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ROLE OF CALCIUM IN ABSORPTION OF MONOVALENT CATIONS^{1, 2}

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The interaction of ions during the absorption process has frequently been exploited to study the mechanism and kinetics of ion absorption (4, 6, 17). Generally ions of similar valence have been used, although Middleton and Russell (14) have proposed a mechanism of ion absorption based primarily on the interaction of Sr and Rb. It has been recognized for some time that polyvalent cations often exert a striking influence on the absorption of monovalent ions. Viets (18) demonstrated that the alkaline earth metals and Al accelerated the accumulation of K and Br by excised barley roots and ascribed the effect to a metabolic or membrane phenomenon. Calcium had either a depressing or stimulating effect on K uptake depending upon the concentration of the latter (16).

Hydrogen ion has been implicated in the Ca effect. Arnon and Johnson (1) found that the deleterious effect of low pH on the growth of lettuce and tomato could be lessened by increasing the Ca content of the nutrient solution. More recently Ekdahl (5) has demonstrated that Ca effectively overcame the harmful influence of low pH on the elongation of root hairs. The work of Fawzy et al (7) indicated that pH was also a factor in the behavior of other polyvalent cations in addition to Ca. The stimulating effect increased markedly percentagewise as the pH of the external solution decreased. Wadleigh and Bower (19) were able to influence the relative Na and K contents of red kidney bean plants by varying the Ca content of the nutrient solution. Kahn and Hanson

(12) suggested that Ca increased the affinity between K and a postulated carrier but decreased the velocity of the metabolic phase of K uptake in a second independent reaction.

In the experiments to be described, the effect of Ca on the absorption of several monovalent cations has been investigated over a wide range of pH. In addition, the relationships of various monovalent cations and pH in the absorption process have been examined.

MATERIALS AND METHODS

Excised roots of barley, California Mariout 1956 crop, were used in these experiments. The plants were grown and the root material harvested as previously described (11). To minimize concentration changes in the solution, absorption experiments were carried out using 7 gm roots in 7 liters of solution. The solutions were aerated with CO₂ free air during the absorption period of 3 hours. In all experiments the pH was held constant during the course of the absorption period by appropriate additions of acid or base. These additions did not materially change the concentration of the solution. All absorption experiments were performed at 25° C. At the conclusion of an experiment, the roots were separated on a Nylon mesh filter and washed for 10 seconds with running distilled water. The washed roots were ignited and the various elements determined in an acidified solution of the ash. Li, Na, K, Rb, and Cs were determined by the flame photometric method.

Previous experiments had shown that after washing this material for 10 seconds, the non-metabolic or physical uptake corresponded to approximately 20% apparent free space (10). This correction was ap-

¹ Received August 10, 1959.

² This paper is based on work performed under contract No. At-(11-1)-34, Project 5, with the Atomic Energy Commission.

