

Influence of Calcium and Magnesium on Manganese Absorption¹

Eugene V. Maas², David P. Moore, and Benjamin J. Mason³

Department of Soils, Oregon State University, Corvallis, Oregon 97331

Received August 16, 1968.

Abstract. Mutual effects between Mn, Ca, and Mg were studied during steady-state absorption experiments with excised barley roots. Calcium appeared to enhance the rate of Mn absorption; whereas, Mg had a highly depressive effect. The combination of both Ca and Mg was even more inhibitory to Mn absorption than Mg alone. Manganese had no effect on the usual negligible Ca absorption by this tissue, but effectively inhibited the absorption of Mg. Although divalent cation absorption from the Ca-Mg-Mn system was essentially nil, K absorption was greatly stimulated in the presence of these cations.

These mutual effects and others reported in the literature are explained by the hypothesis that selectivity in ion absorption results from cation-induced conformational changes in the structure of the carrier molecule.

In a previous paper (10), evidence was presented for the metabolic transport of Mn into excised barley roots. Absorption was relatively rapid and was affected by concentration, pH, *etc.*, much as that of other inorganic cations. To further study the mechanism of Mn absorption and its similarities to that of other metabolically absorbed cations, the influence of several monovalent, divalent and trivalent cations on the absorption of Mn was examined. The effects of 2 of these cations, Ca and Mg, are discussed here.

The role of Ca in regulating the absorption of ions is well recognized. Its effects on the uptake of the monovalent, alkali cations range from highly stimulatory to strongly inhibitory, depending on the ion and the H⁺ concentration (8). Furthermore, Ca has been found to be highly effective in controlling the relative selectivity of absorption of these ions from mixed solutions (4,7). The effect of Ca on the absorption of divalent cations is also diverse. Whereas Ca and Sr are generally considered to be mutually competitive (5), the marked inhibition of Mg absorption by Ca was shown to be non-competitive (15). Although Mg seemingly is unimportant in modifying the selectivity of barley-root tissue (4,7), it exhibits its own peculiar effects. It re-

sembles Ca in stimulating the absorption of K (19), yet contrasts with Ca in having no inhibitory effect on the absorption of Li (7). These diverse regulatory effects, together with others reported here and elsewhere (5), emphasize the importance of the cationic environment in controlling the selectivity of the cell membrane. On the basis of considerable recent evidence that metal ion activation of enzymes involves configurational changes, the hypothesis is promoted that selectivity in ion transport results from cation-induced conformational changes in the structure of the membrane or ion carrier(s).

Materials and Methods

Excised roots of 5-day-old barley seedlings (*Hordeum vulgare* L., var. Trebi) were used in all experiments. The plants were grown and the roots were prepared and treated as described previously (10). The experimental temperature was $25 \pm 0.5^\circ$ and the pH was 5.0 ± 0.2 maintained by small additions of 0.1 N HCl. Distilled water and analyzed reagent-grade chloride salts were used in all experiments. Absorption periods of 1 and 6 hr were chosen on the basis of the previous work so that rates of metabolic absorption occurring during the steady-state phase could be measured (10). Other time-course experiments conducted during the course of this study verified that steady-state conditions did prevail. The rates, calculated from the change in content during the 5-hr interval, are expressed in meq of ion absorbed per kg of fresh roots per 5 hr. Negative values indicate a net efflux during the 5-hr absorption period. The experiments were terminated by collecting the roots on a nylon screen and washing with 3 liters of distilled water. After drying overnight at 70° and wet ashing in nitric-perchloric acid,

¹ This technical paper No. 2528 of the Oregon Agricultural Experimental Station is based on work performed under contract No. AT (45-1)-1547 with the United States Atomic Energy Commission.

² Present Address: United States Salinity Laboratory, USDA, ARS, SWCRD, P. O. Box 672, Riverside, California 92502.

³ Present Address: United States Public Health Service, S. W. Radiological Health Laboratory, 4505 Md., Parkway 00, Las Vegas, Nevada 89101.

the root tissue was analyzed for Mn, Ca, and Mg by atomic absorption spectrophotometry. Calcium and Mg analyses were made in the presence of 1500 ppm Sr. Strontium has been reported to suppress satisfactorily any interferences from phosphate, sulfate, aluminates, or silicates (3). Potassium was determined with a flame emission spectrophotometer. The initial Mn, Ca, Mg, and K contents of the fresh root tissue were <0.1, 2.7, 10, and 20 meq/kg, respectively.

