

UPTAKE AND TRANSLOCATION OF ZINC BY INTACT PLANTS *

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INTRODUCTION

Antagonisms between zinc and other nutrient elements have been reported for intact plants by several investigators. Malavolta *et al.* ⁶ investigated the influence of added cations on zinc content of coffee plants grown in nutrient solution. They found that with addition of copper, the zinc content of the plants was severely reduced. Similar results were obtained when manganese or molybdenum were the competitor ions. Iron showed little effect in this respect. Similar work, by Dunne ¹, showed that the zinc level and yield of cereal grains were influenced by the copper level of the soil on which they were grown. As the soil copper level was increased, the zinc content of the plant tissues, as well as yield, were reduced. In other work (Haag, Schmid, and Epstein, unpublished data) it has been demonstrated that uptake of radioactively labeled zinc by intact barley and bush bean plants is reduced when copper is present in the nutrient solution, and that the effect of added copper is to reduce the amount of zinc being taken up by the plants and not to prevent its translocation internally from the roots to the tops. These results are in agreement with Schmid *et al.* ¹⁰, who have found that copper competitively inhibits zinc transport in excised barley roots.

The present experiments were undertaken to study: 1) the effect of other cationic species on zinc uptake by intact plants, and 2) the

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possible demonstration that some of these cations may exert their effect through interference with zinc translocation from the root to the shoot rather than through uptake into the root.

METHODS

All work was done with bush bean, *Phaseolus vulgaris* L. (variety Topcrop; Burpee). The plants were germinated on cheesecloth over an aerated solution which contained Johnson's solution⁵ macronutrients at 1/10th of the normal concentration. The seeds were grown in a dark chamber at 22° C. When the seedlings were 7 days old, they were transferred to individual 1-liter pots of complete, full strength Johnson's solution. For the remainder of the experiment the plants were kept in a greenhouse. After the plants had been in the full strength Johnson's solution for 7 days, they were transferred (with an intermediate dip of the roots into a 5×10^{-4} M CaCl₂ solution, then into distilled water) to a full strength Johnson's solution (the 24-hour treatments received only the macronutrients at full strength). This latter solution contained labeled zinc chloride (hereafter referred as to Zn*) in a concentration of 5 μ M (radiozinc, Zn⁶⁵Cl₂, was obtained from Oak Ridge National Laboratory). Competing cations were added to the proper concentration. Each treatment consisted of 4 plants in the 24-hour series or 3 plants in the 4- or 7-day series. Each plant in a treatment was grown and maintained in its own pot of solution. All of the experimental plants were selected for uniform appearance. After the plants had been in the labeled solution for the proper length of time (1, 4, or 7 days), they were removed and separated into tops and roots. Top refers to that portion of the plant above the cotyledonary node and root refers to that portion below. The combined tops in each treatment were weighed for total fresh weight. The combined roots in each treatment were rinsed with 2 washes of distilled water, followed by a rinse in a 5×10^{-4} M CaCl₂ solution, and then a final rinse in distilled water. Then the roots were blotted and the total fresh weights determined. The dipping of the roots into CaCl₂ solution was used to displace exchangeadsorbed zinc from the root surfaces. Greenway and Thomas⁴ have used a similar technique to remove extraneous chloride and Schmid *et al.*¹⁰ found such treatment necessary to remove adsorbed zinc from excised barley roots.

After the separated plant materials had been dried at 70° C for at least 48 hours, the dry weights were taken and the dried material was ground in a Wiley mill. Portions of the dried ground material, weighing 0.1 gram each, then were ashed in aluminum planchets in a muffle furnace at 500° C for at least 2 hours. The radioactive content of the ash was measured with a gas flow detector.

RESULTS

Effect of copper

Figure 1 presents the results of an experiment in which copper was the competing ion. The Zn^* -concentration was $5 \mu M$; copper: 0, 5, 10, or $20 \mu M$; the Johnson's solution macronutrients at full strength. It should be noted that the majority of the Zn^* in the plant was present in the roots after a 24-hour period in the labeled nutrient solution. When copper was present at the same concentration as the Zn^* , $5 \mu M$, Zn^* -uptake into the plant was severely reduced, and this reduction was observed in both tops and roots. Proportionally, the same amount of Zn^* was present in the tops and in the roots at the $5 \mu M$ level, and at all succeeding copper levels, as was present at zero copper level. Beyond the $5 \mu M$ copper level, further reduction in total Zn^* -content was very slight.

