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Influence of Essential Elements on Manganese Intoxication

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Information derived from the toxicity data on a single environmental pollutant or toxin cannot always be extrapolated to a whole population as they may be exposed to more than one pollutant or toxic agent simultaneously. It seems worthwhile to determine whether or not the effect of a specific pollutant or compound on biological functions is modified by the presence of, or exposure to other pollutants or compounds (Petering 1978). An excess of essential metals viz. calcium, copper, iron, selenium or zinc reduces the toxicity of heavy metals (Webb 1972; Petering 1978), while their deficiency enhances the toxicity (Six and Goyer 1972; Washko and Cousins 1976).

It has been observed that the accumulation of manganese decreases significantly when given with iron or copper in mice (Chandra et al. 1980) and an excess of zinc protects against testicular injury induced by manganese (Chandra et al. 1975). On the contrary, iron deficiency has been reported to cause increased absorption of manganese from the gastro-intestinal tract in humans and experimental animals (Chandra and Tandon 1973).

With a view to explore the influence of essential metals in manganese intoxication, the effect of calcium, iron or zinc supplementation on the uptake of manganese and on the activity of manganese sensitive enzymes, succinic dehydrogenase and cytochrome oxidase in brain and liver of rat was investigated. The choice of the two mitochondrial enzymes was based on the fact that the mitochondria are the chief site of manganese accumulation and their activity in brain, liver and blood of rats is significantly influenced by manganese (Halacheva and Boyadjiev 1975).

MATERIALS AND METHODS

Ninety male albino rats (200 g) of the ITRC colony maintained on pellet diet (Hindustan Lever Ltd., India)* and water ad libitum were equally divided into five groups and were given daily intraperitoneal administrations for 28 days as follows -

Group I (Control)	- 4 ml/kg, normal saline
Group II	- 6 mg/kg, Mn as $MnCl_2.4H_2O$
Group III	- 6 mg/kg, Mn as $MnCl_2.4H_2O$ + 2 mg/kg, Zn as $ZnSO_4.7H_2O$
Group IV	- 6 mg/kg, Mn as $MnCl_2.4H_2O$ + 6 mg/kg, Ca as $CaCl_2.2H_2O$
Group V	- 6 mg/kg, Mn as $MnCl_2.4H_2O$ + 2 mg/kg, Fe as $FeSO_4.7H_2O$

Six animals from each group were sacrificed after 7, 14 and 28 days. Brain and liver were removed, cleaned free of extraneous material and weighed. Samples of liver and brain were taken for manganese estimation by atomic absorption spectrophotometry (Shearer et al. 1977). The tissues were homogenized in chilled 0.25 M sucrose to obtain 10% (w/v) homogenate. The standard procedure was employed to prepare sub-cellular fractions of liver and brain. The mitochondrial fraction was used for the assay of succinic dehydrogenase and cytochrome oxidase by the methods of Slater and Bonner (1952) and Co-operstein and Lazarow (1951) respectively.

RESULTS AND DISCUSSION

The uptake of Mn increased significantly in liver, with the duration of exposure to Mn alone, while the activity of SDH and cytochrome oxidase decreased significantly only after 4 weeks of Mn administration. The supplementation of Zn or Fe along with Mn significantly reduced the uptake of Mn and restored Mn induced decrease in the activity of enzymes. However, supplementation of Ca failed to affect the uptake of Mn and the Mn induced enzyme inhibition (Fig. 1-3). The level of Mn in brain increased significantly after 1 week of Mn administration, remained unaltered after

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* Composition: Metal content of animal feed (ppm dry weight): Cu 10.0, Mn 55.0, Co 5.0, Zn 45.0, Fe 70.0

2 weeks and increased further thereafter. The activity of SDH and cytochrome oxidase decreased significantly only after 4 weeks of exposure to Mn alone. The supplementation of Zn along with Mn significantly reduced the uptake of Mn at 4 weeks and restored the decreased activity of two enzymes. The supplementation of Fe along with Mn, though significantly reduced the level of Mn at 4 weeks, it could not restore the Mn induced enzyme inhibition. The supplementation of Ca however, could, neither affect the increase in Mn concentration particularly at 4 weeks, nor the Mn induced enzyme inhibition (Fig. 4-6).