Results

In view of the similarity between Mn and Mg uptake (10, 15), the question arose whether these 2 divalent ions were mutually competitive. In separate experiments, the effects of increasing concentrations of 1 ion on the absorption of the other were evaluated. Magnesium absorption was measured from 5.0 meq $MgCl_2$ per liter with Mn varying from 0 to 5.0 meq/liter. Absorption of Mn was determined from 0.05 meq $MnCl_2$ per liter in the presence of 0 to 0.1 meq Mg per liter. The results of these experiments, summarized in tables I and II, show that Mn and Mg had a mutually depressing effect on the metabolic absorption of each other. At equivalent concentrations of Mg and Mn (5.0 meq/liter), the absorption rate of Mg was reduced to only 8 % of its rate from a single-salt solution (table I). In the 0.05 meq/liter range, the rate of Mn absorption was reduced over 50 % by the addition of an equivalent amount of Mg (table II). Even a concentration of the interfering ion only one-tenth that of the ion studied substantially depressed the absorption rate. It is interesting that at equal concentrations of these 2 ions in mixed-salt solutions, Mn absorption considerably exceeded that of Mg.

These results also reveal a striking similarity between the effects of Ca and Mn on the absorption of Mg by excised barley roots. A comparison of table I with the results of Moore, Overstreet, and Jacobson (15) shows that these 2 ions were equally effective in inhibiting Mg absorption. Parallel effects were also found in the response of Mg to

Table I. *Effects of Increasing Mn Concentration on Mg Absorption*

Magnesium concentration was 5.0 meq/liter and the pH was 5.

Mn concn.	Absorption rates	
	Mg	Mn
<i>meq/l</i>	<i>meq kg⁻¹ 5-hr⁻¹</i>	
0.000	14.5	0.0
0.001	14.8	0.0
0.01	14.3	0.2
0.05	13.4	0.5
0.1	11.7	0.7
0.5	4.4	1.8
1.0	2.5	2.6
5.0	1.1	4.7

pre-treatments of Ca and Mn. A 30-min exposure of the excised root material to 5.0 meq $CaBr_2$ per liter had no effect on subsequent absorption of Mg (15). In a similar experiment, we found that pre-treatment in various concentrations of $MnCl_2$ (0.01–10.0 meq/liter) failed to have any appreciable residual effect.

Table II. *Effects of Increasing Mg Concentration on Mn Absorption*

The ambient concentrations of Mn and Ca (where present) were each 0.05 meq/liter. The pH was 5.

Mg concn.	Absorption rates				
	Ca absent Mn	Mg	Mn	Ca present Mg	Ca
<i>meq/l</i>	<i>meq kg⁻¹ 5-hr⁻¹</i>				
0.000	3.6	—0.4	3.9	—0.1	0.2
0.001	3.7	—0.2	3.4	—0.1	0.4
0.005	2.6	0.0	2.4	—0.1	—0.1
0.010	2.7	0.0	1.9	0.1	0.1
0.025	2.0	0.2	1.2	0.7	0.1
0.05	1.7	0.4	0.8	0.8	0.0
0.10	1.5	0.7	0.6	0.8	0.0

The effect of Ca on the rate of Mn absorption is presented in the first column of table III. An appreciable stimulation of Mn absorption resulted from the presence of Ca at concentrations up to 0.5 meq/liter, a Ca:Mn ratio of 10:1. While higher Ca concentrations exerted a depressing effect, competition between Ca and Mn for the same transport site does not appear likely. This follows from both the Ca stimulation of Mn absorption and the nearly complete lack of Ca absorption by this tissue (table III and ref. 14). Even at a Ca concentration of 10.0 meq/liter, only 0.2 meq of Ca/kg was absorbed in 5 hr.

Since Ca markedly inhibited the absorption of Mg by this tissue (15) and slightly stimulated that of Mn, the question immediately arose whether Ca, by blocking Mg absorption, would eliminate Mg inter-

Table III. *Effects of Increasing Ca Concentration on Mn Absorption*

The ambient concentrations of Mn and Mg (where present) were each 0.05 meq/liter. The pH was 5.