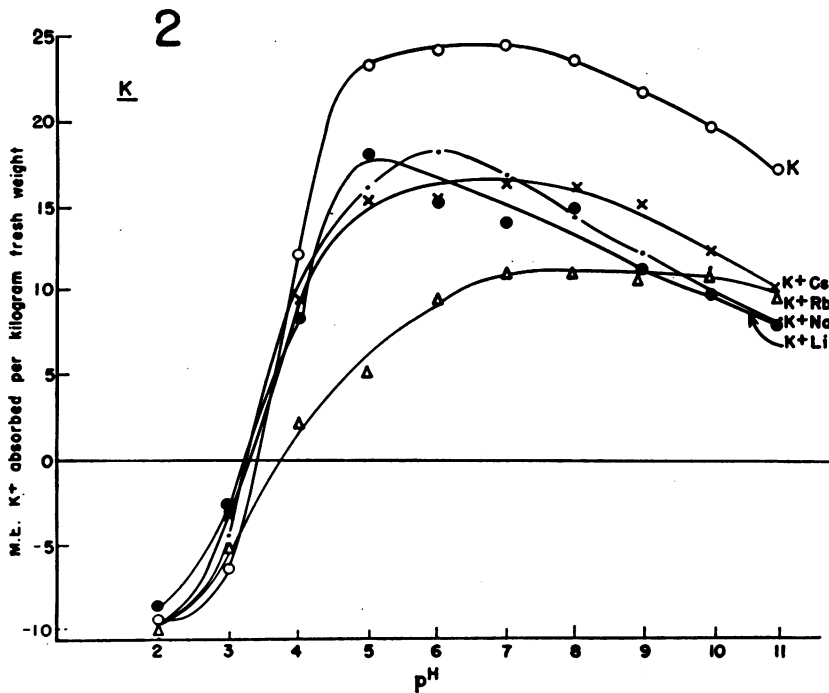
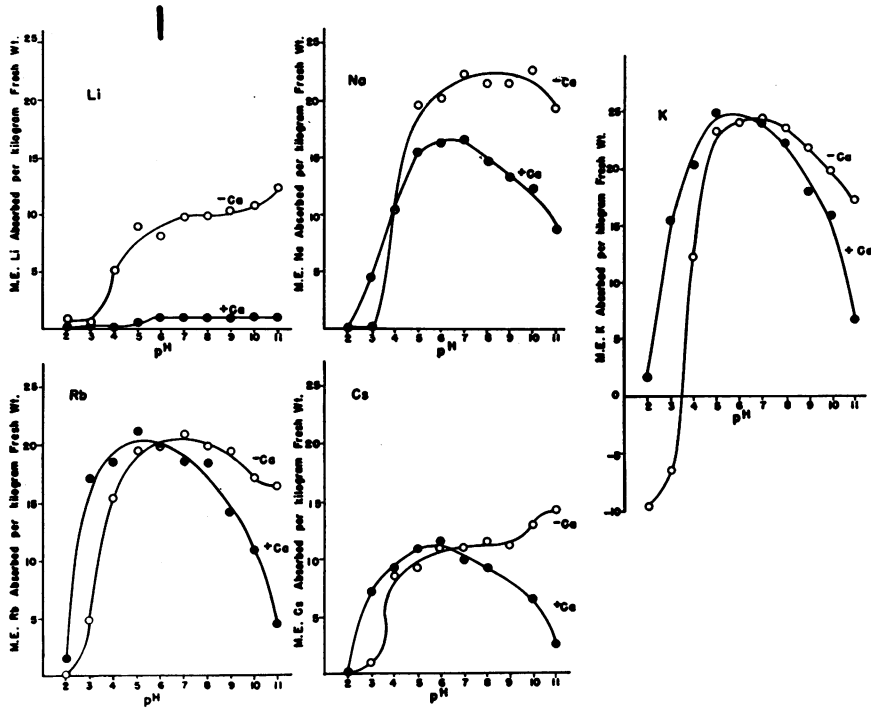


FIG. 1. Absorption of the alkali metal cations as a function of pH in the presence and absence of Ca. All salts present as the bromides at 5 meq/l each. Three hour absorption period.

FIG. 2. Effect of alkali metal cations on the absorption of K as a function of pH. All salts present as the bromides at 5 meq/l each. Three hour absorption period.

plied to all data. In most cases, the correction had relatively little effect on the shape or magnitude of the absorption curves.

RESULTS

In the first experiment, the effect of Ca on the absorption of the alkali metal cations was determined over a broad pH range. The concentration of the solutions was 5 meq/l with respect to the particular cation being studied. The solutions were adjusted to pH values ranging from 2 to 11 by adding HBr to the hydroxide solution of the cation. By this procedure all solutions had identical alkali metal cation concentrations. For each of the elements, the absorption in the absence and presence of added Ca was determined. In addition to the test cation the Ca series contained 5 meq/l of Ca as CaBr_2 otherwise these solutions were prepared similarly to the single salt solutions. It is recognized that the extremes of the pH range studied are not ordinarily considered physiological. During short term experiments, however, the absorption mechanism is still operative at these extremes.

