INFLUENCE OF CALCIUM ON SELECTIVITY OF ION ABSORPTION PROCESS[1, 2]

LOUIS JACOBSON, RAYMOND J. HANNAPEL, DAVID P. MOORE, & MICHAIL SCHAEDLE
Department of Soils & Plant Nutrition, University of California, Berkeley

Introduction

Of the various factors known to influence ion absorption by excised barley roots Ca is distinctive in that its effect depends upon the species of monovalent cation undergoing absorption (6). At a concentration of 5 meq/l, Ca is a very effective inhibitor of Li absorption and a moderately effective inhibitor of Na absorption. However, at the same concentration, Ca stimulates absorption of K, Rb, and Cs, particularly at low pH values. The latter phenomenon has been explained as primarily a blocking of a deleterious H uptake. It has been postulated that Ca modifies the permeability of cytoplasmic surfaces in such a way as to decrease the access of certain monovalent cations to the absorption site. According to this view the overall absorption process of monovalent cations, at least, initially involves a screening at the cell surface presumably non-metabolic in nature and affected by the presence of Ca followed by a metabolic absorption step (6). That the effect is due to a non-metabolic Ca fraction rather than absorbed Ca is suggested by the lack of carryover effect following pretreatment with Ca and by the observation that metabolic absorption of Ca occurs very slowly, if at all, with this material (9).

It has been known for some time that polyvalent cations other than Ca exert an effect similar to that of Ca on K absorption (4, 11). In the present study a comparison is made between the effect of several polyvalent cations and the effect of Ca on the absorption of K and Li.

The absorption of K and Na are affected differently by Ca in solutions of the separate ions (6). Wadleigh and Bower (12) were able to vary the K to Na ratio in intact plants by varying the Ca content of the solution. Epply (3) connected K and Na selectivity in Porphyra with Ca. Preliminary data in this laboratory indicated that in mixtures of K and Na the effect of Ca on the absorption of each ion was qualitatively similar to that obtained in single salt solutions; that is Ca increased the absorption of K and decreased the absorption of Na. Accordingly a more comprehensive study on the interaction of Ca with the K-Na system has now been undertaken.

A protective effect of traces of Ca on plant material has frequently been reported, e.g., Laties (8). The studies reported here include an examination of the effects of very small amounts of Ca on the absorption of various ions.

Finally a brief survey is conducted to see how widespread the effect of Ca is with respect to several plant species.

Materials & Methods

In most experiments excised barley (*Hordeum vulgare* L.) root material was used. Barley seed was soaked in aerated distilled water for 24 hours. The germinated seeds were spread on cheesecloth supported by a tinned and Amercoat (Amercoat Corp.) painted screen. The ends of the cheesecloth dipped into the solution. The seedlings were grown in the dark using a dilute nutrient medium consisting of 0.1 mmole/l each of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. The solution was gently aerated. Four days after transferring the seed to the cheesecloth, the nutrient solution was replaced with fresh solution. Two days later the roots were excised just below the seed, cut into approximately two centimeter lengths, thoroughly washed with distilled water, and centrifuged for 5 minutes at 65 × *g*. A ratio of 1 g root material to 1 liter of solution was employed. In most cases the pH of the treatment solution was adjusted to pH 6.0 and maintained at this pH by additions of the appropriate acid or base, e.g., HBr or KOH in a KBr solution. The concentrations of the test salts were not significantly changed by these additions. Unless otherwise stated the concentrations of the alkali metal salts were 5 meq/l and were present as the bromides. The absorption periods were 3 hours and the solutions were continuously aerated during this period. All experiments were performed at 25° C. At the conclusion of the treatment, the roots were poured on a fine mesh nylon screen and washed for 10 seconds with flowing distilled water. After drying and igniting, the ash was taken up in dilute HCl. The alkali metal cations were determined by means of a flame photometer. Direct titration of Ca or Mg plus Ca in solutions which had been in contact with root material gave erroneous results due to the presence of organic matter. In these cases solutions were first evaporated and digested with concentrated HNO_3 and 30 % H_2O_2. Ca was then determined by titration with sodium ethylenediamine tetraacetate using Calcein indicator (2). Mg plus Ca was determined by Na EDTA titration with Eriochrome Black T as the indicator (1). Mg content was obtained by difference between this value and the Ca content.