Ca concn.	Absorption rates				
	Mg absent Mn	Ca	Mn	Mg present Ca	Mg
<i>meq/l</i>	<i>meq kg⁻¹ 5-hr⁻¹</i>				
0.00	2.9	—0.2	1.3	—0.1	0.1
0.01	3.1	—0.1	0.8	—0.1	0.1
0.05	3.5	0.0	0.6	—0.1	0.7
0.10	3.7	0.2	0.5	—0.1	0.4
0.50	3.3	0.1	0.8	—0.1	0.7
1.0	2.6	0.1	0.9	0.0	0.5
5.0	1.3	0.2
10.0	0.9	0.2

ference with Mn absorption. The results in tables II and III showed that the individual effects of Ca and Mg on Mn absorption were functions of their concentration in the ambient solution. Consequently, it seemed advisable to study increasing concentrations of 1 ion (Ca or Mg) in the presence of the other. In the first experiment, Mg concentrations were varied from 0 to 0.1 meq/liter. The concentrations of Ca and Mn were each 0.05 meq/liter. The results are presented with the previous data in table II. A direct comparison can be made between the data obtained in the absence and presence of Ca as all the data presented in a given table were obtained in 1 experiment. The influence of Mg in the presence of Ca was quite unexpected. Instead of eliminating the Mg interference of Mn absorption, the presence of Ca accentuated inhibition by Mg at all levels studied. Although Ca alone had a slight stimulatory effect on Mn absorption, it is obvious that an exceedingly small amount of Mg was capable of reversing this effect. As little as 0.001 meq of Mg per liter converted an 8% Ca stimulation into an 8% reduction (table II). An important observation in this experiment was the extremely low total cation absorption from the Ca-Mg-Mn solution (table II). At equivalent solution concentrations (0.05 meq/liter), Ca absorption was nil and the sum of the Mg and Mn absorption rates was only 1.6 meq kg^{-1} 5-hr⁻¹. Although Ca absorption by excised barley roots is known to be negligible (14), both Mn and Mg are rapidly absorbed from single-salt solutions (10, 15). It should also be noted that while Mn absorption exceeded that of Mg in the absence of Ca, the rates were the same when Ca was present.

The second experiment was designed to study the effect of increasing concentrations of Ca in the presence of Mg. Manganese absorption from solutions of 0.05 meq MnCl₂ per liter was measured at Ca concentrations varying from 0 to 1.0 meq/liter. Magnesium was present in all treatments at 0.05 meq/liter. Again, the results are shown with previous data (table III). These data show, even more strikingly, the inhibitory effect of Ca when Mg is present. Whereas Ca at 0.1 meq/liter enhanced the absorption of Mn in the absence of Mg, it markedly reduced Mn absorption in the presence of Mg. As previously noted, the total divalent ion absorption from the Ca-Mg-Mn solutions again was extremely low considering the magnitude of absorption of Mg and Mn from single-salt solutions. It is difficult to see how these data can be explained on the basis of competition. The influence of these ions on the absorption of each other appears to be exerted on the carrier(s) at some point other than the transport site. As a result of this interaction, the ability of this tissue to absorb these divalent cations was severely restricted. To determine whether transport, in general, was impaired under these conditions, the effect of Ca, Mg, and Mn on the absorption of K was evaluated. The experiment included both indi-

Table IV. *Effects of Ca, Mg, and Mn on K Absorption*

The concentration of each ion was 5.0 meq/liter with K present in all solutions. The pH was 5.

Treatment	K	Absorption rates		
		Ca	Mg	Mn
<i>meq kg⁻¹ 5-hr⁻¹</i>				
Control	20.1			
Ca	42.0	-0.1		
Mg	36.5		4.5	
Mn	20.9			9.3
Ca + Mg	42.4	-0.2	0.5	
Ca + Mn	22.3	0.0		7.8
Mg + Mn	33.2		0.2	4.1
Ca + Mg + Mn	42.8	-0.2	0.5	0.8

vidual treatments of Ca, Mg, and Mn and various combinations of these ions (table IV). It is immediately apparent from these results that although the absorption of Mn and Mg again was severely inhibited in the Ca-Mg-Mn system, K absorption was greatly enhanced. Both Ca and Mg markedly increased the absorption of K while Mn at this concentration had little or no effect. It might be noted, however, that Mn at lower concentrations also stimulates K absorption (7, 10). Combinations of any 2 divalent cations had varying stimulatory effects. The combined effect of all 3 cations (Ca + Mg + Mn) was comparable to the highly stimulatory effect of Ca alone. This was surprising in view of the negligible absorption of divalent cations from this system.

