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Manganese Absorption by Excised Barley Roots[1]

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Abstract. **Short-term absorption studies with 5-day-old excised barley roots revealed that the basic aspects of Mn absorption were similar to those of other metabolically absorbed cations. Following an initial non-metabolic equilibration with the root, Mn was absorbed for several hours at a slower steady-state rate comparable to that of other inorganic cations. Complete or nearly complete inhibition of the steady-state phase by low temperature, dinitrophenol, and azide provides strong evidence that Mn transport into this tissue was metabolically mediated. Within limits, the rate of transport was strongly dependent upon the concentrations of Mn and the hydrogen ions in the ambient solution. Absorption increased rapidly with increasing concentrations of Mn up to 1 meq per liter. Above this concentration, the rate leveled off, apparently due to a saturation of the transport mechanism. Within the physiological pH range in which Mn is soluble (below pH 7), absorption increased greatly with decreasing hydrogen-ion concentration.**

Although numerous investigations of ion uptake by excised root tissue have been conducted, relatively little attention has been given to Mn. In 1934, Laine (4) studied the absorption of Mn and other ions by decapitated roots of *Phaseolus multiflorus*. Analyses of both the exudate and the roots revealed an accumulation and retention of Mn by the roots. Using slices of carrot tissue, Stiles and Skelding (10) found that Mn uptake occurred in 2 distinct phases, a rapid initial uptake followed by a prolonged slower absorption. This 2-step process, characteristic of the uptake of other cations, was interpreted as consisting of the usual nonmetabolic and metabolic phases, respectively. Subsequent experiments with red beet discs confirmed this interpretation (9). More recently, Page and Dainty (6) have reported that the uptake of Mn by excised oat roots is nonmetabolic. Both the characteristic rapid initial uptake and the following slower steady-state uptake occurred independently of metabolic activity.

In contrast, studies described in this paper have revealed a very active metabolic accumulation of Mn by excised barley roots. Furthermore, the absorption of Mn with respect to the ambient concentration and pH resembled that of other metabolically absorbed cations.

Materials and Methods

Except for some minor modifications, the experimental procedures used were patterned after those of Jacobson *et al.* (2). Trebi barley seeds (*Hordeum vulgare* L.) were soaked in continuously-aerated distilled water for 24 hours. After soaking, the germinated seeds were distributed on cheesecloth-covered stainless steel racks. The racks were placed in fiberglass animal cages containing 3 liters of culture solution, 0.1 mM each in $Ca(NO_3)_2$, KH_2PO_4 and $MgSO_4$. The solutions were aerated throughout the growth period and were renewed after 3 days. Plants were grown in continuous darkness at a temperature of 25 ± 1°. On the fifth day after the seeds began soaking, the roots were harvested. The excised roots were cut into approximately 1 cm lengths and washed several times in distilled water. Excess water was removed by spinning the root material for 5 minutes at 65 × *g* in a perforated stainless steel basket. Seven grams (fr wt) of roots were immersed in 7 liters of test solution which was aerated continuously throughout the absorption period. The temperature was 25 ± 0.5° and, except where noted, the pH was maintained at 5.0 ± 0.2. When required, downward adjustments in pH were made with small additions of 0.1 N HCl. To obtain solutions with a pH of 6.0 or greater, nylon bags containing analytical grade Bio-Rad AG1-X8 anion exchange resin in the OH^- form were dipped into the solution (7). All salts used were chlorides of analyzed reagent grade.

At the end of the absorption period, the roots were collected on a nylon screen and washed by pouring 3 liters of distilled water over them. After drying overnight at 70°, the roots were digested in nitric and perchloric acids and diluted to 100 ml.

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Manganese was determined directly by atomic absorption spectrophotometry. Potassium analyses were made on a flame emission spectrophotometer.

Results and Discussion

The uptake of Mn from 0.05 meq $MnCl_2$ per liter (approx 1.4 ppm) is shown as a function of time in figure 1. Following a rapid initial uptake, Mn was absorbed at a steady-state rate up to 10 hours. The characteristic 2-phase uptake process was very similar to that of several other actively absorbed cations such as Mg and the alkali metals. Both the magnitude of the absorption rate (0.5 meq kg^{-1} hr^{-1}) and the steady-state nature of the second phase suggested that a metabolic mechanism was involved. However, in a comparable time course of uptake by 4-week-old oat roots, Page and Dainty (6) found that Mn uptake was virtually unaffected by metabolic inhibitors.

