Absorption of Copper, Zinc, and Manganese by Sugarcane Leaf Tissue[1]

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Abstract. The absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} by leaf tissue of 4-month old sugarcane plants (*Saccharum officinarum* L., var. H53-263) has been investigated. After the "apparent free space" fraction was desorbed, the absorption of Cu^{2+}, Mn^{2+}, and Zn^{2+} yielded a curve typical of many ion uptake processes when measured as a function of the external concentration. However, only 1 absorption mechanism was evident for each cation. The pH optimum for Cu^{2+} and Zn^{2+} uptake was 5.0 to 6.0, whereas that for Mn^{2+} absorption was 4.5 to 6.0. Absorption was competitively inhibited by H^+, and this inhibition was reversible when 0.5 mM Ca^{2+} was present. Cu^{2+} and Zn^{2+} were absorbed through the same carrier sites, as concluded from their mutually competitive activities. Mn^{2+} was absorbed through a second, independent mechanism. Uptake of each cation was strongly inhibited by uncouplers of oxidative phosphorylation, by Amytal and Nembutal[2], by 5×10^{-2} M succinate, and by ADP and P_i. Absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} was concluded to be coupled to oxidative phosphorylation, and specifically to energy-conservation Site I.

Although the literature abounds with reports related to various aspects of ion uptake by plant tissues, it has only been recently that attention has been directed toward the micro-nutrient elements (1, 3, 14, 18, 22). The dearth of data on micro-nutrient absorption is no doubt due at least in part to the fact that the physiological concentrations of these elements are quite low, as are their rates of absorption. Further, reversible adsorption is a major factor in the uptake of divalent cations and must be taken into consideration. However, as emphasized by Schmid *et al.* (22), these problems can be overcome largely through the application of carefully designed experimental procedures and techniques.

It was reported by Broda (3) that the uptake of Zn^{2+} by barley roots and *Chlorella* was primarily a passive process and was insensitive to metabolic inhibitors. Page and Dainty (18) published similar findings for the absorption of Mn^{2+} by oat roots. However, both of these reports have recently been refuted (14, 22). The data in this paper likewise will be shown to support the contention that the uptake of Zn^{2+} and Mn^{2+} is under metabolic control, as is the absorption of Cu^{2+}.

This study was undertaken to characterize the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} by sugarcane leaf tissue. Effort was directed also toward the elucidation of the specific site(s) in the metabolic processes to which the uptake of these cations is coupled.

Materials and Methods

Plant Material. Sugarcane seedlings (*Saccharum officinarum* L., var. H53-263) were cultured hydroponically in the greenhouse as described earlier (1). The nutrient solutions were renewed at weekly intervals for the first month and at 4-day intervals thereafter. When the plants were 4 months old, the blades of leaves 3 and 4 (the youngest fully-expanded leaves) were removed and immediately brought to the laboratory for use in the experiments.

Nutrient Solutions. The composition of the base nutrient solution was in millimoles/liter: $Ca(NO_3)_2 \cdot 4H_2O$, 3.0; $CaCl_2$, 4.5; KNO_3, 3.0; KH_2PO_4, 1.0; and $MgSO_4 \cdot 7H_2O$, 2.0. In addition, 5 mg/liter of Fe and 1 mg/liter each of Mn, Cu, and Zn were provided as chelates of EDTA. Boron, as H_3BO_3, was provided at a final concentration of 1 mg/liter. All plants to be used in the Cu^{2+} absorption experiments were grown in solutions lacking Cu^{2+} so that the Cu^{2+} concentration of the tissue would be minimized. Similarly, Zn^{2+} and Mn^{2+} were each omitted from the solutions for the plants used in the respective uptake studies. At no time however, did any deficiency symptoms appear on the plants that could be attributed to the absence of these elements. Although no Cu^{2+}, Zn^{2+}, and Mn^{2+} was purposely added to the respective nutrient solutions, the final concentration of each was, in mg/liter: Cu^{2+}, 0.20; Zn^{2+}, 0.12; and Mn^{2+}, 0.14. No purification of the solutions was attempted. The pH was adjusted to 5.7 immediately prior to use.

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[2] Abbreviations: Amytal, 5-ethyl-5-(3-methylbutyl)-barbituric acid, Na salt; Nembutal, 5-ethyl-5-(2-pentyl)-barbituric acid, Na salt; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP, 2,4-dinitrophenol.