The supplementation of Zn or Fe along with Mn reduced the uptake of Mn significantly in brain and liver of rats. The former restored the manganese induced decrease in the activity of SDH and cytochrome oxidase in brain and liver, while the latter could do so in liver only. The protective effect of Zn has been more marked than Fe particularly in brain. The protective effect of Zn and Fe against the uptake of Mn and its toxic effects might be attributed to a decreased absorption of Mn from the gastro-intestinal tract or prevention of the deleterious effects of Mn on mitochondrial respiration. The supplementation of Fe or Zn along with Mn is expected to cause a competition for the binding sites in the body and it appears from the present results that the biological ligands have preferential binding affinity for Fe or Zn than for Mn. The possible binding of Fe or Zn at the active sites of enzymes and bioligands will reduce the accumulation of manganese and thus its toxicity. Chandra et al. (1975, 1980) have also observed protection against Mn intoxication by Fe, Cu or Zn supplementation.

The supplementation of Ca, however, failed to influence either the accumulation of Mn or the altered activity of two enzymes. High dietary Ca decreases Pb (Kostial et al. 1971) and Cd absorption (Kello et al. 1979) probably by blocking the -SH groups of proteins, the active binding sites for Cd and Pb (Ono et al. 1973). Manganese on the contrary, appears to have a low affinity for -SH groups and alternatively binds to groups containing N and O to exert its toxic effects (Tandon and Khandelwal 1982). This may partly explain the inability of Ca to affect the absorption of Mn and the decrease in the activity of SDH and cytochrome oxidase caused by Mn. The present study however, suggests the usefulness of Zn or Fe supplementation in prevention of manganese intoxication.

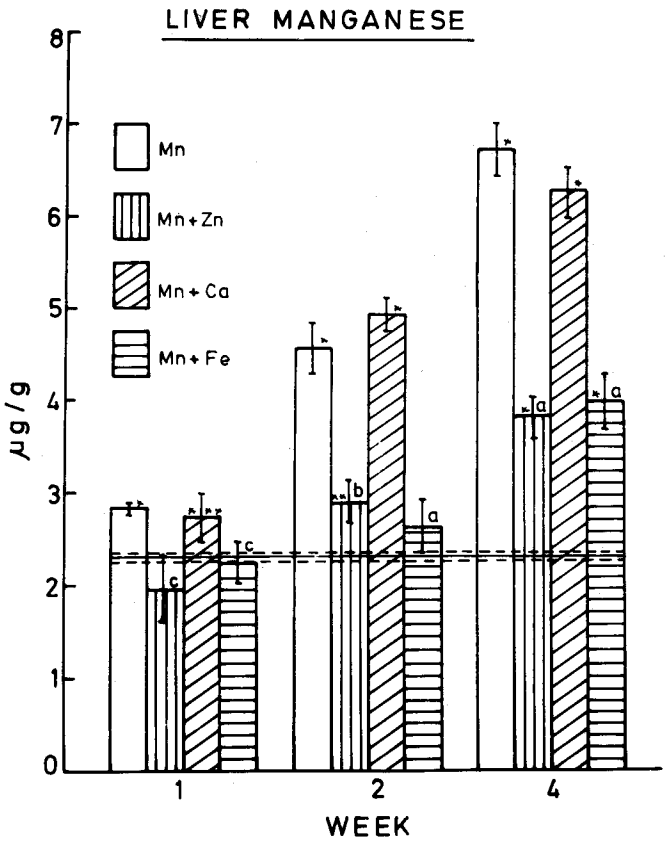

Fig. 1 : Effect of Zn, Ca or Fe supplementation on the uptake of manganese in liver of rats. Each value represents mean ± S.E. of 6 values.
$^{*}P < 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$, when compared to control (Horizontal line).
$^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$, when compared to manganese group using student's 't' test.

