K remaining after exposure of the tissue to ice-cold 10^{-2} M $CaCl_2$ for the indicated period.

FIG. 4 (*middle right*). The time course of K accumulation by K-EDTA and KCl pretreated roots.

FIG. 5 (*lower left*). The time course of K accumulation by normal (not pretreated) soybean root segments.

FIG. 6 (*lower right*). Time course calcium accumulation by KCl and K-EDTA treated soybean root tissue.

Table V

Effect of Presence of Metabolic Inhibitors During Absorption on Potassium Absorption

Pretreatment	Absorption conditions	Potassium absorbed				
		Total	Accumulated	% of control	Exchangeable	% of control
	Atmosphere			μmoles/g fr wt		
2 × 10^{-3} M KCl	air	3.99	2.33	...	1.67	...
	N_2	2.98	1.47	63	1.52	91
10^{-3} M K-EDTA	air	7.97	4.53	...	3.44	...
	N_2	4.83	2.37	52	2.47	80
	additive					
2 × 10^{-3} M KCl	none	4.27	2.53	...	1.79	...
	10^{-4} M DNP	3.75	2.08	82	1.68	94
	10^{-3} M DNP	1.95	1.09	43	0.86	48
10^{-3} M K-EDTA	none	8.64	5.01	...	3.61	...
	10^{-4} M DNP	5.98	3.28	65	2.70	75
	10^{-3} M DNP	1.94	0.75	15	1.20	33

and Mg was thought to expose the RNA to the ribonucleases. However, in the current experiments no evidence was found that activation of endogenous ribonuclease was involved in the increased K absorption due to short term K-EDTA pretreatment; a 30 minute treatment which removes two-thirds of the Ca from the 0.5 to 1.5 cm section of the tissue (fig 2) did not produce a significant degradation of RNA. If after the 30 minute pretreatment in K-EDTA the root tissue was transferred to sucrose for periods up to 2 hours, no change could be found in subsequent K accumulation. Only when the tissue is continuously in K-EDTA is there evidence of catabolism deleterious to ion uptake (fig 1).

The increased potassium accumulation following K-EDTA pretreatment might be due to an increase in slowly exchangeable ions. However, the time course of exchange is the same for KCl and K-EDTA pretreated tissue (fig 3), and is essentially complete in 30 minutes. The exchange solution volume (4 vs. 20 ml), temperature (0 vs. 20°), and composition (10^{-2} M KCl vs. 10^{-2} M $CaCl_2$) were checked and found to have no effect on the rate of exchange as shown. The increase in nonexchangeable ion due to 30 minutes pretreatment with K-EDTA appears to be due to accumulation beyond some exchange barrier.

The time course of potassium accumulation is biphasic, with an initial period of rapid accumulation that is complete in less than one hour, followed by an extended period of slow accumulation (fig 4). The accelerated accumulation by the K-EDTA treated roots occurs in the first hour. The subsequent rate of accumulation is the same as with KCl pretreated roots.

The time course of accumulation in non-pretreated tissues (fig 5) reveals that these soybean roots normally have a biphasic time course of K accumulation. In the initial period of accumulation the ion is rapidly taken into some phase from which it cannot be readily exchanged. The amount of initial accumulation and the length of time needed for its accomplishment depends upon the relative age of the cells concerned.

Table VI

Reversal of K-EDTA Effect on Potassium Absorption with the Addition of 5 × 10^{-3} M $CaCl_2$ or $MgCl_2$ to the Absorption Solution

Pretreatment	Absorption solution	Potassium absorbed		
		Total	Accumulated	Exchangeable
	additive		μmoles/g fr wt	
2 × 10^{-3} M KCl	none	3.64	2.16	1.48
	$CaCl_2$	1.89	1.40	0.49
10^{-3} M K-EDTA	none	6.33	3.87	2.46
	$CaCl_2$	1.51	0.97	0.54
2 × 10^{-3} M KCl	none	2.66	1.46	1.35
	$MgCl_2$	2.18	0.70	1.48
10^{-3} M K-EDTA	none	4.10	2.15	1.95
	$MgCl_2$	1.22	0.58	0.65

Table VII

Effect of a Second Pretreatment with Various Ions on Potassium Absorption by K-EDTA Pretreated Roots

The first pretreatment in 10^{-3} M K-EDTA was for 40 minutes, the second pretreatment was for 30 minutes.