Preparation of Tissue Sections. Since the terminal 10-cm section of the leaf blade contained unusually high concentrations of Cu^{2+} and Zn^{2+} as compared to the remainder of the blade, this portion was routinely discarded. After removal of the midrib, the leaf blade was cut into sections measuring 300 μ × 12 mm (1, 24). The leaf sections (50 mg, fr wt), in cheesecloth "teabags" (5), were placed in aerated distilled water for about 2 hr. Immediately before an experiment the tissue was transferred to constantly aerated 0.5 mM $CaCl_2$ solution for 30 min at the experimental temperature, usually 30°. The maximum elapsed time between leaf collection and the start of an experiment was 3 hr, during which time the tissue was continually in aerated water or 0.5 mM $CaCl_2$ solution.

Experimental Methods. Copper as $CuSO_4$, and Zn as $ZnCl_2$, were varied over the range of 0.002 to 0.50 mM. In preliminary experiments it was observed that the absorption of Cu^{2+} and Zn^{2+} was virtually the same whether the counter-ion was SO_4^{2-} or Cl^-. It would have been desirable to increase the upper limit of this range with the aim of investigating the possible occurrence of multiple absorption mechanisms, but external Cu^{2+} and Zn^{2+} concentrations in excess of 0.5 mM caused the influx of high concentrations of Cu^{2+} and Zn^{2+} into the tissue that were inhibitory to photosynthetic O_2 evolution and respiratory O_2 uptake, respectively. Nevertheless, this expands the range of Zn concentrations 50-fold over that studied by Schmid, Haag, and Epstein (22). Mn^{2+} as $MnCl_2$ was varied from 0.002 to 5.0 mM.

All solutions used in these experiments contained 0.5 mM $CaCl_2$, except when Ca^{2+} was being studied as a variable. The solutions were constantly aerated during the 30-min absorption period. Only when time was the variable did the absorption period exceed 30 min. The initial pH was 5.7, unless the effect of pH was under investigation. The pH change during each experiment was less than 0.3 units and was considered to be insignificant in view of the pH optima reported below. To avoid significant changes in the ion concentration of the solutions during the absorption period, a high solution volume to tissue weight ratio (500 or 1000 ml: 50 mg fr wt) was maintained. The temperature was 30 ± 0.5° unless stated otherwise. Under these conditions the rate of uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+} by sugarcane leaf tissue was linear for at least 2 hr.

These experiments were terminated by rinsing the tissue 3 times for 1-min each in cold (8°) 0.5 mM $CaCl_2$. The leaf sections were transferred to 0.5 mM $CaCl_2$ at 8° for 30 min under constant aeration to desorb reversibly-accumulated ions. At the end of the desorption period, the tissue was rinsed in distilled water and drained. Lastly, the tissue was dried for 24 hr at 70°, weighed, and ashed overnight at 480°.

Analytical Methods. The ash was dissolved in 10 ml of 4 N HCl by warming slightly on a hot plate. Dilutions, when necessary, were made with distilled water. The concentrations of Cu^{2+}, Zn^{2+}, and Mn^{2+} in the acid solutions were determined with a Perkin-Elmer Model 290B atomic absorption spectrophotometer. The data reported are the averages of at least 4 replications in all cases. In every case the data have been corrected for the concentrations of Cu^{2+}, Zn^{2+}, and Mn^{2+} originally present in the tissue.

Kinetic Analysis. The graphical methods of Lineweaver and Burk (13) and Hofstee (8) were used for the estimation of the kinetic constants *Km* and Vmax for the absorption processes and to establish the specific mechanisms of inhibition where applicable.

Statistical Analysis. Linear and curvilinear regression techniques were used to plot the curves on the graphs. Student's "t-test" was employed for making statistical comparisons between treatments (25).

Reagents. ADP, succinate, malate, pyruvate, DNP, and NaN_3 were purchased from Nutritional Biochemicals, Cleveland, Ohio. Amytal was the product of Eli Lilly, Indianapolis, Indiana; and Nembutal, a product of Abbott Laboratories, North Chicago, Illinois. DCMU was obtained from E. I. DuPont de Nemours, Wilmington, Delaware. All other chemicals were reagent-grade products of J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Results and Discussion

Absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} as a Function of Time. Figure 1, Curve A depicts the absorption of Cu^{2+} from a 0.1 mM Cu^{2+} solution as a function of