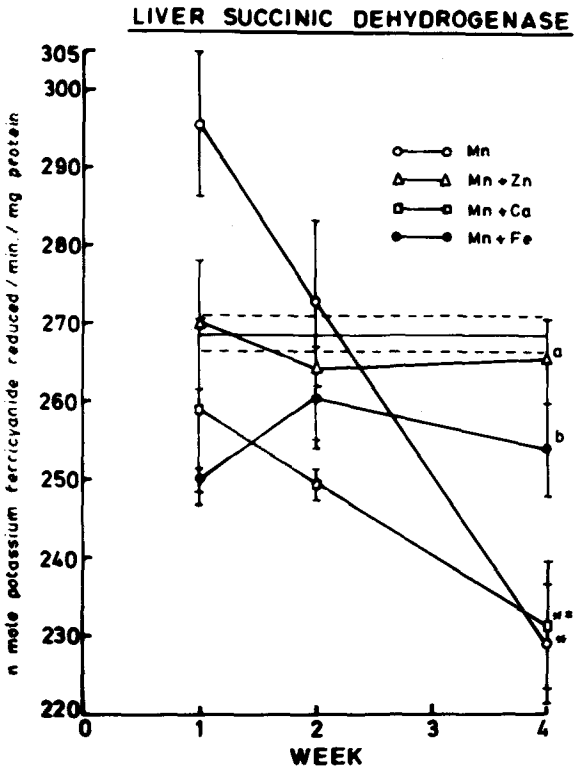

Fig. 2 : Effect of Zn, Ca or Fe supplementation on the activity of succinic dehydrogenase in liver of rats.
Each value represents mean ± S.E. of 6 values.
$^{*}P < 0.001$, $^{**}P < 0.01$, when compared to control (Horizontal line).
$^{a}P < 0.01$, $^{b}P < 0.05$, when compared to manganese group using student's 't' test.

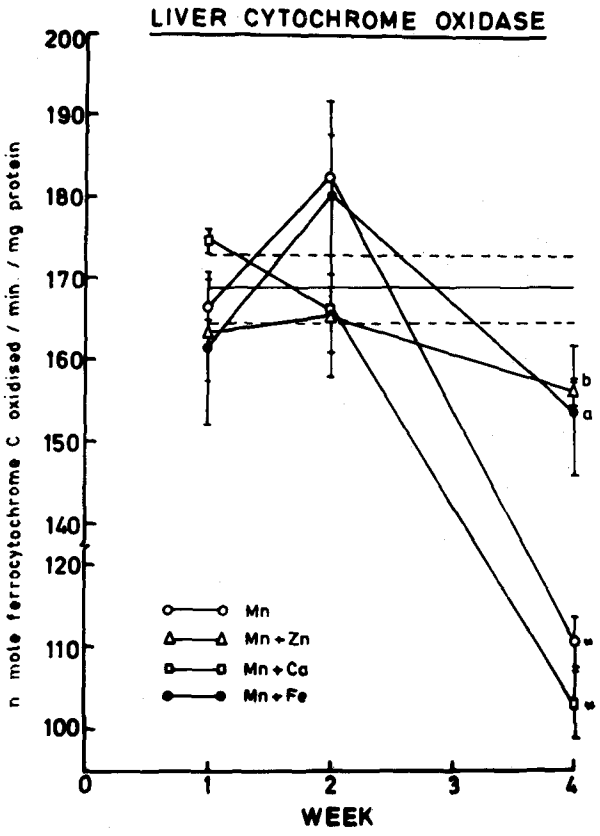

Fig. 3 : Effect of Zn, Ca or Fe supplementation on the activity of cytochrome oxidase in liver of rats.
Each value represents mean ± S.E. of 6 values.
*$P < 0.001$, when compared to control (Horizontal line).
$^{a}P < 0.001$, $^{b}P < 0.05$, when compared to manganese group using student's 't' test.