In the data reported here no corrections for non-metabolic uptake have been applied. Since the pres-

---

[1] Received July 9, 1960.

[2] This paper is based on work performed under Contract No. AT-(11-1)-34, project 5 with the United States Atomic Energy Commission.

ence of polyvalent cations may modify the nonmetabolic uptake of the accompanying monovalent cations, it was thought best not to attempt a correction. In any event the correction, based on previous experience with barley root material, would not be greater than 0.9 meq/Kg fresh weight (7). In most cases the correction would not be significant and in no case would the application of this correction alter the conclusions drawn from the data.

RESULTS

In the first experiment the effect of various polyvalent cations on the absorption of Li and K was examined. These experiments were performed in single salt solutions of LiBr and KBr. The concentrations of the salts were 10 and 5 meq/l, respectively. Polyvalent cations were added as the bromide salts at a concentration of 1 meq/l except Mg at 2 meq/l. For most of the tests the pH was maintained at 6.5, but because of the limited solubility of Mn and Al ions at this pH, these two ions were tested at pH 4.7. At both pH values measurements of Li and K absorptions in the absence of any added polyvalent cation were included for purposes of comparison.

The uptake data, expressed as meq/Kg fresh weight, are given in table I. With the exception of Mg all polyvalent cations tested reduced the absorption of Li sharply. Mg, at the concentration used, had a relatively minor inhibitory effect on Li absorption. All polyvalent cations examined caused an appreciable increase in K absorption. In contrast to previous experiments (6) the variety of barley used in this experiment (Arrivat 1958 crop) showed an increase in K uptake resulting from the addition of Ca at pH 6.5.

TABLE I

EFFECT OF VARIOUS POLYVALENT CATIONS ON ABSORPTION OF Li & K BY BARLEY ROOTS

POLYVALENT CATION	ABSORPTION meq/Kg FRESH WT* Li**	K***
None	14.7	24.3
Mg	13.0	29.4
Ca	4.6	27.2
Sr	4.9	27.2
La	7.1	30.1
None†	12.3	22.7
Mn†	5.3	26.6
Al†	6.9	25.8

* Three hour absorption period.

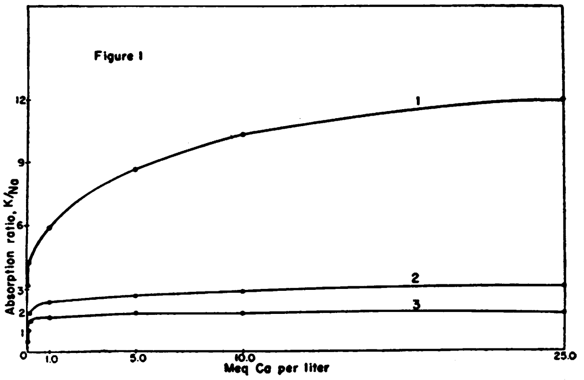
** From LiBr at 10 meq/l + 1 meq/l polyvalent cation except Mg at 2 meq/l.

*** From KBr at 5 meq/l + 1 meq/l polyvalent cation except Mg at 2 meq/l.

† pH = 4.7, all others at pH 6.5.

Since earlier experiments suggested that Ca might control the relative amounts of Na and K absorbed from a mixture of the two, an experiment was undertaken to explore this behavior more fully. Barley root material (Tennessee Winter 1958 crop) was exposed to three series of solutions. All solutions contained 5 meq/l KBr. In one series 1 meq/l of NaBr was present. The second and third series contained 5 and 15 meq/l NaBr. In each series the Ca was varied from no Ca added to 25 meq/l. The pH was maintained at 6.0.