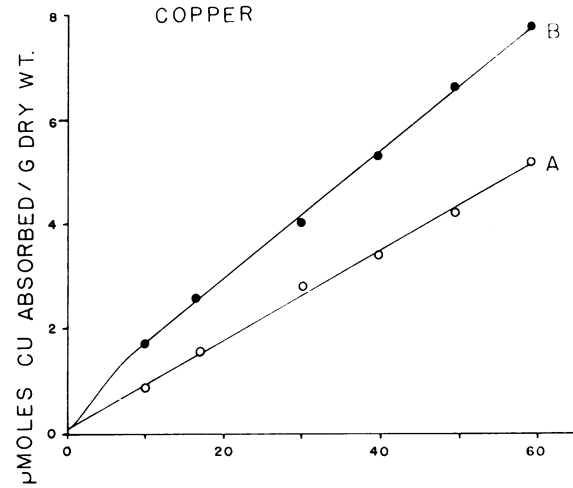

FIG. 1. Absorption of Cu^{2+} from a 0.1 mM Cu^{2+} solution containing 0.5 mM $CaCl_2$. Absorption period 60 min at pH 5.7 and 30°. (Curve A) Tissue desorbed in 0.5 mM $CaCl_2$ at 8° for 30 min. (Curve B) Tissue rinsed 3 times in water at 8° for 1 min each. Curve A represents Cu^{2+} accumulation into the "inner space", whereas Curve B represents total Cu^{2+} uptake including reversible adsorption.

time. Since the tissue was rinsed for 30 min in 0.5 mM $CaCl_2$ after the absorption period, the ions remaining in the tissue were considered to represent the irreversibly-accumulated, or "inner space", fraction. Figure 1, Curve B is plotted from data obtained by rinsing the tissue for 3 1-min intervals in cold (8°) water only. Therefore, fraction B represents the total Cu^{2+} accumulated. By comparing Curves A and B it is readily seen that there was a significant amount of reversible adsorption even when 0.5 mM Ca^{2+} was present during the absorption period. These data thus emphasize the necessity for adequate desorption if only the "inner space" fraction (Fraction A) is to be considered. The patterns of fractions A and B were similar for Zn^{2+} and Mn^{2+}, differing only in the final concentrations of the ions in the tissues. Fraction A—the non-exchangeable "inner space" fraction—will be the subject of this paper.

Absorption as a Function of External Concentration. When the uptake of Cu^{2+} and Zn^{2+} by sugarcane leaf tissue was measured as a function of the external Cu^{2+} and Zn^{2+} concentrations over the range of 0.002 to 0.5 mM, curves typical of other ion uptake systems were observed (fig 2). The tissue levels increased sharply with increasing exogenous Cu^{2+} and Zn^{2+} from 0.002 to 0.1 mM. The absorption system(s) were apparently saturated at 0.1 mM since further increases in external concentrations up to 0.5 mM resulted in virtually no further increases in the Cu^{2+} and Zn^{2+} content of the leaf tissue. The uptake of Mn^{2+} was similar to that of Cu^{2+} in every respect, with the additional observation that the exogenous Mn^{2+} concentration could be increased to 5 mM without causing any further influx of Mn^{2+} into the cells (fig 2). This is similar to the absorption of Mn^{2+} by barley roots as reported recently by Maas *et al.* (14).

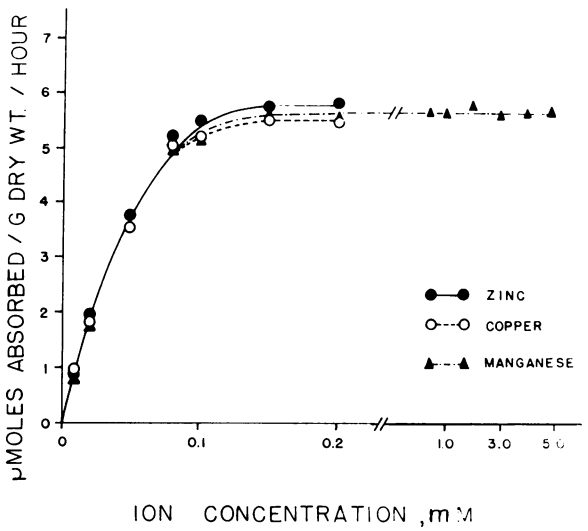

FIG. 2. Uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+} by excised sugarcane leaf tissue as a function of the external concentration of the respective ions. $CaCl_2$ concentration = 0.5 mM. Absorption period, 30 min; pH 5.7; temperature, 30°.

Absorption Kinetics. The kinetic parameters Vmax (μmoles ion absorbed/g dry wt/hr) and *Km* as determined from double reciprocal plots (13) for the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} are summarized in table I.