As all of the above data were obtained at pH 5, it seemed advisable to evaluate the Ca-Mg-Mn interaction at other H⁺ concentrations. The importance of the hydrogen ion in interrelationships between ions was demonstrated by Jacobson, Moore and Hannapel (8). An experiment was designed to study the effects of Ca and Mg individually and together on the absorption of Mn in the pH range of 4 to 7. Although the data obtained at pH 7 are open to question due to possible precipitation of Mn in the ambient solution (10), the data were included to give some indication of the relative effects of the treatments (Fig. 1). As found previously (10), Mn absorption increased rapidly with decreasing H⁺ concentration up to pH 6 (Fig. 1). In the presence of Mg, Mn absorption was also pH dependent; however, the effect of pH was greatly reduced as Mg had an even greater relative inhibitory effect at pH 6 than at pH 5. Unlike the results in tables II and III, the stimulatory Ca effect was not evident in this experiment, neither at pH 5 nor at other H⁺ concentrations, with the possible exception of pH 7. In view of the depressive effect on Mn absorption by exceedingly small amounts of Mg in the presence of Ca, the absence of a stimulatory Ca effect may have been due to a critical concentration of Mg in the treatment solutions. Slight contamination or a greater than usual loss of indigenous Mg from the root tissue could have contributed to the threshold level. A loss

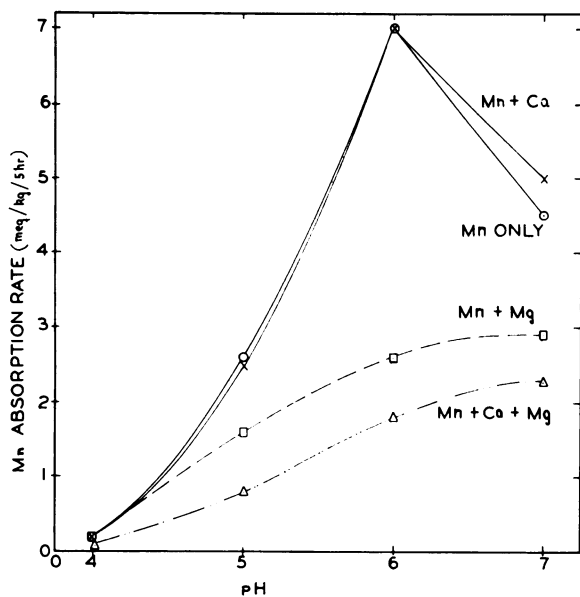


FIG. 1. Effects of Ca and Mg on the absorption of Mn at various pH values. The concentration of each cation was 0.05 meq/liter.

of only 1 meq/kg would increase the Mg concentration of the ambient solution by 0.001 meq/liter. As seen in table III, this could have been sufficient to eliminate any stimulatory effect from Ca. Nevertheless, Mn absorption as a function of pH was affected similarly in both the absence and presence of Ca.

Discussion

In this investigation, as well as in many others, one becomes increasingly aware of the many varied and diverse effects that cations exert on the absorption of one another. In some cases, the regulatory effects are relatively nonspecific, in others, they are highly specific. Whereas polyvalent cations react similarly in stimulating K and Rb absorption, they react quite differently with respect to other ions. An excellent example is the difference in effects of Ca and Mg on Mn absorption. Calcium slightly stimulated the absorption of Mn (table III), whereas Mg had a marked inhibitory effect (table II). The contrasting effects of Ca and Mg were also emphasized in studies of Li absorption (7,8). In that case, the effects of Ca and Mg were essentially reversed from their effects on Mn. Calcium almost completely blocked Li transport, yet Mg had surprisingly little effect. Additional evidence of the contrasting behavior of Ca and Mg was shown for the selective absorption of Na, K, and Rb (4,7). A very small amount of Ca effectively increased the K/Na and Rb/Na absorption ratios by enhancing the absorption of K or Rb and decreasing that of Na. In contrast, Mg was relatively ineffective. A com-

parison of these polyvalent cation effects vividly illustrates that the regulatory effects of cations are often peculiar to the ion involved. Yet none of these contrasting effects can be explained by mutual competition for the same transport site. The regulatory action, therefore, appears to have resulted from binding at some site on the carrier other than the actual absorption site.