The data for the five elements are presented in figure 1. For K the stimulating effect of Ca is present at pH values below about 6.5. The percentage stimulation increased markedly with decreasing pH as observed by Fawzy et al (7). Above pH 6.5 Ca exerts a depressing effect on K absorption; the effect increases with pH. At pH 11 the absorption is reduced by 60% when Ca is present. The qualitative effect of Ca on Rb and Cs absorption is similar to that on K although the absorption pattern for Cs in the absence of Ca is different from that of K and Rb. Over most of the pH range the absorption rate of Cs is about half that of K or Rb and at high pH values Cs absorption increases appreciably whereas K and Rb absorption decreases.

This root material absorbs Na almost as rapidly as it does K, but the effect of Ca on the two is quite different. In contrast to its effect on K absorption, Ca exerts a marked depressing effect on Na absorption over most of the pH range. Only at pH values below 4 does the presence of Ca show a stimulating effect and even then it is relatively small.

Of the five alkali metal cations studied in single salt solutions, Li is absorbed the least. Slightly less Li is absorbed than Cs, otherwise the two absorption curves are similar. Ca has a very striking effect on Li absorption, the presence of Ca almost completely inhibiting the absorption of Li over the entire pH range.

In figure 2 data for the effect of Li, Na, Rb, and Cs on the absorption of K at various pH values are

TABLE I

RETENTION OF POTASSIUM BY BARLEY ROOTS AS FUNCTION OF pH IN PRESENCE AND ABSENCE OF CALCIUM

pH*	K CONTENT** (MEQ/KG FRESH WEIGHT)	
	+ Ca***	- Ca
5	13.6	13.6
4	13.0	8.28
3	10.3	2.07
2	2.67	0.83

* pH adjusted and maintained with HBr for duration of experiment, i.e., 3 hours.