Effect of pH. The optimum pH for the absorption of Cu^{2+} and Zn^{2+} was 5.0 to 6.0, whereas the optimum for Mn^{2+} uptake was 4.5 to 6.0. Absorption decreased rapidly as the pH was varied beyond these limits. Attention was turned toward attempts to elucidate the specific effects of pH on the absorption system(s). It has been reported (19) that H^+ ion exerted a competitive effect upon Rb^+ uptake by barley roots through 2 mechanisms—A) an irreversible injury mechanism in the absence of Ca^{2+}, and B) a competition between H^+ and Rb^+ for the carrier sites when Ca^{2+} was added to the absorption system. Since the present experiments were all conducted in the presence of 0.5 mM Ca^{2+}, the former effect was presumably minimized. The absorption of each ion—Cu^{2+}, Zn^{2+}, and Mn^{2+}—was studied as a function of the external concentration at pH 4.0 and 5.7. When the data were plotted according to Lineweaver and Burk (13), the effect of H^+ was seen to have the characteristics of competitive inhibition (fig 3, for Zn^{2+}). The Michaelis constants for the competing H^+ were, for Cu^{2+}, 2.46×10^{-5}; for Zn^{2+}, 3.09×10^{-5}; and for Mn^{2+}, 1.37×10^{-5} M. On the basis of the *Km* and Ki values for the primary ion (Cu^{2+}, Zn^{2+}, and Mn^{2+}) and the H^+ ion, respectively, the affinity of these sites for H^+ is about one-half their affinity for Cu^{2+}, one-third that for Zn^{2+}, and about equal to their affinity for Mn^{2+}.

Table I. *Apparent Km and Vmax Values for Uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+} by Sugarcane Leaf Tissue at pH 5.7 and 30° in the Presence of 0.5 mM $CaCl_2$.*

Ion	Km	Vmax
	M	*μmoles ion absorbed/g dry wt/hr*
Cu^{2+}	1.45×10^{-5}	5.37
Zn^{2+}	1.11×10^{-5}	5.88
Mn^{2+}	1.61×10^{-5}	5.35

To this point it has been assumed that the presence of Ca^{2+} has indeed eliminated any irreversible disruption of cellular processes that otherwise may be induced by H^+. This assumption was validated in the following experiment. Before the absorption experiment the leaf sections were pre-treated in a 0.5 mM $CaCl_2$ solution at pH 4.0 for 30 min. The leaf tissue was removed, blotted dry, and placed in the experimental solutions at pH 5.7. These latter solutions contained 0.5 mM Ca^{2+} in addition to one of the following at 0.1 mM: Cu^{2+}, Zn^{2+}, or Mn^{2+}. Controls were pre-treated and run at pH 4.0 and 5.7.

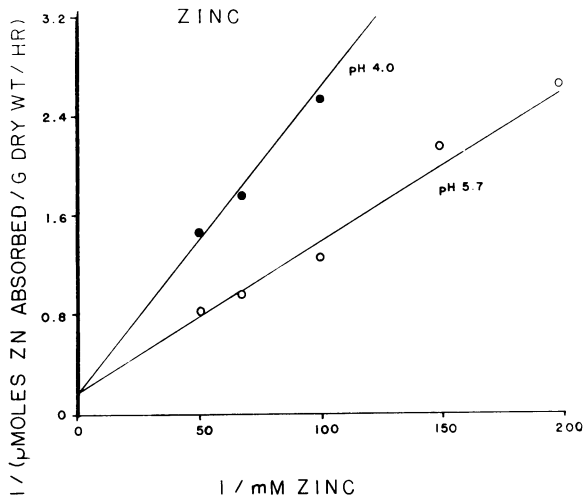

FIG. 3. Double reciprocal plot for the absorption of Zn^{2+} over the external concentration range of 0.005 to 0.02 mM at pH 4.0 and 5.7. Other conditions as for figure 2.

The uptake of Cu^{2+} under these conditions will be discussed as a case in point. At pH 4.0 Cu^{2+} absorption was reduced to 53.4 % of that in the pH 5.7 control. The competitive inhibition of Cu^{2+} uptake by H^+ was almost completely reversible, however. Copper absorption in the tissue pre-treated at pH 4.0 upon re-adjustment to 5.7 was 4.96 μmoles Cu^{2+} absorbed/g dry weight/hr, whereas that of the control pre-treated and run at pH 5.7 was 5.11 μmoles Cu^{2+} absorbed/g dry weight/hr. Thus it was concluded that the decreased uptake of Cu^{2+} due to H^+ was effected through a reversible mechanism when Ca^{2+} was present.

As compared to the pH 5.7 control, at pH 4.0 the uptake of Zn^{2+} and Mn^{2+} was reduced to 52.2 % and 43.9 %, respectively. Upon re-adjustment to pH 5.7 the absorption of each ion once again approached that of the pH 5.7 control: Zn^{2+}, 94.8 % of the control, and Mn^{2+}, 97.9 %.