Other non-competitive interactions between the divalent cations, Ca, Mg, and Mn lend further support to this conclusion. As noted in the results, the absorption characteristics of Mg and Mn were very similar. Both cations were rapidly absorbed from single-salt solutions and both behaved similarly as a function of pH (10,15). However, on the basis of the nearly complete mutual inhibition of each other's absorption (tables I and II), 2 apparently different transport sites were involved. This was also indicated by the contrasting effect of Ca on Mn (table III) and on Mg absorption (15). Whereas, Mn absorption became more rapid upon addition of Ca, Mg absorption was greatly reduced. A plausible explanation of these results is a cation-induced modification of the ion carrier(s) so that in the Mg-Mn system, neither Mg nor Mn were readily accommodated. Calcium, added to single-salt solutions of these ions, affected these 2 sites differently: Mg sites became less accessible and Mn sites became more accessible.

The strongest evidence for cation-induced changes in the carrier(s) comes from the combined effect of Ca and Mg on Mn absorption. As mentioned above, Mn absorption was non-competitively inhibited by Mg and stimulated by Ca, respectively. In view of the inhibitory effect of Ca on Mg absorption (15), one would expect increased Mn absorption upon addition of Ca to the Mg-Mn system: that is, by blocking Mg, Ca would prevent Mg interference of Mn transport while exerting its own stimulatory influence. Several parallel relationships exist in which Ca exerts this very effect. For instance, by inhibiting the absorption of Li, Ca completely eliminated Li interference with K absorption (8). Calcium has also been found to reduce competition between K and Na (4,7) and between K and Mg (13). In both cases, K absorption was enhanced while that of Na and Mg was reduced or abolished. However, the effect of Ca on Mn absorption in the presence of Mg was just diametrically opposite of that expected. As illustrated in table II, Mg inhibition of Mn absorption was greatly accentuated in the presence of Ca. Obviously, the combination of Ca and Mg had a greater inactivating effect on the Mn carrier than did Mg alone. Furthermore, absorption of Mg was also slight in this system because of the inhibitory effects of both Ca and Mn. Ca absorption, as usual, was completely absent. Nevertheless, while this tissue was nearly incapable of absorbing divalent cations from the Ca-Mg-Mn system, its capacity to absorb K was certainly not impaired. As shown in table IV, K absorption was greatly

enhanced in the presence of Ca, Mg, and Mn. This observation is especially important as it emphasizes the fact that both stimulatory and inhibitory effects may be exerted by the same cations at the same time and in neither case were mutually competitive effects involved. These highly regulatory effects, together with other non-competitive interactions reported in the literature, clearly illustrate that selectivity cannot be attributed to any one ion. Rather selective transport of ions is a function of the nature and concentrations of all ions present in the ambient solution and perhaps more precisely, at the membrane-solution interface. Viets (19) suggested years ago that the regulatory influence of cations resulted from their direct effects upon the protoplasmic membrane. This explanation has been further elaborated on in subsequent work by Jacobson and co-workers (7, 8, 15). They postulated that Ca and other polyvalent cations acted by altering the structural configuration of surface groups thus changing the permselective properties of the cell surface.