In Table 1 are shown the results of an experiment in which Zn^* -uptake was measured as a function of both copper concentration and time. Zn^* was present at $5 \mu M$, copper at 0, 10, or $20 \mu M$, and the Johnson's solution nutrients at full strength. The time that the

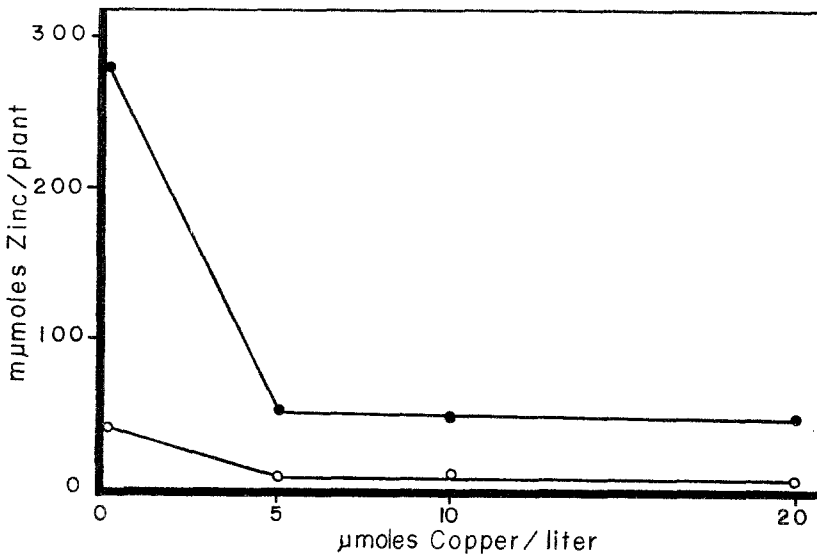


Fig. 1. Zn^* -content of tops and roots of bean plants grown in nutrient solution cultures to which copper ($CuCl_2$) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn^* -level was $5 \mu M$. Closed circles represent the roots and open circles represent the tops

TABLE 1

Zn*-content of tops and roots of bean plants grown in nutrient solution cultures to which copper (CuCl ₂) has been added as a competing ion. Plants were in labeled solution for either 4 or 7 days. Zn*-level was 5 μ M. All data on a 'per plant' basis									
Competitor ion concentration	Days	Tops				Roots			
		Fresh weight	Dry weight	Zn*-content †	% of total Zn*	Fresh weight	Dry weight	Zn*-content †	% of total Zn*
0.0 μ M	4	4.0 g	0.5 g	266.0	19	4.0 g	0.3 g	1149.3	81
0.0	7	6.2	0.6	432.6	27	4.8	0.2	1161.1	73
10.0	4	2.9	0.4	36.7	28	3.1	0.2	95.4	72
10.0	7	5.3	0.5	81.6	19	3.8	0.3	347.1	81
20.0	4	3.6	0.4	14.3	11	3.5	0.3	119.5	89
20.0	7	3.9	0.4	27.1	24	3.0	0.2	88.0	76

† Zn*-content expressed in nanomoles of zinc/plant.

plants were in nutrient solution containing Zn* was 4 or 7 days.

The data depicted in Table 1 show that the average fresh weights and dry weights of the plants remained reasonably uniform. Results obtained for the plants grown in nutrient solution containing added copper for 4 and 7 days were essentially the same as those obtained for the one-day treatment. The percent of Zn* distributed between the roots and the shoots for each of the copper levels tended not to change markedly within each of the time intervals.

Effect of zinc

In order to have a basis of comparison in examining the effects of other cations, an experiment utilizing added zinc as the competing cation was set up. It is apparent in Figure 2 that when zinc was present at 5 μ M, the reduction in Zn* uptake was not as severe as when copper was present at this level (*cf* Figure 1). In addition, the slope of the line gradually dropped off at the higher zinc levels. Zinc competition on Zn*-uptake was, then, unlike copper competition in that copper caused a more rapid, initial reduction in uptake rate.