The inhibition of uptake of these cations above pH 6.0 was similarly investigated. In these cases the tissue was pre-treated in 0.5 mM $CaCl_2$ at pH 7.5 for 30 min, and then absorption was measured from 0.1 mM solutions of the respective ions at pH 5.7. All solutions contained 0.5 mM $CaCl_2$. As compared to the pH 5.7 control, the percents inhibition in the tissue pre-treated at pH 7.5 were, for Cu^{2+}, 87.5; Zn^{2+}, 81.2; and for Mn^{2+}, 84.3. No pH 7.5 control could be included since the ions were heavily precipitated at this pH. Irreversible injury was implicated as the cause of the reduced uptake since the absorption by the pH 7.5 pre-treated tissue upon re-adjustment to pH 7.5 was strongly inhibited as compared to the pH 5.7 control.

Effect of Temperature. The absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} was strongly temperature-dependent. The rates of uptake from 0.1 mM solutions at 30° were, in μmoles absorbed/g dry weight/hr, for Cu^{2+}, 5.11; Zn^{2+}, 5.80; and Mn^{2+}, 5.23. The absorption rates at 10° however, were greatly reduced, averaging 1.94, 1.88, and 2.01 μmole absorbed/g dry weight/hr for Cu^{2+}, Zn^{2+}, and Mn^{2+}, respectively. These data indicate the mediation of metabolism in the absorption processes.

To determine whether or not the temperature-induced reduction in cation absorption was reversible, the following experiment was performed. The leaf sections were pre-treated for 30 min in an aerated 0.5 mM $CaCl_2$ solution at 10°. Absorption of each cation was then measured after re-adjustment of the temperature to 30°. Controls were pre-treated and run at 10° and 30°. In the case of each cation—Cu^{2+}, Zn^{2+}, and Mn^{2+}—absorption in the cold pre-treated tissue after re-adjustment to 30° was within 10 % of the uptake rate in the 30° control. Therefore, it must be concluded that the reduction in cation absorption by low temperature during the absorption period is essentially a reversible inhibition. Schmid *et al.* (22) have reported a similar effect of low temperature (4.5°) upon Zn^{2+} uptake by barley roots, and interpreted this finding as evidence for the metabolic control of Zn^{2+} absorption. However, the reversibility of the temperature effect was not investigated by these workers (22).

Effect of Ca. In the experiments discussed thus far Ca^{2+} has routinely been added at a concentration of 0.5 mM. This is in accordance with the well-known fact that Ca^{2+} is essential for the maintenance of selective ion transport systems in cellular membranes (19). In the experiments to be discussed in this section the uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+} was studied as a function of the external Ca^{2+} concentration. Absorption was terminated by rinsing the tissue in cold (8°) $CaCl_2$ for 3 1-min periods, fol-

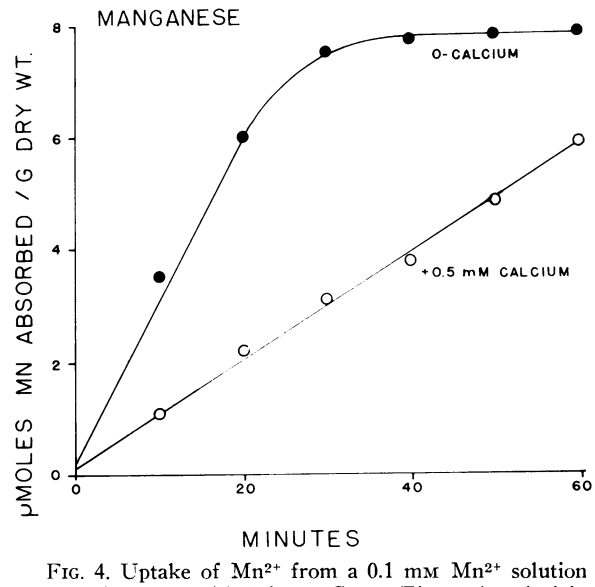

FIG. 4. Uptake of Mn^{2+} from a 0.1 mM Mn^{2+} solution without Ca^{2+} and with 0.5 mM Ca^{2+}. Tissue desorbed in 0.5 mM $CaCl_2$ (8°) for 30 min. Absorption period, 60 min. Other conditions as for figure 2.

lowed by a 30-min desorption period in 0.5 mM $CaCl_2$ at 8°.

In the absence of Ca^{2+} a large influx of each ion was observed as compared to the controls containing 0.5 mM Ca^{2+} (fig 4, for Mn^{2+}). It has been established above that in the presence of Ca^{2+} absorption of each cation from a 0.1 mM solution was linear for at least 2 hr. In the absence of Ca^{2+}, however, the absorption of Mn^{2+}, for example, was non-linear after approximately 30 min. An analogous response was obtained for the effect of Ca^{2+} on Cu^{2+} and Zn^{2+} absorption. Schmid *et al.* (*22*) reported similar data for Zn^{2+} absorption by excised barley roots with and without 0.5 mM Ca^{2+}, although the Zn^{2+} concentration they used was much lower.