Second pretreatment solution	Potassium absorbed		
	Total	Accumulated	Exchangeable
		μmoles/g fr wt	
1 % sucrose	7.49	5.68	2.65
+ 10^{-2} M NaH_4Cl	8.83	4.87	3.96
+ 10^{-2} M NaCl	8.37	4.39	3.97
+ 10^{-2} M RbCl	8.33	3.79	4.04
+ 10^{-2} M KCl	8.31	4.74	3.60
+ 10^{-2} M LiCl	6.15	1.99	4.16
+ 10^{-2} M $AlCl_3$	6.28	3.77	2.41
+ 10^{-2} M $MgCl_2$	4.75	3.28	1.46
+ 10^{-2} M $CaCl_2$	2.11	1.00	1.11
+ 0.05 mg/ml protamine	6.65	3.95	2.96
+ 0.05 mg/ml protamine + 10^{-2} M $CaCl_2$	1.96	0.79	1.11

The densely cytoplasmic tip section has the largest initial accumulation: it is also the section showing the largest K-EDTA response (table II).

Since in these experiments we are following the accumulation of radioactive tracer, it does not follow that a net accumulation of potassium is involved. An active exchange of tracer Rb^{86} for K (or other cation) lying beyond an exchange barrier will produce the same result. In the initial stage of accumulation the tracer is rapidly moved into a phase which is soon saturated, probably the cytoplasm. When these rapidly labeled sites are filled, a second stage becomes apparent, probably accumulation into the vacuole. The assumed vacuolar accumulation rates per gram of tissue are about the same in the 0 to 0.5 cm and 0.5 to 1.5 cm sections (last 30 min, fig 5). Extrapolation of the final 30-minute rate to zero time gives intercepts of 3.1 and 0.8 μmoles of labeled K per gram. This fourfold difference in the assumed cytoplasmic accumulation corresponds to a fourfold difference in protein content (table III).

The additional initial-phase accumulation induced by K-EDTA pretreatment is under metabolic control (table V). Anaerobic conditions and DNP have qualitatively similar effects in reducing accumulation in both KCl and K-EDTA pretreated tissue. On a percentage basis the reductions are greater in the K-EDTA pretreated tissue. The decline in exchangeable K with lowered metabolism has been previously reported by Barber and Russell (1).

The stimulation of K accumulation by K-EDTA pretreatment is readily reversed by the presence of Ca or Mg in the absorption solution (table VI). The accumulation of K is actually reduced to a lower level in the K-EDTA pretreated tissue. If the root sections are exposed to various cations during a second pretreatment period and then placed in the absorption solution, the Ca ion is most effective in reversal (table VII). In general, divalent and polyvalent cations are more effective than monovalent ions, with the exception of Li. All monovalent ions tended to increase the amount of exchangeable K found during subsequent absorption.

Calcium Uptake following Calcium Removal. Since a K-EDTA pretreatment removes Ca from the tissue it was expected that the subsequent accumulation of Ca^{45} would be increased. It was not (table VIII). However, there was an increase in exchangeable Ca. Pretreatment in KCl also increased exchangeable Ca.

Compared to the KCl control, Ca accumulation (fig 6) after K-EDTA pretreatment is rapid for about 10 to 20 minutes, then declines. The prior removal of Ca by K-EDTA apparently opens the tissue to a rapid accumulation of Ca onto sites which are quickly saturated. However, subsequent accumula-

Table VIII

Effect of K-EDTA Pretreatment on Subsequent Calcium Absorption

Pretreatment	Calcium absorbed		
	Total	Accumulated	Exchangeable
		μmoles/g fr wt	
1 % sucrose	2.99	1.24	1.75
+ 2 × 10^{-3} M KCl	3.35	1.28	2.07
+ 10^{-3} M K-EDTA	3.57	1.18	2.39