** Initial K content = 13.6 meq/kg fresh weight.

*** Concentration of added CaBr_2 = 5 meq/l.

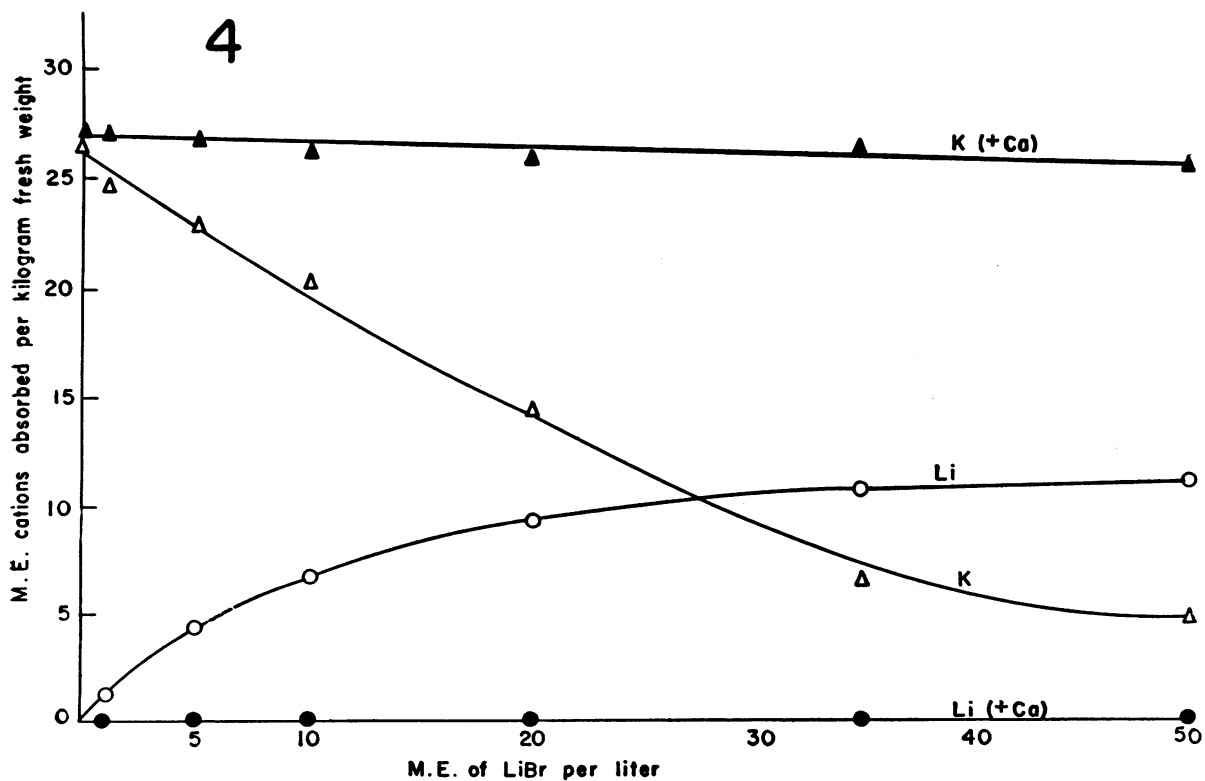
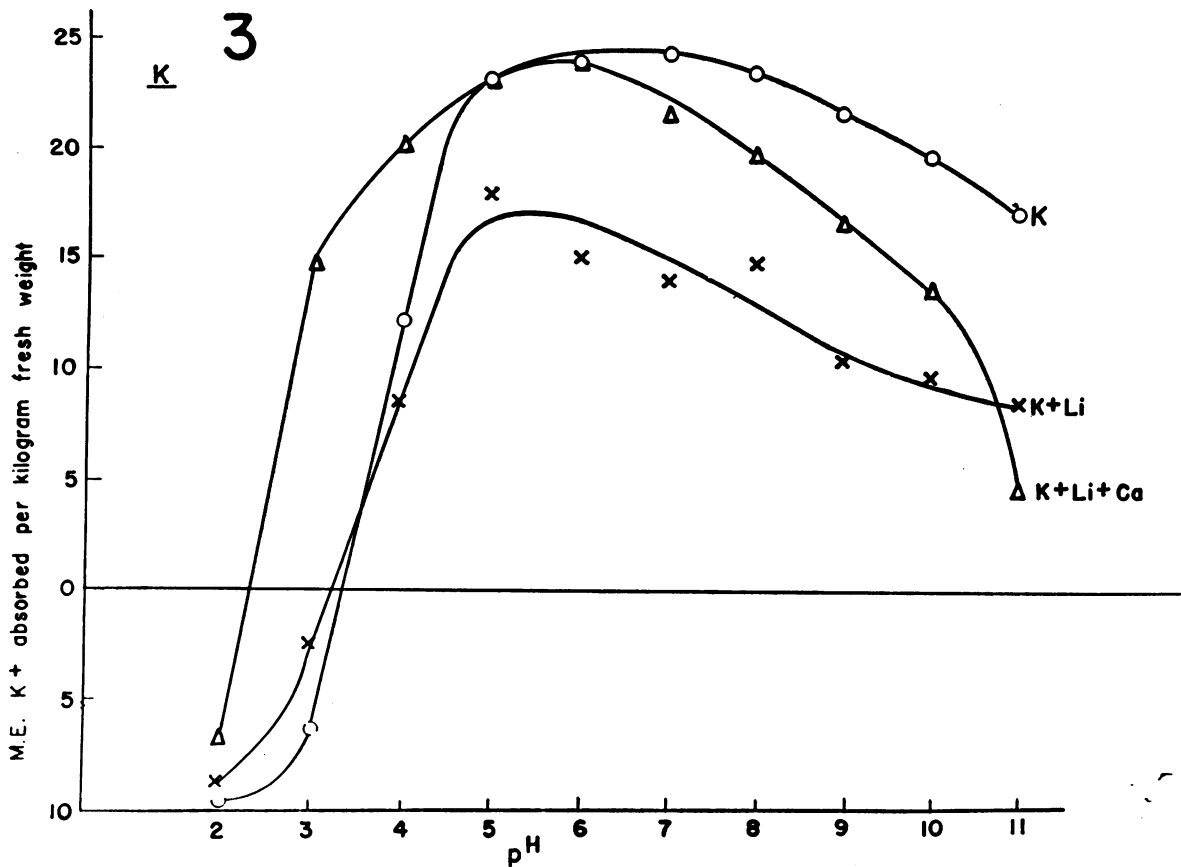
given. The solutions contained 5 meq/l each of K and the test cation. The anion was Br and the pH adjusted and controlled as previously described. All four of the test cations significantly reduced K absorption: Li, Na, and Cs to approximately the same degree and Rb to a greater extent at the lower pH values. Other data showed the depressing effect to be mutual, i.e., the absorption of the test cation also was decreased in the presence of K. The necessity of relating ion competition to pH is clearly illustrated. At pH 5, Rb decreases the absorption of K by about 80%. At pH 7 the decrease at the same concentration of Rb is about 55%.