Interactions in the Uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+}. The possible occurrence of interactions and mutual competitions in the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} was investigated in a series of factorially-designed experiments. Each solution contained 0.5 mM Ca^{2+} and 1 or more of the above 3 cations at 0.1 mM.

Initially the uptake of Cu^{2+}, Zn^{2+}, and Mn^{2+} was examined as a function of time in the presence of each of the other cations (table II). Mn^{2+} was without any significant effect on the absorption of Cu^{2+} and Zn^{2+}, whereas the uptake of the latter 2 cations manifested mutual interferences. Further experiments showed that Cu^{2+} and Zn^{2+} competitively inhibited the absorption of each other. When the Mn^{2+} concentration was increased to 2 mM, Mn^{2+} still had no significant effect on Cu^{2+} and Zn^{2+} uptake. It was therefore concluded that the uptake of Cu^{2+} and Zn^{2+} is effected through the same absorption mechanism, *i.e.*, the same carrier sites. Mn^{2+} is absorbed through a second, independent mechanism. It is interesting to note that these data for sugarcane leaf tissue are the same as those found for root tissue of barley (*22*).

Effect of Other Cations. Several additional cations were studied with regard to possible competition for the Cu^{2+}-Zn^{2+} and the Mn^{2+} absorption sites. Potassium stimulated the rate of Cu^{2+} and Zn^{2+} uptake, but this effect could not be reproduced consistently. Leggett and Gilbert (11) reported that an efflux of K^+ accompanied Mg^{2+} absorption by soybean roots under low-Ca^{2+} conditions, but the situation was reversed upon addition of Ca^{2+}. Thus, the inconsistent results obtained with K^+ in the present study may have been due to variable K^+ nutrition and content of the tissue used.

Table II. *Mutual Effects of Cu^{2+}, Zn^{2+}, and Mn^{2+} in Their Absorption by Excised Sugarcane Leaf Tissue*

The concentration of each ion was 0.1 mM; Ca^{2+}, 0.5 mM; pH 5.7 and temperature, 30°.

Ions present	Absorption Cu^{2+}	Zn^{2+}	Mn^{2+}
	μmoles/g dry wt/hr		
Cu^{2+}	5.14	...	...
Zn^{2+}	...	5.28	...
Mn^{2+}	...	...	5.91
Cu^{2+} + Zn^{2+}	4.28	4.05	...
Cu^{2+} + Mn^{2+}	5.10	...	5.87
Zn^{2+} + Mn^{2+}	...	5.22	5.84
Cu^{2+} + Zn^{2+} + Mn^{2+}	4.34	3.98	5.80

Table III. *Effects of Metabolic Inhibitors, Respiratory Substrates, P_i, and ADP on the Absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} by Excised Sugarcane Leaves (External Cation Concentration, 0.1 mM)*

Ca^{2+}, 0.5 mM; pH 5.7 and temperature, 30°.

Additive	Absorption Cu^{2+}	Zn^{2+}	Mn^{2+}
	μmoles/g dry wt/hr		
Control (no additive)	5.11	5.23	5.80
10^{-6} M DNP	3.37	3.25	4.00
10^{-5} M DNP	1.08	1.27	0.89
10^{-4} M NaN_3	3.51	3.53	3.67
10^{-3} M CN	4.40	4.35	4.59
10^{-2} M Arsenate	3.69	3.66	3.71
10^{-5} M Amytal	0	0	0
10^{-5} M Nembutal	0	0	0
10^{-5} M DCMU	5.09	5.27	5.77
0.05 M Succinate	0.28	0.13	0.09
0.05 M Malate	5.48	5.47	5.99
0.05 M Pyruvate	5.42	5.48	5.88
0.05 M Oxalacetate	5.40	5.44	6.02
0.05 M Glycerate	5.28	5.30	5.94
0.01 M Glucose	5.19	5.31	5.89
0.01 M Fructose	5.23	5.30	5.89
0.01 M Mannose	5.15	5.26	5.82
10^{-3} M P_i	2.68	2.74	2.94
5×10^{-5} M ADP	0.36	0.36	0.40

The following ions at 0.1 mM had no influence on the absorption from 0.1 mM solutions of any of the 3 ions studied: Na^+, Li^+, Cs^+, Ag^+, Co^{2+}, Cr^{2+}, Al^{3+}, Mg^{2+}, Ba^{2+}, and NH_4^+. Cu^{2+} and Zn^{2+} uptake were significantly inhibited (1 % confidence level) by Fe^{3+}, but Mn^{2+} absorption was not affected.