Table IX

Reversal of K-EDTA Effect on Phosphate Absorption with Calcium and Magnesium

Pretreatment	Second pretreatment or absorptive solution	Phosphate absorbed		
		Total	Accumulated	Exchangeable
	additive		μmoles/g fr wt	
Reversal with 10^{-2} M $CaCl_2$ due to second pretreatment for 30 min				
2×10^{-3} M KCl	none	0.756	0.558	0.198
	$CaCl_2$	0.778	0.578	0.200
10^{-3} M K-EDTA	none	0.642	0.324	0.318
	$CaCl_2$	0.827	0.567	0.260
Reversal with 5×10^{-3} M $CaCl_2$ during absorption				
2×10^{-3} M KCl	none	0.701	0.523	0.177
	$CaCl_2$	1.262	0.797	0.465
10^{-3} M K-EDTA	none	0.706	0.298	0.409
	$CaCl_2$	1.258	0.777	0.482
Reversal with 5×10^{-3} M $MgCl_2$ during absorption				
2×10^{-3} M KCl	none	0.690	0.422	0.268
	$MgCl_2$	1.238	0.638	0.600
10^{-3} M K-EDTA	none	0.702	0.235	0.467
	$MgCl_2$	1.300	0.600	0.694

tion is blocked, possibly by some destructive catabolism resulting from the K-EDTA pretreatment.

Anion Uptake Following Calcium Removal. Total phosphate absorption is unaffected by the short term K-EDTA pretreatment, but there is a change in the relationship between the accumulated and exchangeable fractions (fig 7). The accumulated ion decreases, while the exchangeable ion increases. This change occurs in 30 minutes, the time required for K-EDTA to remove the largest portion of Ca from the tissue (fig 2). Apparently, the substitution of the potassium from K-EDTA for the Ca on certain cytoplasmic sites during the 30 minutes of pretreatment makes absorbed phosphate readily exchangeable. "Exchangeable" is here defined in an operational sense; the phosphate may in good part be arising from free space.

If the tissue is supplied with Ca during a second pretreatment, and then placed in the absorption solution, the ability of the tissue to accumulate phosphate is brought to control levels (table IX). When present in the absorption solution, Ca and Mg increase

Table X

Calcium Stimulation of Phosphate Absorption in the Presence of Anaerobic Conditions and Low Temperature

Pretreatment	Absorption conditions		Phosphate absorbed		
			Total	Accumulated	Exchangeable
	additive*	atmosphere		μmoles/g fr wt	
2×10^{-3} M KCl	none	air	0.375	0.351	0.024
		N_2	0.135	0.090	0.045
	$CaCl_2$	air	0.844	0.683	0.161
		N_2	0.442	0.245	0.197
10^{-3} M K-EDTA	none	air	0.316	0.127	0.189
		N_2	0.262	0.105	0.157
	$CaCl_2$	air	0.881	0.571	0.310
		N_2	0.476	0.324	0.152
		temp.			
2×10^{-3} M KCl	none	25°	0.495	0.429	0.066
		3°	0.067	0.032	0.035
	$CaCl_2$	25°	0.966	0.749	0.217
		3°	0.139	0.044	0.095
10^{-3} M K-EDTA	none	25°	0.448	0.248	0.200
		3°	0.146	0.014	0.132
	$CaCl_2$	25°	1.028	0.672	0.356
		3°	0.302	0.072	0.230

* 5×10^{-3} M $CaCl_2$.

Table XI

Chloride Absorption in the Presence of 5 × 10⁻⁴ M Calcium Chloride

Pretreatment	Absorption solution	μmoles Cl/g fr wt		
		Total	Accumulated	Exchangeable
KCl (90 min)	— Ca	0.407	0.278	0.129
	+ Ca	0.616	0.386	0.231
K-EDTA	— Ca	0.305	0.086	0.219
	+ Ca	0.500	0.203	0.298

both accumulated and exchangeable phosphate (table IX). With both methods of supplying Ca, the shift from accumulated to exchangeable phosphate caused by K-EDTA pretreatment is eliminated.

Low temperature and gassing with N_2 reduce phosphate accumulation both in KCl and K-EDTA pretreated tissues (table X). There is also a reduction in exchangeable ion in the K-EDTA pretreated tissue. The Ca stimulation of phosphate absorption occurs even under low temperature and a nitrogen atmosphere, though to a lesser degree.