Table 2 presents the effect of added zinc on Zn*-uptake over longer experimental times. Again, the same slow reduction in uptake is noticed with increasing levels of zinc. The 4-day treatment revealed no real change in distribution of Zn* between the tops and the roots. However, there was a change shown in the distribution pattern of

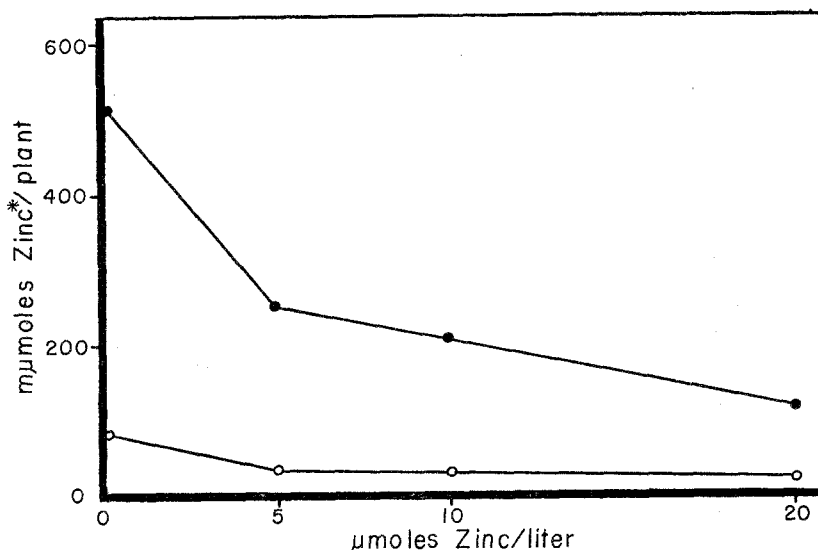


Fig. 2. Zn*-content of tops and roots of bean plants grown in nutrient solution cultures to which zinc (ZnCl_2) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn* level was $5 \mu\text{M}$. Closed circles represent the roots and open circles represent the tops.

TABLE 2

Zn*-content of tops and roots of bean plants grow in nutrient solution cultures to which zinc (ZnCl_2) has been added as a competing ion. Plants were in labeled solution for either 4 or 7 days. Zn*-level was $5 \mu\text{M}$. All data on a 'per plant' basis									
Competitor ion tration	Days	Tops				Roots			
		Fresh weight	Dry weight	Zn*- con- tent †	% of total Zn*	Fresh weight	Dry weight	Zn*- con- tent †	% of total Zn*
0.0 μM	4	4.8 g	0.5 g	186.2	27	3.2 g	0.2 g	491.8	73
	7	7.7	0.8	501.4	31	4.2	0.3	1131.7	69
10.0	4	3.5	0.3	83.7	24	2.4	0.1	270.1	76
	7	7.7	0.8	368.8	29	4.5	0.2	892.5	71
20.0	4	4.7	0.4	94.5	24	3.2	0.1	295.6	76
	7	6.0	0.6	212.2	20	3.8	0.3	866.1	80

† Zn*-content expressed in nanomoles of zinc/plant.

the Zn* in the 7-day treatment. As the unlabeled zinc level was increased, a higher percentage of the total Zn* (contained in the intact plant) remained in the roots. That this may be due to zinc toxicity was not discounted although no toxicity symptoms were evident.

Effect of manganese

In the experiment shown in Figure 3, the effect of manganese on Zn*-uptake is depicted. It is apparent from this figure that manganese had very little effect on Zn*-uptake or translocation internally in the bush bean plant.