Uptake in the Light vs. Dark. The contrariety of the literature regarding the effects of light on ion absorption by various plant species, tissues, and organelles (2, 6, 9, 10, 15, 16, 17, 20, 21, 23) prompted an investigation of the possible effect of light on the Mn^{2+} and the Cu^{2+}-Zn^{2+} absorption systems in sugarcane leaves. The light intensity within the cheesecloth bags was 6000 lumen·m^{-2}, an intensity shown by Rains (21) to produce maximal K^+ uptake by corn leaves. No increase in the net accumulation of Cu^{2+}, Zn^{2+}, or Mn^{2+} in the leaf tissue attributable to light was observed.

Effect of Metabolic Inhibitors. The effects of several metabolic inhibitors upon the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} from 0.1 mM solutions were studied. Uptake of each ion was strongly inhibited by 10^{-6} M DNP, 10^{-4} M NaN_3, 10^{-3} M cyanide, and 10^{-2} M arsenate (table III). The decreased cation uptake induced by each of these inhibitors was statistically significant at the 1 % confidence level. Absorption of each ion was completely inhibited by 10^{-5} M Amytal and 10^{-5} M Nembutal (table III).

DCMU at 10^{-5} M had no effect on the uptake of any of these cations.

Effect of P_i and ADP. Although P_i has been demonstrated to be essential for Ca^{2+} uptake by isolated mitochondria when the absorption process is coupled to substrate oxidation (7), no similar requirement could be shown for the intact leaf tissue used in this study. Presumably, any requirement for P_i, if such does exist in the sugarcane plant, would be fulfilled by endogenous P_i. However, P_i, as KH_2PO_4, at concentrations as low as 0.2 mM competitively inhibited absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} from 0.1 mM solutions. When the P_i concentration was increased to 1.0 mM, the resultant inhibition approximated 50 % for each cation (table III); complete cessation of absorption occurred at 5 mM P_i.

ADP at 1 μmole/liter also competitively inhibited the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+}. Since these ions are absorbed through 2 independent mechanisms, both of which are competitively inhibited by P_i and ADP, it would appear that P_i and ADP are acting at a site removed from the carrier molecule itself, presumably through a non-phosphorylated intermediate of oxidative phosphorylation. This conclusion is in general agreement with the data of Rains (21) for K^+ uptake and Springer-Lederer and Rosenfeld (26) for Rb^+.

Action of Respiratory Substrates. To further localize the site of coupling of ion uptake to oxidative phosphorylation, uptake was measured as a function of various respiratory substrates (table III). Since excised leaf sections are not amenable to quantitative depletion of endogenous substrates as are mitochondria, additions of rather high concentrations of substrates were necessary. Uptake of each of the 3 cations studied was virtually abolished by the addition of 5×10^{-2} M succinate, although O_2 uptake was actually enhanced somewhat.

Of the 3 molecules of ATP generated incident to the flow of electrons from NADH to O_2, 1 arises during electron transport from NADH to flavoprotein Fp_{D1}, in the terminology of Chance, Bonner, and Storey (4) as applied to mammalian mitochondria. The formation of the other 2 ATP's is associated with the cytochrome chain (27). When succinate is the respiratory substrate, electrons flow through flavoprotein Fp_S to Fp_{D2}, bypassing the Fp_{D1}-coupled energy conservation Site I (4). Since 5×10^{-2} M succinate strongly inhibited absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+}, as did the uncouplers of oxidative phosphorylation, it is suggested that the absorption of these 3 cations is mediated specifically through energy conservation Site I. Further support for this conclusion is to be gained from the complete inhibition of cation uptake by Amytal and Nembutal, since these barbituric acid derivatives are known to intercept electron transport between Fp_{D1} and Fp_{D2} (4), thus inhibiting Site I oxidative phosphorylation. Inherent to this and other conclusions in this paper is the assumption that the sites of inhibition of the various inhibitors employed are correct as reported in the literature. Furthermore, this discussion is based upon the respiratory electron transport pathway and oxidative phosphorylation sites as elucidated for mammalian mitochondria. It does appear however, that these processes are basically the same in mitochondria of higher plants (12).

Glucose, fructose, and mannose, each at 10^{-2} M, were slightly stimulatory to cation absorption, but the response was not statistically significant. A statistically-significant (5 % confidence level) increase in absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} was noted upon inclusion of the following in the reaction medium: 5×10^{-2} M pyruvate, 5×10^{-2} M oxaloacetate, 5×10^{-2} M L-malate, and 10^{-2} M glycerol (table III).

Conclusion

The inhibition of cation uptake by uncouplers of oxidative phosphorylation, the strong inhibitory activity of succinate, and the complete inhibition of uptake by Amytal and Nembutal are interpreted as evidence that the absorption of Cu^{2+}, Zn^{2+}, and Mn^{2+} is coupled to energy conservation Site I. The specific sites of Cu^{2+} and Zn^{2+} uptake and that of Mn^{2+} differ, however. Cu^{2+} and Zn^{2+} are transported across the cellular membrane through a common mechanism as evidenced by the mutually competitive action of each cation upon the absorption of the other. On the other hand, Mn^{2+} is absorbed through a second, independent mechanism. A similar delineation of cation absorption systems has been observed in barley roots (22).