Not only is Ca effective in enhancing the absorption of K, but it is equally effective in decreasing the loss of previously absorbed K when roots are subjected to low pH. The data in table I were obtained from an experiment in which roots without prior treatment were placed for 3 hours in solutions at different pH values. One series consisted of distilled water adjusted to various pH values with HBr. The other series contained 5 meq/l of Ca as CaBr_2 in addition. The initial K content of the roots was 13.6 meq/Kg fresh weight. At pH 5 no K was lost in either series. As the pH decreased considerably greater losses of K took place in the minus Ca series as compared to the plus Ca series.

The interrelationship between K, Li, and Ca was examined more thoroughly. The absorption of K from solutions of KBr, KBr + LiBr, and KBr + LiBr + CaBr_2 , all at 5 meq/l was determined as a function of pH. The data for the three solutions are summarized in figure 3. As shown above, Li represses the absorption of K but the addition of Ca to the K-Li system increases the K absorption to a level comparable to that obtained from a K-Ca system. At no pH value was there any significant uptake of Li from the K-Li-Ca system. Apparently Ca, in mixtures of K and Li, exerts independent effects upon the

FIG. 3. Absorption of K as influenced by Li and Li + Ca at various pH values. All salts present as bromides at 5 meq/l each. Three hour absorption period.

FIG. 4. Absorption of K from 5 meq/l KBr in the presence of increasing amounts of LiBr with and without the addition of 5 meq/l of CaBr_2 . Three hour absorption period, pH 6.5.



two ions. Just as in single salt solutions, K absorption is stimulated at the lower pH values and Li absorption is abolished in the presence of Ca.

The absorption of K in the presence and absence of Ca from solutions containing various ratios of K to Li was compared. One set of solutions contained 5 meq/l of KBr and increasing amounts of LiBr up to 50 meq/l. The other set was similar but contained 5 meq/l of Ca as CaBr_2 . In both sets the pH was controlled at 6.5 and the absorption period was 3 hours. In this particular root material and at these concentrations, Ca has a negligible effect on the absorption of K at pH 6.5. The results are given in figure 4. In mixtures of K and Li, the absorption of K is reduced with increasing concentration of Li. At 50 meq/l of Li, K absorption falls to about 20% of its absorption in the absence of Li. When Ca is present Li essentially exerts no effect even at Li-K ratios of 10:1.

Hydrogen is present on root surfaces in an exchangeable form (20). Burström (3) related the rate of hydrolysis of sucrose by wheat roots to the H adsorbed on the root surfaces. He varied both the hydrolysis and the adsorbed H with equimolar mixtures of NaCl and CaSO_4 . Since there appeared to be a strong relation between Ca and H, an attempt was made to correlate the stimulating effect of Ca and other polyvalent cations with their ability to compete with H on the root surface. A relatively high root to solution ratio of 20 gm roots to 100 ml solution was used. The pH of the solutions of various bromide salts was adjusted to 4.00. Roots with no treatment other than the usual preparatory washing were placed in the solution and the pH measured. To avoid complications induced by metabolic absorption only the pH values at the end of 2 minutes were considered. Contact between the roots and the electrodes was avoided by shielding the latter with a stainless steel screen suspended about 1 cm from the electrodes. The pH values obtained are listed in table II. Although 2 minutes may not have been sufficient time for equilibration, the polyvalent cation treatments did result in lower pH values indicating that these ions were more effective in preventing H sorption than the monovalent cations. In other experiments at higher pH values evidence was obtained suggesting that the untreated roots contain exchangeable H and the polyvalent cations displace more of this H than monovalent cations. In a typical case roots were placed in 0.005 N KBr at pH 5.25. In 2 minutes the pH fell to 4.78. When placed in 0.005 N CaBr_2 the pH dropped to 4.49 in the same time period. In the absence of salts, the pH remained constant at 5.25.