The data for the three series are presented in figure 1. In order to clearly show the effect of Ca on the relative absorption of Na and K, the ratios of absorptions of the two ions are plotted against the Ca added to the solution. It is immediately apparent that Ca exerts a large degree of control over the relative



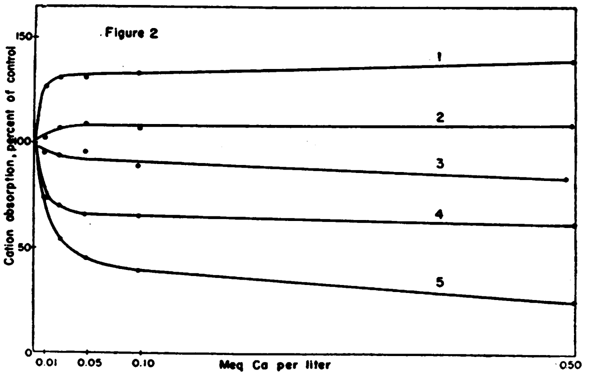


FIG. 1. Effect of Ca on the ratio of absorption of K to Na by barley roots. All solutions contained 5 meq KBr/l. In addition, treatments 1, 2, and 3 contained 1, 5, and 15 meq NaBr/l, respectively. The absorption period was 3 hours and the pH was maintained at 6.0.

FIG. 2. Effect of small amounts of Ca on the absorption of Li, Na, and K by barley roots. 1. K from NaBr + KBr. 2. K from KBr. 3. Na from NaBr. 4. Na from NaBr + KBr. 5. Li from LiBr. All alkali metal salts were present at 5 meq/l. The control for each curve is the absorption in the absence of added Ca. The absorption period was three hours and the pH was maintained at 6.0.

absorption of the two ions. It is also evident that the major effect occurred at the lower concentrations of added Ca. The total absorption values, i.e., Na+K, in the absence of added Ca were 30.1, 35.7, and 41.8 meq/Kg fresh weight from the K solutions containing 1, 5, and 15 meq/l Na, respectively. The corresponding total absorption values for Na+K from solutions containing 25 meq/l Ca were 40.2, 40.0, and 43.4 meq/Kg.

A study was made of the effect of Ca concentrations below 0.5 meq/l. The effect of Ca concentration on the absorption of Na and K from single salt solutions at 5 meq/l and from mixtures of the two at 5 meq/l each were examined. In addition to these ions, the effect of very low concentrations of Ca on the absorption of Li was studied. In these experiments Tennessee Winter 1958 crop barley was used.

The results are presented in figure 2. Because of the small amounts of Ca added, the Ca liberated by the root material into the test solution was a significant contribution to the Ca concentration of the solution. For this reason samples of the test solution were taken at the end of the absorption period and analyzed for Ca as described above. No allowance was made for the Mg released by the roots since the amount was too small to have an appreciable effect. Even without added Ca the solutions were not completely Ca free due to Ca release from the roots and the presence of Ca impurity in the salts. With no added Ca, the Ca concentrations at the end of the absorption period were 0.0011, 0.0015, 0.003, and 0.0019 meq/l for the NaBr, KBr, NaBr + KBr, and the LiBr solutions, respectively. The absorption curves of figure 2 have been plotted as percent of control. In each case the control is the absorption with no added Ca. The curves bring out the fact that Ca has a considerably greater effect on Na and K absorptions from a mixture of the two ions than from single salt solutions of the same ions. Comparing curves 1 and 2, with Ca at the 0.025 meq/l level, K absorption is 130 % of the control when absorption occurs from a mixture of Na and K. From KBr solution the absorption is only 106 % of the control. At the same Ca level, curves 3 and 4, Na absorption is reduced to 70 % of the control in the mixture and to 93 % in NaBr solution. The absorption values for the controls were 20.1 meq Na/Kg from NaBr, 23.0 meq K/Kg from KBr, 12.4 meq Na/Kg, and 16.5 meq K/Kg from the mixture. The total absorption, i.e., Na + K, from the mixture varied from 28.9 meq/Kg with no added Ca to 30.7 meq/Kg with 0.5 meq/l Ca. Curve 5 in figure 2 shows the absorption of Li from LiBr as a function of Ca concentration. The control for this curve was 10.1 meq Li/Kg. The high degree of sensitivity of Li absorption to minute concentrations of Ca is well demonstrated by this curve.