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Literature Cited

1. Bowen, J. E. 1968. Borate absorption by excised sugarcane leaves. Plant Cell Physiol. 9: 467–78.
2. Briggs, G. E., A. B. Hope, and R. N. Robertson. 1961. Electrolytes and Plant Cells. F. A. Davis, Philadelphia, Pennsylvania. p 168–74.
3. Broda, E. 1965. Mechanism of uptake of trace elements by plants (experiments with radiozinc). Isotopes Radiation Soil-Plant Nutr. Studies, Proc. Symp., Ankara 1965: 207–15.
4. Chance, B., W. D. Bonner, Jr., and B. T. Storey. 1968. Electron transport in respiration. Ann. Rev. Plant Physiol. 19: 295–320.
5. Epstein, E., W. E. Schmid, and D. W. Rains. 1963. Significance and technique of short-term experiments on solute absorption by plant tissue. Plant Cell Physiol. 4: 79–84.

6. Hoagland, D. R. and A. R. Davis. 1923. Further experiments on the absorption of ions by plants, including observations on the effect of light. J. Gen. Physiol. 6: 47–62.
7. Hodges, T. K. and J. B. Hanson. 1965. Calcium accumulation by maize mitochondria. Plant Physiol. 40: 101–09.
8. Hofstee, B. H. J. 1952. On the evaluation of the constants *Vm* and *Km* in enzyme reactions. Science 116: 329–31.
9. Jeschke, W. D. 1967. Die cyclische und die nichtcyclische Photophosphorylierung als Energiequellen der lichtabhängigen Chloridionenaufnahme bei *Elodea*. Planta 73: 161–74.
10. Kylin, A. 1960. The accumulation of sulfate in isolated leaves as affected by light and darkness. Botan. Notiser 113: 49–81.
11. Leggett, J. E. and W. A. Gilbert. 1967. Localization of the Ca-mediated apparent ion selectivity in the cross-sectional volume of soybean roots. Plant Physiol. 42: 1658–64.
12. Lieberman, M. and J. E. Baker. 1965. Respiratory electron transport. Ann. Rev. Plant Physiol. 16: 343–82.
13. Lineweaver, H. and D. Burk. 1936. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56: 658–66.
14. Maas, E. V., D. P. Moore, and B. J. Mason. 1968. Manganese absorption by excised barley roots. Plant Physiol. 43: 527–30.
15. MacRobbie, E. A. C. 1965. The nature of the coupling between light energy and active ion transport in *Nitella translucens*. Biochim. Biophys. Acta 94: 64–73.
16. MacRobbie, E. A. C. 1966. Metabolic effects of ion fluxes in *Nitella translucens*. I. Active fluxes. Australian J. Biol. Sci. 19: 363–70.
17. Nobel, P. S. and L. Packer. 1965. Light-dependent ion translocation in spinach chloroplasts. Plant Physiol. 40: 633–40.
18. Page, E. R. and J. Dainty. 1964. Manganese uptake by excised oat roots. J. Exptl. Botany 15: 428–43.
19. Rains, D. W., W. E. Schmid, and E. Epstein. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. Plant Physiol. 39: 274–78.
20. Rains, D. W. 1967. Light-enhanced potassium absorption by corn leaf tissue. Science 156: 1382–83.
21. Rains, D. W. 1968. Kinetics and energetics of light-enhanced potassium absorption by corn leaf tissue. Plant Physiol. 43: 394–400.
22. Schmid, W. E., H. P. Haag, and E. Epstein. 1965. Absorption of zinc by excised barley roots. Physiol. Plantarum 18: 860–69.
23. Smith, F. A. 1966. Active phosphate uptake by *Nitella translucens*. Biochim. Biophys. Acta 126: 94–99.
24. Smith, R. C. and E. Epstein. 1964. Ion absorption by shoot tissue: technique and first findings with excised leaf tissue of corn. Plant Physiol. 39: 338–41.
25. Snedecor, G. W. 1948. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State College Press, Ames, Iowa.
26. Springer-Lederer, H. and D. L. Rosenfeld. 1968. Energy sources for the absorption of rubidium by *Chlorella*. Physiol. Plantarum 21: 435–44.
27. White, A., P. Handler, E. L. Smith, and D. Stetton, Jr. 1959. Principles of Biochemistry, 2nd Ed. McGraw-Hill, New York, New York. 1149 p.