Pretreatment with K-EDTA for 30 minutes had no reliable effect on Cl accumulation. However, a 90-minute pretreatment reduced Cl accumulation and increased exchangeable Cl (table XI). Ca in the absorption solution increased Cl absorption and partially reversed the effect of EDTA pretreatment, much as in phosphate absorption.

Ribonuclease and Ultraviolet Pretreatments. We tried to confirm Tanada's experiments with ultraviolet light (20) and ribonuclease (21), which he found to give responses similar to those of acid or salt pretreatment. Ribonuclease pretreatment did not affect Rb^{86} or $P^{32}O_4$ absorption in the 0.5 to 1.5 cm root section; ultraviolet light had only a small effect. However, by using the 0.5 cm root tip we were able to confirm the effect of these pretreatments on salt absorption (tables XII, XIII). The general effects of ribonuclease and UV light pretreatment on the subsequent absorption of Rb^{86} and $P^{32}O_4$, and the action of Ca in the absorption solution were somewhat similar to those obtained with K-EDTA pretreatment. Potassium absorption was promoted. The

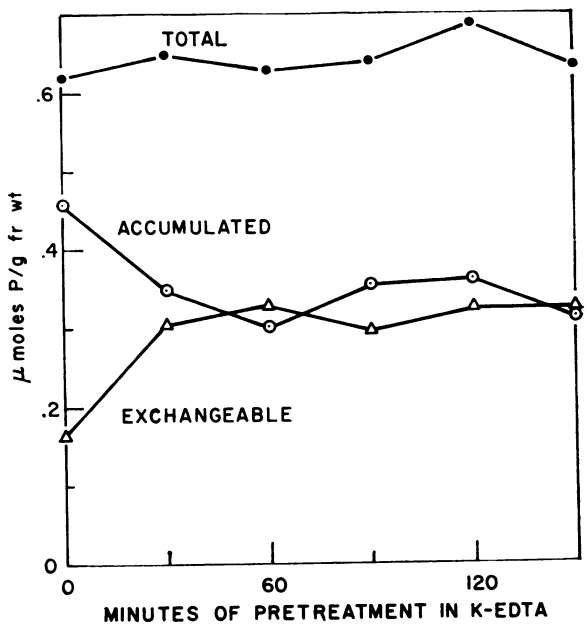


FIG. 7. Phosphate absorption by KCl and K-EDTA pretreated soybean root tissue.

Table XII

Effect of Ultraviolet Pretreatment on Potassium and Phosphate Absorption in the Presence and Absence of 5 × 10⁻³ M $CaCl_2$

The tip 0.5 cm of the soybean root tip was used.

Pretreatment	Absorption solution	Salt absorbed		
		Total	Accumulated	Exchangeable
			μmoles K/g fr wt	
1 % sucrose	none	5.18	3.34	1.83
	$CaCl_2$	2.18	2.12	0.05
+ UV light	none	9.57	6.78	2.78
	$CaCl_2$	0.82	0.64	0.18
			μmoles P/g fr wt	
1 % sucrose	none	0.62	0.61	0.01
	$CaCl_2$	1.56	1.24	0.32
+ UV light	none	0.38	0.24	0.14
	$CaCl_2$	1.20	0.98	0.22

Table XIII

Effect of Ribonuclease Pretreatment on Potassium and Phosphate Absorption in the Presence and Absence of 5×10^{-3} M $CaCl_2$

The tip 0.5 cm of the soybean root tip was preincubated for 40 minutes at room temperature.

Pretreatment	Absorption solution	Salt absorbed		
		Total	Accumulated	Exchangeable
			μmoles K/g fr wt	
1 % sucrose	none	4.97	3.57	1.40
	$CaCl_2$	2.49	2.21	0.28
+ 1 mg/ml Ribonuclease	none	8.77	6.90	1.86
	$CaCl_2$	0.62	0.50	0.12
			μmoles P/g fr wt	
1 % sucrose	none	0.57	0.52	0.05
	$CaCl_2$	1.79	1.45	0.34
+ 1 mg/ml Ribonuclease	none	0.46	0.51	− 0.05
	$CaCl_2$	1.26	0.95	0.31

presence of calcium eliminates this promotion, and strongly inhibits potassium absorption by the pretreated root tips. The pretreatments did not markedly depress phosphate accumulation except in the presence of Ca.