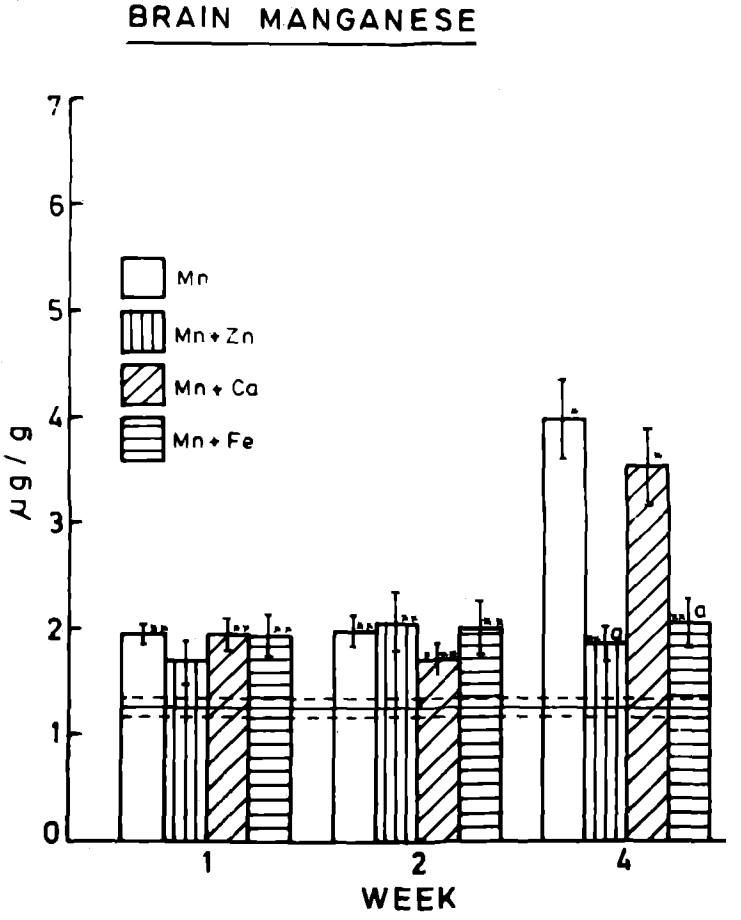

Fig. 4 : Effect of Zn, Ca or Fe supplementation on the uptake of manganese in brain of rats. Each value represents mean ± S.E. of 6 values.
*P < 0.001, **P < 0.001, ***P < 0.05, when compared to control (Horizontal line).
aP < 0.001, when compared to manganese group using student's 't' test.