DISCUSSION

The absorption of cations by barley roots cannot be dissociated from hydrogen ion effects. Injury and competition with H and other cations are readily observed (8, 11, 15). The conclusion of Sutcliffe (17) that absorption is insensitive to external pH is not tenable in view of the results reported here and the

above cited references. The effect of pH on the absorption of the alkali metal cations is similar qualitatively but differs quantitatively. These differences in specific behavior appear to be involved when absorption from mixtures of monovalent cations occur. At equimolar concentrations of K and an accompanying monovalent cation the ratio of K absorbed from KBr solution to K absorbed from the mixture varies with pH. This may be quite pronounced in some cases and emphasizes the importance of rigorous pH control during competitive experiments. Quantitative relationships between two competing cations may be determined largely by the pH of the solution. With the material used here there is little interference with K absorption at pH 3.5 or 4 by the added monovalent cations with the exception of Rb. At pH 5 or higher all of the added cations cause a marked depression of K absorption. An examination of figure 2 indicates that in order to draw meaningful conclusions from mixtures of two salts it is necessary to pay considerable attention to pH relationships because of the three way interaction which exists involving H and the two cations.

The relationship between pH and absorption is particularly striking in mixtures of mono and polyvalent cations. The effect of Ca on the absorption of the monovalent cation is both a function of the pH of the external solution and the particular monovalent cation involved. In solutions of K, Rb, and Cs, the presence of Ca may enhance, depress, or have no effect on the absorption of the alkali metal cation depending upon the pH of the solution. The absorption of Na is strongly depressed by Ca over much of the pH range studied. The effect of Ca on Li absorption is

TABLE II
EFFECT OF BARLEY ROOTS ON pH OF VARIOUS SALT SOLUTIONS INITIALLY ADJUSTED TO pH 4.00

ADDED SALT*	pH**
None	4.79
LiBr	4.71
NaBr	4.72
NH_4Br	4.71
KBr	4.69
RbBr	4.70
CsBr	4.74
BeBr_2	4.30
MgBr_2	4.65
CaBr_2	4.59
SrBr_2	4.64
BaBr_2	4.60
AlBr_3	4.00
LaBr_3	4.31
ThBr_4	4.28

* 1 meq/l of indicated salt added to solution of 1 meq/l KBr.

** pH measured 2 minutes after addition of 20 g roots to 100 ml solution. Electrodes separated from roots by wire mesh.

unique in that the presence of Ca almost completely inhibits the absorption over the entire pH range.

These differences in the effect of Ca on the absorption of the various alkali metal cations cannot be correlated with relative rates of absorption of the cations from single salt solutions. The three ions Na, K, and Rb are absorbed at approximately the same rate by the barley root material yet Ca affects the absorption of Na quite differently than it does that of K and Rb. The absorption rates of Cs and Li are similar in the absence of Ca but react very differently to the presence of Ca. These observations and the fact that the Ca effect is a function of pH indicate that a valid explanation must depend upon the interaction of Ca, H, and the specific monovalent cation being absorbed.

If the absorption from mixtures of K and Li is examined, it is observed that there is a mutual influence of one cation upon the other. When the K concentration is maintained constant and the Li concentration is increased, the absorption of K decreases considerably and the Li absorption increases. The presence of Ca completely destroys this relationship: K absorption proceeds as if there were no Li present, and Li absorption is abolished. In order to minimize the complications resulting from the Ca-H interaction with K absorption, this experiment was carried out at pH 6.5 where Ca has little effect upon the absorption of K from KBr solutions with this material. However, the data show that at any pH the addition of Ca to mixtures of K and Li results in absorption approximating that obtained from mixtures of K and Ca at the same pH. It seems reasonable to assume that Li interferes with K absorption at the absorption reaction site and that the action of Ca is to prevent access of Li to this step. It is interesting that this behavior is not confined to barley since an almost identical K-Li-Ca interrelationship was observed with excised pea roots grown and prepared in a similar fashion as the barley roots. Preliminary data also indicated that the partial blocking of Na absorption by Ca results in an increased absorption of K from mixtures of Na and K.