To determine the role of metabolism in the absorption of Mn by 5-day-old barley roots, Mn uptake was measured in the presence of 10^{-5} M 2,4-dinitrophenol (DNP), 10^{-4} M sodium azide, and at a temperature of 0.5°. In order to evaluate the effect of these metabolic inhibitors on the steady-state phase, absorption periods of 1 and 6 hours were used. The rate of absorption, calculated from the change in Mn content during this 5-hour interval, is expressed in meq of Mn absorbed per kg of fresh roots per 5 hours. As shown in table I, both DNP and low temperature completely inhibited the absorption of Mn after the first hour. Sodium azide was only slightly less effective in inhibiting Mn absorption. That this effect was more than a depressive effect of Na on Mn absorption is indicated by a considerably greater inhibition by sodium azide than by an equivalent concentration of NaCl.

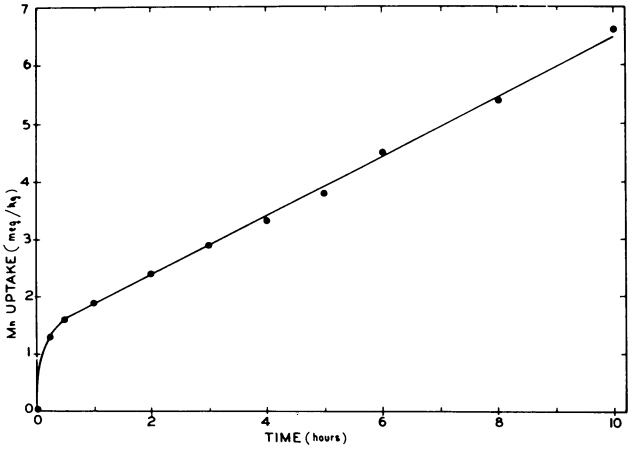

FIG. 1. Uptake of Mn as a function of time from 0.05 meq $MnCl_2$ per liter at pH 5.

Table I. *Effect of Metabolic Inhibitors on Mn Uptake*

The concentration of $MnCl_2$ was 0.05 meq/l and the pH was 5.

Treatment	Uptake 1 hr	Uptake 6 hr	Absorption rate
	meq/kg		*meq* kg^{-1} 5 hr^{-1}
Control	2.0	4.6	2.6
DNP (10^{-5} M)	1.9	1.9	0.0
0.5°	1.5	1.5	0.0
NaN_3 (10^{-4} M)	1.6	2.0	0.4
NaCl (10^{-4} M)	1.6	3.4	1.8

Although the metabolic inhibitors also exhibited slight effects during the first hour, the results are difficult to interpret since uptake undoubtedly included both non-metabolic and metabolic components. The effectiveness of these inhibitors during the second phase is strong evidence that Mn transport is a metabolically-mediated process. A similar observation has been made with excised 6-day-old wheat roots in which DNP depressed the absorption of Mn after 1 hour (1, p 86). These findings demonstrate that Mn absorption by excised roots is a process which is basically the same as that for other divalent cations, such as Mg and Zn (5, 8), as well as that for the monovalent, alkali cations. The conclusion that Mn absorption is a metabolic process directly contradicts that obtained by Page and Dainty (6). A possible reason for this discrepancy is the difference in the age and physiological condition of the roots. In the study described here, roots 8 to 12 cm long were readily obtained from 5-day-old barley seedlings. In contrast, the 4-week-old oat roots used by Page and Dainty (6) were only 3 to 8 cm long. Since their oat plants were grown the entire 4 weeks in a nonaerated medium without Mn, we question whether physiologically healthy tissue was obtained.

The influence of hydrogen-ion concentration on the rate of Mn absorption during the steady-state phase from 1 to 6 hours is shown in figure 2. The sharp reduction in the rate of Mn absorption at pH 7.0 was due to the rapid oxidation and precipitation of Mn which occurred in the test solution at this and higher pH values. Consequently, the direct influence of H^+ can only be evaluated at pH 6 or less. In the physiologically favorable pH range, the rate of Mn absorption increased rapidly with decreasing H^+ concentration. This relationship is very comparable to that found for the metabolically absorbed alkali metals and Mg (3, 5). The similarity between the transport of Mn and these cations is also revealed by the effect of concentration on the rate of Mn absorption as shown in figure 3. As before, the rate of absorption was determined for the period between 1 and 6 hours. Consequently, the influence of free space and non-metabolic uptake is not involved in the data presented. Like other cations, the absorption rate for