TABLE II

EFFECT OF Ca ON ABSORPTION BY DIFFERENT PLANT SPECIES

	ABSORPTION, meq/Kg FRESH WT*					
	Na**		K**		Li***	
	−Ca	+Ca†	−Ca	+Ca†	−Ca	+Ca†
Barley, *Hordeum vulgare*	12.4	6.4	16.5	24.5	9.9	1.3
Corn, *Zea mays*	12.9	8.7	1.8	10.6	5.9	1.3
Onion, *Allium cepa*	11.4	3.3	−3.7	−0.7	9.8	1.0
Pea, *Pisum sativum*	8.0	4.1	−0.2	1.8	7.2	1.5
Squash, *Cucurbita maxima*	21.8	5.0	8.0	34.8	17.1	1.5
Sunflower, *Helianthus annuus*	18.8	8.4	−5.2	1.5	21.6	1.5

* Three hour absorption period.
** From mixture of 5 meq/l each of NaBr and KBr.
*** From 5 meq/l LiBr.
† Ca added at 5 meq/l as CaBr$_2$.

The above experiments were performed using barley as a test material. In order to see if the Ca effect was specific for barley or was a more general phenomenon, the effect of Ca on absorption was determined for six different species of plants. The results are listed in table II. The roots were from 6 day old plants grown and prepared as described for barley. The solutions consisted of LiBr and a mixture of NaBr and KBr: each without added Ca and with 5 meq/l Ca. To a greater or lesser degree all species responded to the addition of Ca. The response of squash roots to Ca is especially noteworthy. Additional tests using a level of 0.05 meq/l Ca likewise indicated a response to Ca. Attempts to use potato disks were unsuccessful because of the very large ratio of free space uptake to active absorption.

DISCUSSION

Other polyvalent cations share with Ca the property of altering the selectivity of barley root tissue to the absorption of monovalent cations. Of the polyvalent cations tested Mg was the only exception. It has been postulated that Ca modifies the selectivity of the cell membrane for monovalent cations (6, 9) presumably by reacting with the membrane. Since most of the polyvalent cations have a similar effect on selectivity, either the reaction is non-specific or these ions exert an effect via a Ca interaction. It is possible that polyvalent cations exchange for Ca in the root. If the polyvalent cation satisfies exchange sites which normally compete for Ca with the active binding sites that control selectivity, then even the small amounts of Ca liberated from inactive sites might be highly effective.

In a previous article (6) it was shown that the presence of Ca affected the absorption of Na and K from single salt solutions in quite different ways. Absorption of the former was reduced and that of the latter increased by the addition of Ca. The data reported here show that the same holds true for absorption from mixtures of Na and K. The ratio of absorption of the two ions is strongly influenced by the presence of Ca even if present in low concentration.

The absorption system is exceedingly sensitive to very small concentrations of Ca in the external solution. The addition of as little as 0.001 meq/l of Ca to solutions of LiBr and NaBr + KBr consistently reduces absorption of Li or Na about 3 %. Perhaps if it were possible to work with truly Ca free systems even greater sensitivity might be attained. It is interesting that Ca at a concentration of about 10^{-6} M is as good or better an inhibitor of Li and Na absorption than many metabolic inhibitors.

In a mixture of Na and K salts, the effect of Ca is considerably amplified compared to its effect in single salt solutions. A reduced access of Na and an increased access of K caused by the presence of Ca would result in presenting to the absorption site an increased ratio of K to Na. It is this increased ratio which may cause the enhancement of the Ca effect in Na and K mixtures. Inherent in this view is the supposition that Na and K compete for the same metabolic ion carrier. It is suggestive that in the presence of varying amounts of Ca the sum of the absorptions of Na and K remained constant within about 6 % although the ratio of absorption of the two ions varied from 1.33 to 3.00. This is the type of behavior one would expect if a common carrier were involved.