Discussion

The increased Rb^{86}-labeled potassium uptake induced by a short term pretreatment in K-EDTA is largely due to enhancement of an initial rapid phase of accumulation (fig 4). The subsequent linear phase of accumulation is unaffected. (There is some increase in freely exchangeable K as well, but this must largely result from removal of Ca from pectates of the wall and middle lamella, and is not of major interest here.) Since the extent of initial phase accumulation is correlated with protein content, (table III, fig 5), it is likely into the cytoplasm. The initial-phase accumulation is probably limited because the determining parameter is the amount of immobile cytoplasmic anion. The cytoplasm may come to a Donnan equilibrium with the external cation.

It should be noted that the accumulation of Rb^{86} labeled potassium into this assumed cytoplasmic phase is governed by metabolism. Although "accumulation" is defined in this report in an operational sense (absorbed ion which cannot be readily exchanged from the tissue), the ions are accumulated in the sense of active transport as well (5). Factors known to interfere with active ion uptake reduce accumulation (tables V, X). The amount of readily exchangeable K is lowered by DNP, in agreement with the findings of Barber and Russell (1). Hence, the amount of cation bound within and without some exchange barrier (probably the outer cell membrane) can be deuced to be a function of metabolism involving high energy phosphate. Recent reports showing increased Ca binding in mitochondria at the expense of ATP are of interest in this respect (2, 3, 6, 24). We have confirmed these reports for corn shoot mitochondria (T. K. Hodges and J. B. Hanson, unpublished). There is some direct evidence, then, that cation binding to membranes can fluctuate with the supply of metabolic energy.

MacDonald and Laties (18) suggest that penetration of ions into the cytoplasm is metabolically implemented by a different mechanism from that causing movement into the vacuole. Waisel (26) considers entry into the cytoplasm to be a diffusion-limited step. As indicated above, metabolism would appear to be involved at some level, if only to alter the permeability of the outer membrane to diffusing ions.

The increased initial-phase accumulatoin of Rb^{86} following EDTA pretreatment can best be explained as follows: during pretreatment, K from K-EDTA replaces Ca, and to some extent Mg, in the cytoplasm. Metabolic activity is required for the exchange, for low temperatures are inhibitory (table I). The result is a cytoplasm nearly saturated with K. In the subsequent absorption period this K exchanges with the Rb^{86}-labeled potassium. Exchange is more likely than net accumulation of K, since there appears to be a limit on the K held in the initial (cytoplasmic) phase. Metabolic activity is required to move the exchanging ions across the outer membranes. Alternatively, if one prefers not to visualize active exchange transport, the permeability of the membrane can be considered to be regulated by metabolism. The short term pretreatment with EDTA does not affect the steady state of K accumulation, which we assume to be into the vacuole. Hence the cytoplasmic sites freed of Ca and Mg by the EDTA do not appear to be involved in transport of K from cytoplasm to vacuole. Only with extended pretreatment does the K transport mechanism fail.

It is perplexing that K-EDTA pretreatment, which removes Ca (table IV, fig 2) does not result in a large subsequent accumulation of Ca^{45}. One would think that sites voided of Ca would be preferentially reoccupied by the ion. Calcium does in fact

enter very rapidly after EDTA pretreatment (fig 6), but after 10 to 20 minutes accumulation levels off. Only exchangeable Ca is increased. The cytoplasm thus appears to be extremely permeable to Ca after K-EDTA pretreatment, so that Ca enters readily and exchanges readily. However, the second phase of accumulation is impaired.