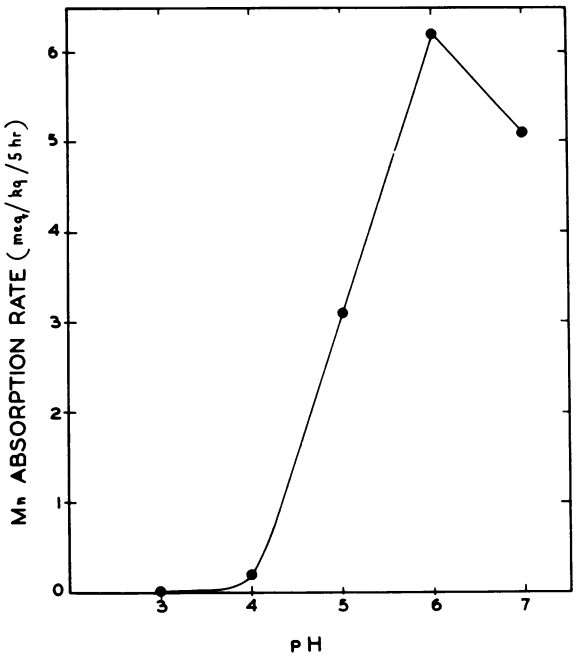

FIG. 2. The effect of pH on the absorption of Mn from 0.05 meq $MnCl_2$ per liter.

Mn was essentially a logarithmic function of the ambient concentration. Manganese absorption increased rapidly with initial increases in concentration up to 1.0 meq per liter. With additional increases, the rate approached a maximum and became essentially independent of the ambient concentration above 5.0 meq per liter. Although absorption reduced the 2 lowest ambient concentrations by about 20 %, this had little effect on the shape of the curve since the concentrations of the other treatment solutions were essentially unchanged. These results suggest that the transport mechanism became saturated at about 3.0 meq per liter. Although Mn toxicity might be suspected at the high Mn concentrations, no injurious effects were found at concentrations up to 1.0 meq Mn per liter in the short-term experiments. This conclusion was based on the effects of Mn on the K-absorbing capacity, the rate of respiration, and the leakiness of the root tissue. Neither the absorption of K from 5.0 meq KCl per liter nor the rate of respiration was impaired by additions of Mn up to 1.0 meq per liter. In fact, K absorption was enhanced at lower concentrations of Mn (table II). This stimulatory

Table II. *Effect of Increasing Mn Concentration on K Absorption and Loss*

Mn conc	K[1] Absorption rate	K[2] Loss
meq/l	*meq kg^{-1} 5 hr^{-1}*	*meq kg^{-1} 6 hr^{-1}*
0.000	33.6	...
0.001	...	1.1
0.01	...	0.4
0.1	35.8	0.6
0.5	37.3	...
1.0	33.6	0.6
5.0	26.3	...
10.0	19.8	1.3

[1] K absorption from 5.0 meq KCl per liter at pH 5.
[2] Loss of indigenous K from excised barley roots to single-salt $MnCl_2$ solutions at pH 5. Initial K content = 18.8 meq/kg fresh weight.

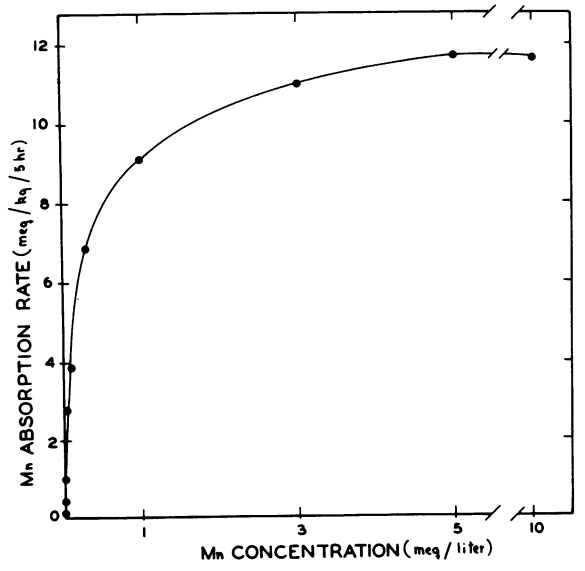

FIG. 3. Absorption of Mn as a function of increasing concentrations of $MnCl_2$ at pH 5.

effect of Mn is in agreement with the observation of Jacobson *et al.* (2). Of course, the reduction in K absorption does not imply toxicity even at higher Mn concentrations since competitive or other effects are likely explanations. Furthermore, the integrity of the cell membranes, as assayed by the loss of indigenous K, was not disrupted by Mn (table II). On the contrary, Mn appeared to function like Ca in maintaining membrane integrity. This effect of Mn has also been observed by Van Steveninck (11). Even in the presence of 10 meq Mn per liter, the root tissue lost less than 7 % of the indigenous K during the entire 6-hour treatment period (table II). Appreciably greater K losses to NaCl and $MgCl_2$ solutions of equivalent concentration were observed.

The influence of various monovalent and polyvalent cations on the absorption of Mn has also been evaluated. Regulatory effects of most of these cations resulting in inhibition, reduction, or stimulation of Mn absorption will be discussed in subsequent papers.

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