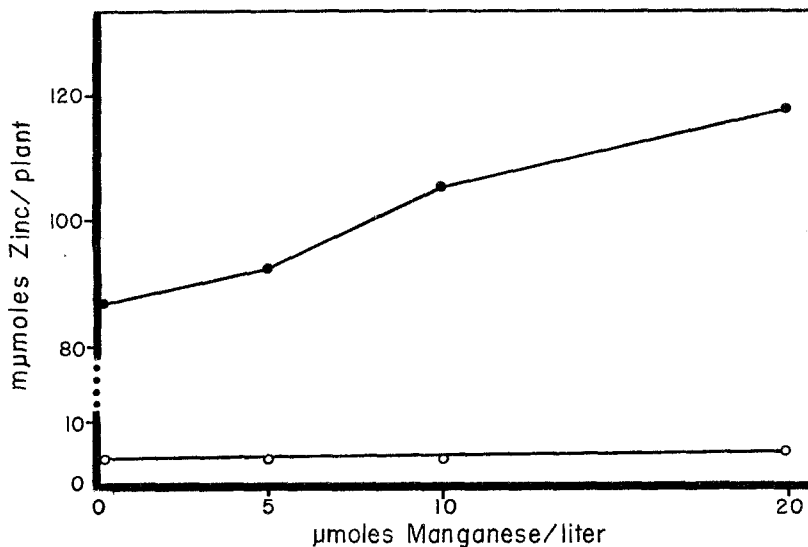


Fig. 3. Zn*-content of tops and roots of bean plants grown in nutrient solution cultures to which manganese (MnCl_2) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn*-level was $5 \mu\text{M}$. Closed circles represent the roots and open circles represent the tops .

TABLE 3

Zn*-content of tops and roots of bean plants grown in nutrient solution cultures to which manganese (MnCl_2) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn*-level was $5 \mu\text{M}$. All data on a 'per plant' basis								
Competitor ion concentration	Tops				Roots			
	Fresh weight	Dry weight	Zn*-content †	% of total Zn*	Fresh weight	Dry weight	Zn*-content †	% of total Zn*
0.0 μM	4.1 g	0.4 g	55.2	16	4.0 g	0.2 g	286.7	84
30.0	3.5	0.3	27.3	22	3.4	0.1	94.8	78
60.0	3.8	0.3	25.4	28	3.3	0.1	66.8	72
90.0	2.8	0.2	14.2	22	3.0	0.1	51.1	78

† Zn*-content expressed in nanomoles of zinc/plant.

Interference in zinc transport was observed at high concentrations of manganese relative to the Zn^* -concentration (Table 3). When manganese was present at $30 \mu M$ (6 times the concentration of Zn^*) there was a reduction in Zn^* -uptake to about half of the amount found in the control. However, there was little change in the percentage of Zn^* found in the roots versus that in the shoots whether manganese was absent or present.

Effect of cadmium

Figure 4 and Table 4 present the results of experiments in which cadmium was added as the competing ion. From Figure 4, it will be seen that cadmium had basically the same effect as did copper. In both cases there was a severe reduction in Zn^* -uptake at the $5 \mu M$ added competitor level. With either copper or cadmium, the 10- and $20\text{-}\mu M$ concentrations of competitor ion induced little additional change from the situation at the $5\text{-}\mu M$ level. In regard to the distribution pattern of Zn^* within the plant, there was a change at the $20\text{-}\mu M$ cadmium level but not with the same copper level. At the

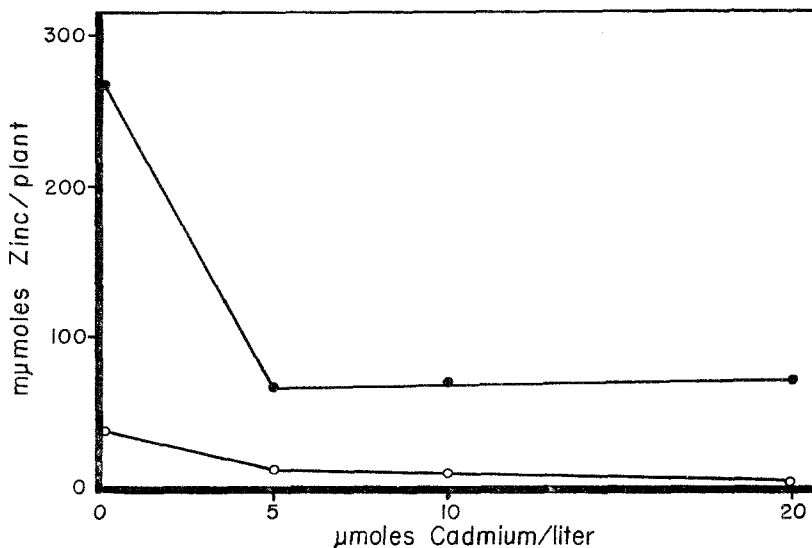


Fig. 4. Zn^* -content of tops and roots of bean plants grown in nutrient solution cultures to which cadmium ($Cd(NO_3)_2$) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn^* level was $5 \mu M$.