Probably the most striking effect of Ca removal is in the inhibition of phosphate or chloride accumulation. (The fact that K-EDTA reduces anion accumulation shows that the general processes of ion accumulation are not accelerated, and lends support to the belief that increased uptake of Rb^{86}-labeled K represents nothing more than a metabolically activated exchange into the cytoplasm). The simplest explanation of the phosphate experiments is that phosphate normally enters the root on anionic sites, with Ca or Mg forming the requisite salt bridge. In the extreme, one could say that phosphate enters the cell as a Ca or Mg salt. The recent reports of Brierley et al. (2, 3) showing phosphate accumulation by mitochondria to be associated with Mg or Ca uptake can be interpreted in this fashion, although these authors assumed insoluble phosphates were formed. In Ca-depleted K-saturated roots phosphate is absorbed freely, but not held against an exchange solution. Such exchangeable anion, as operationally defined here, would be considered as arising from free space, that part of the tissue into which solute and solvent penetrate readily (4). The substitution of K for Ca and Mg presumably extends the free space in depth into the cytoplasm but does not permit active accumulation. In the presence of Mg or Ca there is a further increase in exchangeable phosphate which probably represents binding to cell surfaces through cationic bridges.

The work reported here turns out to be but an extension of observations initially made by Tanada (20). In the important respects we have confirmed his observations on the effects of ultraviolet light and ribonuclease. The removal of Ca, treatment with ultraviolet light or ribonuclease, and sulfhydryl inhibitors all produce very much the same result on salt uptake. Tanada deduces that a ribonucleoprotein containing SH groups is involved (23). It would appear that Ca (or Mg) must be requisite to the functioning of this carrier. It is not clear why altering a carrier with ultraviolet light or ribonuclease should produce the same acceleration of potassium uptake as does Ca removal. We can suggest that such treatments appear to have a common effect in making cell membranes more permeable. Perhaps it is in altered permeability that the common response is to be found. The sensitive site may be a ribonucleoprotein which binds divalent ions.

Summary

Treating soybean root tissue with ethylenediamine tetraacetic acid for 30 to 40 minutes removes two-thirds of the calcium and results in increased labeled potassium accumulation. Longer treatment depresses accumulation. The effectiveness of the EDTA treatment is temperature dependent. The increased accumulation appears in the initial phase of a biphasic curve and is interpreted as an activated exchange of radioactive label for cytoplasmic cations. The second linear phase of accumulation, presumably into the vacuole, is unaffected. The initial phase of labeled calcium accumulation is promoted, but the second phase is inhibited.

Phosphate and chloride accumulation are depressed by calcium removal, but the amount of freely exchangeable anion is increased. Calcium or magnesium will reverse the inhibition and actually increase accumulation. Anion uptake appears to be critically linked to the presence of divalent ions.

Ultraviolet irradiation or ribonuclease treatment of root tips will produce effects similar to calcium removal.

Literature Cited

1. Barber, D. A. and R. S. Russell. 1961. The relationship between metabolism and the exchangeability of ions in plant tissues. J. Exptl. Botany 12: 255–60.
2. Brierley, G. P., E. Bachmann, and D. E. Green. 1962. Active transport of inorganic phosphate and magnesium ions by beef heart mitochondria. Proc. Natl. Acad. Sci. 48: 1928–35.
3. Brierley, G. P., E. Murer, and D. E. Green. 1963. Participation of an intermediate of oxidative phosphorylation in ion accumulation by mitochondria. Science 140: 60–62.
4. Briggs, G. E. and R. N. Robertson. 1957. Apparent free space. Ann. Rev. Plant Physiol. 8: 11–30.
5. Briggs, G. E., A. B. Hope, and R. N. Robertson. 1961. Electrolytes and Plant Cells. Blackwell Scientific Publications. Oxford.
6. DeLuca, H. F. and G. W. Engstrom. 1961. Calcium uptake by rat kidney mitochondria. Proc. Natl. Acad. Sci. 47: 1744–50.
7. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437–44.
8. Epstein, E., D. W. Rains, and W. E. Schmid. 1962. Course of cation absorption by plant tissue. Science 136: 1051–52.
9. Fischer, H. 1956. Ionenwirkung. Encylopedia of Plant Physiology. II. 706–46.
10. Hanson, J. B. 1960. Impairment of respiration, ion accumulation, and ion retention in root tissue treated with ribonuclease and ethylene diamine tetraacetic acid. Plant Physiol. 35:372–79.
11. Hanson, J. B. and H. R. Swanson. 1962. The role of basic proteins in the declining respiration of senescing corn scutellum. Biochem. Biophys. Res. Commun. 9: 442–46.
12. Heilbrunn, L. V. 1956. Dynamics of Living Protoplasm. Academic Press. New York.
13. Jacobson, L., D. P. Moore, and R. J. Hannapel. 1960. The role of calcium in absorption of monovalent cations. Plant Physiol. 35: 352–58.
14. Jacobson, L., R. J. Hannapel, D. P. Moore, and M. Schaedle. 1961. Influence of calcium on selectivity of ion absorption process. Plant Physiol. 36: 58–61.
15. Jacobson, L., R. J. Hannapel, M. Schaedle, and D. P. Moore. 1961. Effect of root to solution