The particular Ca reaction which controls the relative selectivity has not been ascertained. There is no scarcity of substances in cellular membranes which are capable of reacting with Ca (10). The problem appears to be which substances and what mechanisms are involved. It has been suggested that RNA in cell membranes is involved in ion accumulation and that divalent ions protect the RNA from degradation by endogenous enzymes (5). It is possible that membrane RNA is involved in the Ca effect, but the inhibitory effect on Na and Li absorption appears to preclude the role of Ca as a simple protective agent.

It is probable that the type of absorption response to Ca described here is widespread among plant species. All root material thus far tested behaves in an essentially similar fashion. It is reasonable to assume that Ca has a great deal to do with the preference that many plants exhibit for K over Na.

SUMMARY

Several polyvalent cations affect absorption similarly to Ca. Mg appears to be an exception. It is suggested that either the particular mechanism involved is not specific for Ca or that the polyvalent cations act via a Ca interaction.

Ca was found to have the property of drastically altering the ratio of absorption of Na and K from a mixture of the two. Using Li and Na + K solutions it was shown that the absorption system was extremely sensitive to small concentrations of Ca. As little as 10^{-6} M Ca was sufficient to cause a detectable change in absorption rates. The effect of Ca is enhanced in mixtures of Na and K compared to its effect in single salt solutions.

The essentially constant sum of the absorptions of Na and K in spite of large changes in the ratio of absorbed Na and K caused by the presence of Ca suggests a common metabolic carrier.

The controlling behavior of Ca was found in the roots of six different species of plants. This may indicate a widespread occurrence.

LITERATURE CITED

1. CHENG, K. L. and R. H. BRAY. 1951. Determination of calcium and magnesium in soil and plant material. Soil Sci. 72: 449–458.
2. DIEHL, H. and J. L. ELLINGBOE. 1956. Indicator for titration of calcium in presence of magnesium using disodium dihydrogen ethylenediamine tetraacetate. Anal. Chem. 28: 882–884.
3. EPPLEY, R. W. and C. C. CYRUS. 1960. Cation regulation and survival of the red alga, *Porphyra perforata,* in diluted and concentrated sea water. Biol. Bull. 118: 55–65.
4. FAWZY, H. R., R. OVERSTREET, and L. JACOBSON. 1954. The influence of hydrogen ion concentration on cation absorption by roots. Plant Physiol. 29: 234–237.
5. HANSON, J. B. 1960. Impairment of respiration, ion accumulation, and ion retention in root tissue treated with ribonuclease and ethylenediamine tetraacetic acid. Plant Physiol. 35: 372–379.
6. JACOBSON, L., D. P. MOORE, and R. H. HANNAPEL. 1960. Role of calcium in absorption of monovalent cations. Plant Physiol. 35: 352–358.
7. JACOBSON, L., R. H. HANNAPEL, and D. P. MOORE. 1958. Non-metabolic uptake of ions by barley roots. Plant Physiol. 33: 278–282.
8. LATIES, G. G. 1959. The generation of latent-ion-transport capacity. Proc. Nat. Acad. Sci. U. S. 45: 163–172.
9. MOORE, D. P. 1960. Uptake of calcium and magnesium by excised barley roots. Ph.D. dissertation, University of California, Berkeley.
10. ROTHSTEIN, A. 1959. Role of the cell membrane in the metabolism of inorganic electrolytes by microorganisms. Bacteriol. Rev. 23: 175–201.
11. VIETS, F. G. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466–480.
12. WADLEIGH, C. H. and C. A. BOWER. 1950. The influence of calcium ion activity in water cultures on the intake of cations by bean plants. Plant Physiol. 25: 1–12.