Closed circles represent the roots and open circles represent the tops.

20- μM cadmium level, a higher percentage of the total Zn* remained in the roots.

TABLE 4

Zn*-content of tops and roots of bean plants grown in nutrient solution cultures to which cadmium ($Cd(NO_3)_2$) has been added as a competing ion. Plants were in labeled solution for 24 hours. Zn*-level was 5 μM . All data on a 'per plant' basis								
Competitor ion concentration	Tops				Roots			
	Fresh weight	Dry weight	Zn*-content †	% of total Zn*	Fresh weight	Dry weight	Zn*-content †	% of total Zn*
0.0 μM	2.5 g	0.3 g	19.3	13	2.6 g	0.2 g	127.6	87
15.0	2.6	0.3	6.3	12	2.7	0.2	46.6	88
30.0	3.2	0.4	7.4	13	3.1	0.2	48.2	87
60.0	2.7	0.3	1.9	3	3.0	0.2	60.5	97

† Zn*-content expressed in nanomoles of zinc/plant.

DISCUSSION

This study has been done to determine the effect of added ions on zinc uptake and translocation in intact plants. Fried and Shapiro³ have suggested two active steps involved in the movement of ions from the substrate to the shoot. They proposed that an active accumulation of ions occurs at the root surface where the ion combines with the carrier and that another active process occurs within the root where the ion is deposited into the vascular system. The purpose of the present study was to determine if the presence of other cations would influence the absorption and/or translocation of zinc. If an inhibition or enhancement of zinc translocation as well as absorption should occur, it might be in line with Fried and Shapiro's proposal of the two sites being involved in ion absorption.

By employing ion competitors, it can be determined if absorption or translocation of zinc is influenced. If the tops are separated from the roots of the plant and analyzed for Zn*-content, then examination of the total Zn* in tops plus roots will reveal any effect on total Zn* taken up. Comparison of the percent distribution between the tops and the roots will reveal any changes in translocation. Recently, Sakaguchi⁸ has used such an analysis of experimental results to examine the effects of various anions on the uptake and translocation of strontium.

If a competitor interferes with uptake only, it will be apparent as a reduction in total Zn^* . If it interferes with internal translocation this will be made apparent by changes in the percent composition of the tops versus the roots. An inhibitor would increase the percent Zn^* in the roots and decrease it in the tops. A transport-enhancing ion or compound would have the reverse effect.

Effect of copper

Inhibition of Zn^* uptake in bush bean plants was found to occur when copper was added to the nutrient solution in low concentrations, but at these same levels copper did not interfere with the translocation on Zn^* within the plant. These results concur with those cited earlier from Malavolta *et al.*⁶ in coffee; Dunne¹ in cereal grains; Haag *et al.* (unpublished data) with barley and bush beans; and Schmid *et al.*¹⁰ with excised barley roots.

Increasing the period of time in which the plants were in the copper-containing solution had no further effect over the one-day treatment and at the same concentrations. But, in other work it was observed that when higher copper levels were used (0, 15, 30, and 60 μM), and the Zn^* -concentration was maintained at 5 μM , the distribution pattern of Zn^* absorbed changed, even over only 24 hours of exposure. As the concentration of copper was increased, the amount of Zn^* being translocated internally from the root to the shoot was decreased. At the 60- μM copper level there was almost a complete reduction of Zn^* in the tops (only 0.5 per cent of the total Zn^* being moved to the tops).

Schmid and Gerloff⁹ and Tiffin and Brown¹¹ demonstrated that iron occurs in a complexed form in its translocation from the root to the shoot. Possibly zinc also is bound in a complexed form in its ascent within the plant and copper in high concentration may displace it. The possibility that copper is exerting a toxic influence and thus interfering indirectly is not discounted by our findings.