The most intriguing mechanism to explain selectivity in ion transport is that the regulatory effects of cations result from cation-induced conformational changes in the structure of the carrier(s). The action of the cations is visualized to be at some point other than the actual transport site. By binding to critical reactive sites on the carrier molecule, the cation or cations conceivably bring about a change in the shape or conformation of the carrier itself. As a consequence of this conformational change, access to potential transport sites would be permitted for some ions and denied to others. Either the number of available transport sites or the affinity of the carrier for a particular ion would increase or decrease as the case may be. This concept, developed earlier (9) and held by Bange and Meijer (1) and Rains and Epstein (17) is particularly attractive in view of the growing evidence that cation-activation of enzymes arises from cation-induced structural and configurational changes in the enzyme protein. Evans and Sorger (6) have summarized the work supporting this function for the univalent cations. Other investigators, utilizing techniques of nuclear magnetic resonance and ultraviolet difference spectroscopy, have shown that Mn and other divalent cations induce conformational changes in pyruvate kinase (11, 12, 18). Bygrave (2) has pointed out still other examples of conformational changes that accompany cation activation of enzymes and has discussed the importance of the cationic environment and its role in regulating metabolism *via* this mechanism.

While this hypothetical mechanism is based *a priori* on the existence of carriers, evidence for such membrane components is accumulating. Pardee (16), in summarizing the current status of such research, has reported the isolation and crystallization of a sulfate-binding protein that is strongly implicated in sulfate transport in bacteria.

Literature Cited

1. BANGE, G. G. J. AND C. L. C. MEIJER. 1966. The alkali cation carrier of barley roots: A macromolecular structure? *Acta Botan. Neerl.* 15: 434-50.
2. BYGRAVE, F. L. 1967. The ionic environment and metabolic control. *Nature* 214: 667-71.
3. DAVID, D. J. 1960. The determination of exchangeable sodium, potassium, calcium, and magnesium in soils by atomic-absorption spectrophotometry. *Analyst* 85: 495-503.
4. EPSTEIN, E. 1961. The essential role of calcium in selective cation transport by plant cells. *Plant Physiol.* 36: 437-44.
5. EPSTEIN, E. 1962. Mutual effects of ions in their absorption by plants. *Agrochimica* 6: 293-322.
6. EVANS, H. J. AND G. J. SORGER. 1966. Role of mineral elements with emphasis on the univalent cations. *Ann. Rev. Plant Physiol.* 17: 47-76.
7. JACOBSON, L., R. J. HANNAPEL, D. P. MOORE, AND M. SCHAEDELE. 1961. Influence of calcium on selectivity of ion absorption process. *Plant Physiol.* 36: 58-61.
8. JACOBSON, L., D. P. MOORE, AND R. J. HANNAPEL. 1960. Role of calcium in absorption of monovalent cations. *Plant Physiol.* 35: 352-58.
9. MAAS, E. V. 1966. Manganese absorption by barley roots. Ph.D. Dissertation, Oregon State University.
10. MAAS, E. V., D. P. MOORE, AND B. J. MASON. 1968. Manganese absorption by excised barley roots. *Plant Physiol.* 43: 527-30.
11. MILDVAN, A. S. AND M. COHN. 1965. Kinetic and magnetic resonance studies of the pyruvate kinase reaction. I. Divalent metal complexes of pyruvate kinase. *J. Biol. Chem.* 240: 238-46.
12. MILDVAN, A. S. AND R. A. LEIGH. 1964. Determination of co-factor dissociation constants from the kinetics of inhibition of enzymes. *Biochim. Biophys. Acta* 89: 393-97.
13. MOORE, D. P. 1960. Uptake of calcium and magnesium by excised barley roots. Ph.D. Dissertation, University of California, Berkeley.
14. MOORE, D. P., L. JACOBSON, AND R. OVERSTREET. 1961. Uptake of calcium by excised barley roots. *Plant Physiol.* 36: 53-57.
15. MOORE, D. P., R. OVERSTREET, AND L. JACOBSON. 1961. Uptake of magnesium and its interaction with calcium in excised barley roots. *Plant Physiol.* 36: 290-95.
16. PARDEE, A. B. 1968. Membrane transport proteins. *Science* 162: 632-37.
17. RAINS, D. W. AND E. EPSTEIN. 1967. Sodium absorption by barley roots: Role of the dual mechanisms of alkali cation transport. *Plant Physiol.* 42: 314-18.
18. SUELTER, C. H. 1966. Studies on the interaction of substrate and monovalent and divalent cations with pyruvate kinase. *Biochemistry* 5: 131-39.
19. VIETS, F. G., JR. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. *Plant Physiol.* 19: 466-80.