The effect of Ca on the absorption of K, Rb, Cs, and to a lesser extent on Na, at low pH values may be considered in an analogous manner. The absorption of K decreases as the concentration of either H or Li in the solution increases. In both cases Ca is effective in overcoming the depressing effect of the interfering ions to a considerable extent. Roots are known to take up H in an exchangeable form. As might be expected, the higher the valence of the added cation, the more effectively the uptake of H is blocked and the more readily it is displaced. The charge is more important than the species of ion. This is similar to the effect of polyvalent cations on the absorption of K at low pH values (7) and may explain the lack of specificity in stimulation. Since the changes in H concentration occurred within 2 minutes, the site of the exchange reaction is most likely at or near the cell surface. Because of possible changes in the chemical potential of ions adsorbed on surfaces of

cells (13), it does not appear feasible to interpret the results quantitatively.

When untreated roots are placed in dilute acid solution irreversible injury occurs and there is a leakage of cell contents (11). The presence of Ca in the external solution greatly lessens the leakage of K from the roots again suggesting that Ca decreases the uptake of H.

The absorption behavior of an anion may have an appreciable effect on the absorption rate of the accompanying cation (9). The change in Br concentration of the external solution resulting from the addition of CaBr_2 might therefore have influenced the absorption of the cation. If such an effect does exist here, however, it is small compared to the Ca effect. Increasing the Br concentration several fold (as Li Br) in the presence of Ca had essentially no effect on the rate of K absorption. Furthermore a similar effect has been observed even when salts of a poorly absorbed anion were used, i.e., SO_4 salts.

The data of figure 4 can neither be explained on the basis of relative binding affinities of Li, K, and Ca to an absorption site or carrier, nor on the basis of multiple absorption sites. At pH 6.5 Ca almost completely represses Li absorption but has little or no effect on K absorption in the presence or absence of Li, yet Li markedly depresses K absorption in the absence of Ca at this pH. In order to reconcile these observations, it is postulated that the effect of Ca is at some point other than the actual absorption site, possibly at or near the cell surface. Ca is known to alter the properties of cell surfaces. It has been shown, for example, that Ca decreases the charge on root surfaces (2). For an ion to be absorbed it must first cross the barrier imposed by the presence of Ca and then react at the absorption site. The Ca modified surface acts as an efficient barrier for H and Li, less so for Na, and least if at all for K and presumably Rb and Cs. In such a system the interaction between two monovalent cations in the absorption process would be a function of the selectivity of the barrier and the relative rates of absorption of the cations reaching the absorption site. It would thus appear that the stimulation of K absorption by Ca is in reality a blocking of an interfering cation such as H or Li.

SUMMARY

Hydrogen ion has been shown to influence the absorption of monovalent cations in single salt solutions. This influence is reflected in the absorption behavior from mixtures of the monovalent cations and is instrumental, in part, in determining the relative absorption rates. The effect of Ca on absorption is likewise related to pH as well as the specific monovalent cation. The absorption of K, Rb, and Cs is enhanced at low pH by Ca; Na only slightly. Li absorption is repressed almost completely by Ca at all pH values. In mixtures of Li and K the presence of Ca very effectively blocks Li absorption and eliminates the Li interference with the absorption of K. The H-Ca interaction is similar to the Li-Ca inter-

action and accounts for the stimulating effect at low pH. A correlation exists between the charge of a cation and its effectiveness in displacing H on the root surface.

To explain the interrelationships in the Li-K-Ca system or the H-K-Ca system, it is postulated that the presence of Ca in the solution creates a barrier probably at the cell surface. This barrier which is particularly effective in blocking Li and H determines the relative amounts of the cations reaching the absorption site. The stimulating effect of Ca is considered to be essentially a blocking of interfering ions.

ACKNOWLEDGEMENTS

The writers are indebted to Mr. C. K. Stromberg for preliminary work on the Li-Ca system and to Mr. H. M. King for technical assistance throughout this study.

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