Effect of zinc

Comparing the effects of added copper and added zinc on Zn^* -transport and -translocation reveals some similarities in their influences, at least when one examines the results from the viewpoint of Zn^* only. In reality, the effect of added zinc is one of dilution and the labeled zinc, being diluted, represents a far larger amount of

zinc actually being accumulated. If one were to calculate the amounts of zinc actually being transported into the plants, the curve depicted in Figure 2 would show an increase rather than a decrease. It has been shown by other workers (*e.g.* ^{2 10}) that with increasing substrate ion concentration the amount of ion transported behind the diffusion barrier in a given time increases and finally reaches a saturation level. Thus one would expect the curve for total zinc in Figure 2 to show some increase as the external zinc concentration is increased. The purpose in presenting Figure 2 is to give a comparison when the effects of other ions are examined.

Further examination of the results presented indicates that the addition of unlabeled zinc had some effect on the internal distribution of Zn* (7-day treatments; Table 2). This is unusual since once the zinc has been taken up it should be distributed in the same ratios and without discrimination throughout the plant. No competition on Zn*-translocation should be expected.

Effect of manganese

In their work with coffee, Malavolta *et al.*⁶ reported that manganese seemed to have a competitive effect on zinc uptake. Schmid *et al.*¹⁰ were unable to demonstrate competition in zinc uptake in excised barley roots, even when the manganese level was ten times as high as the zinc level. The results obtained here reveal that Zn*-uptake is inhibited by high manganese levels. When the manganese level is increased to six times that of the Zn*-concentration, that is to 30 μM , there is an inhibition of Zn*-absorption.

The results depicted in Figure 3 seem to indicate an enhancement of Zn*-uptake by the roots as the manganese level is increased. It would appear also that the amount of Zn* is increasing in the root in relation to the amount in the shoot. However, this is not the case. Careful examination of Figure 3 will reveal that only a slight percentage increase actually does occur. It has been found that the percentage distribution of labeled zinc in the root or shoot remains the same at each of the manganese levels. At these lower concentrations, manganese had very little effect on Zn*-absorption. Furthermore it did not have any effect on Zn*-translocation at any of the concentrations used. Similar results were obtained in 4-day and 7-day treatments with manganese.

Effect of cadmium

The possibility that cadmium might show some effect on Zn*-uptake or translocation was tested. It was thought that this element which is immediately beneath zinc in the periodic table, might indeed interfere with Zn*-absorption or translocation. Basically, the effect of cadmium was found to be similar to that of copper. Although a possibility of an interference in translocation was noted only at a very high concentration of cadmium, 60 μM , the inhibition of uptake was quite similar to that of copper. Instead of being competitive there is a possibility that cadmium exerts a generally toxic effect upon the plant. Perkins⁷ found that a toxicity similar to that of mercury occurs when cadmium is present in a biological system.

General

Generally, evidence has been presented in this study which indicates that for the most part, ions which compete with zinc in plants exert their effects at the site of transport into the plant and not very much in the translocation mechanism unless high concentrations of the competing ions are employed.

Indeed, the effect of added ions need not necessarily be in terms of interfering with translocation within the plant but rather be in terms of the fact that certain biochemical balances may be altered by the presence of the added ions. In this latter case the effect observed as less Zn* moving to the tops might be one of tying up the Zn* in the roots rather than competing with a translocation mechanism.

SUMMARY

Certain cations were added to nutrient solution cultures, in which intact bean plants were being grown, in an attempt to establish whether they influenced either entry of radiozinc (Zn*) into these plants or translocation of this element from the roots to the tops, or both. The Zn*-level in the culture solution was maintained at 5 μM and the concentrations of the added cations were varied from zero through 60 μM in the highest treatments.

When copper was present as the added cation at the Zn*-level (5 μM), Zn*-uptake was severely reduced but internal translocation was not especially altered.

When zinc was present as the added cation it exerted the expected competition on Zn*-uptake and in addition exerted an unexpected, suppressing effect on Zn* being translocated to the tops.

Manganese, as an added cation, has an effect on Zn*-uptake only at high concentrations (30 μM or more) but it did not alter the internal Zn* distribution.

Cadmium behaved similarly to copper in its influence on Zn*-uptake and translocation.

It is concluded that the general effect of added cations on Zn*-uptake and translocation in intact bean plants is predominantly to inhibit uptake, not internal distribution.

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