ratio in ion absorption experiments. Plant Physiol. 36: 62–65.
16. KAHN, J. S. AND J. B. HANSON. 1957. The effect of calcium on potassium accumulation in corn and soybean roots. Plant Physiol. 32: 312–16.
17. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193: 265–75.
18. MACDONALD, I. R. AND G. G. LATIES. 1963. Kinetic studies on anion absorption by potato slices at OC. Plant Physiol. 38: 38–44.
19. MARINOS, N. G. 1962. Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. Am. J. Botany 49: 834–41.
20. TANADA, T. 1955. Effects of ultraviolet radiation and calcium and their interaction on salt absorption by excised mung bean roots. Plant Physiol. 30: 221–25.
21. TANADA, T. 1956. Effect of ribonuclease on salt absorption by excised mung bean roots. Plant Physiol. 31: 251–53.
22. TANADA, T. 1956. Effect of sulfhydrl inhibitors on rubidium absorption by excised mung bean roots. Plant Physiol. 31: 403–06.
23. TANADA, T. 1962. Localization and mechanism of calcium stimulation of rubidium absorption in the mung bean root. Am. J. Botany 49: 1068–72.
24. VASINGTON, F. D. AND J. V. MURPHY. 1962. Ca^{++} uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. J. Biol. Chem. 237: 2670–77.
25. VIETS, F. G. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466–80.
26. WAISEL, Y. 1962. The effect of Ca on the uptake of monovalent ions by excised barley roots. Physiol. Plantarum 15: 709–24.

Sugar Gradients and Translocation of Sucrose in Detached Blades of Sugarcane [1, 2]

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Studies of the translocation of C^{14}-photosynthate in entire plants of sugarcane grown under normal conditions of climate and nutrition have been summarized (12). Another paper (11) reports defoliation tests which indicated that a major force in translocation resides within the leaf. The present paper describes experiments with detached blades which were started in 1960 (9). Harvesting and processing entire plants of sugarcane involve considerable time and work. This study with detached blades was undertaken to simplify the translocation system and to speed the investigation of the mechanism of translocation in sugarcane.

Materials and Methods

Two varieties were used in these studies: H37-1933 (a complex interspecific hybrid involving *Saccharum officinarum* L., *S. spontaneum* L., and *S. robustum* Brandes and Jeswiet ex Grassl.) and H50-7209 (a hybrid involving *S. officinarum* L., *S. spontaneum* L., and possibly others). The blades were taken from plants grown in the field at the Experiment Station.

Blades were cut from the plants and immediately recut twice under water, then taken to the photosynthesis room, transferred under water to jars containing water, and preilluminated at 2000 ft-c for at least 10 minutes. Preliminary tests indicated that blades cut from the plant and fed $C^{14}O_2$ at a uniform, moderate intensity of light gave better results than plants fed outdoors, at high intensities of light, attached to the plant. High intensities of light, e.g. 8,000 ft-c. decreased translocation in detached blades.

The methods used in the studies reported herein were the same as those reported previously (12), except that the blade was detached from the plant before being fed $C^{14}O_2$. $C^{14}O_2$ (10 μc) was fed to a 20-cm length of blade for 5 minutes at 2000 ft-c, using the chamber described previously (12). All treatments were initiated immediately after removing the feeding chamber. After translocation, the blade was cut into sections, dried, weighed, milled, and counted at infinite thickness.

C^{14} results are expressed as: relative specific activity, which is the net count per minute as infinite thickness; as relative total counts, which is the relative

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