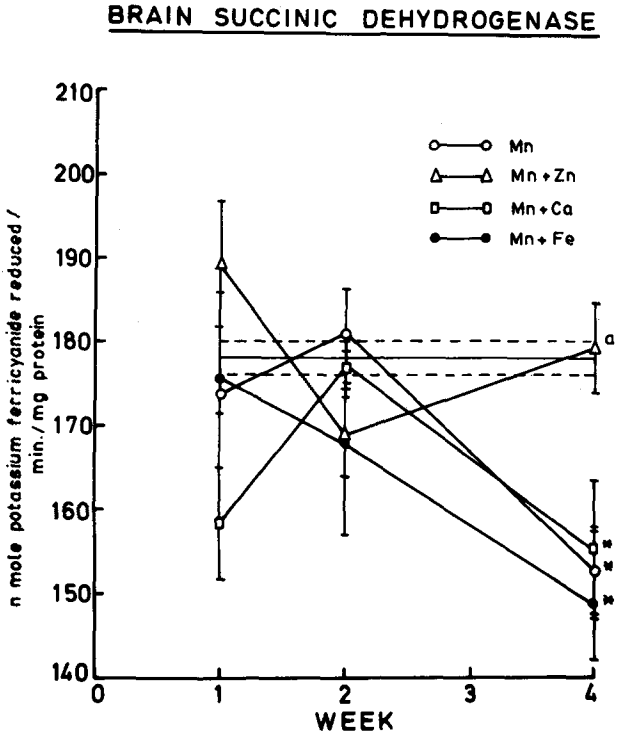

Fig. 5 : Effect of Zn, Ca or Fe supplementation on the activity of succinic dehydrogenase in brain of rats.
Each value represents mean ± S.E. of 6 values.
*$P < 0.05$, when compared to control (Horizontal line).
$^{a}P < 0.01$, when compared to manganese group using student's 't' test.

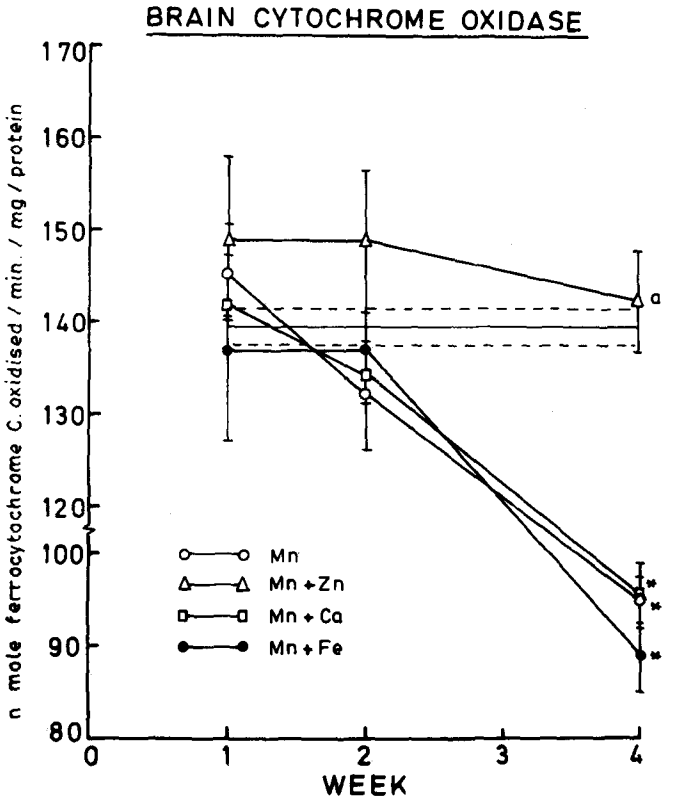

Fig. 6 : Effect of Zn, Ca or Fe supplementation on the activity of cytochrome oxidase in brain of rats.
Each value represents mean ± S.E. of 6 values.
*$P < 0.001$, when compared to control (Horizontal line).
$^{a}P < 0.001$, when compared to manganese group using student's 't' test.

REFERENCES

Chandra SV, Tandon SK (1973) Enhanced manganese toxicity in iron deficient rats. Environ Physiol Biochem 3: 230-235

Chandra SV, Saxena DK, Hasan Mz (1975) Effect of zinc on manganese induced testicular injury in rats. Ind Health 13: 51-56

Chandra SV, Shukla GS, Srivastava RS, Gupta SK (1980) Combined effect of metals on biogenic amines and their distribution in the brain of mice. Arch Environ Contam Toxicol 9: 79-85

Co-operstein SJ, Lazarow A (1951) Microspectrophotometric method for the determination of cytochrome oxidase. J Biol Chem 189: 665-670

Halacheva L, Boyadjiev V (1975) Changes in the activity of SDH and cytochrome oxidase in experimental manganese poisoning. Scr Sci Med 12: 167-171

Kello D, Debonic D, Kostial K (1979) Influence of sex and dietary calcium on intestinal cadmium absorption in rats. Arch Environ Health 34: 30-33

Kostial K, Simonovic I, Pisonic M (1971) Reduction of lead absorption from the intestine in new born rats. Environ Res 4: 360-363

Ono T, Wada O, Nagahashi M, Yamaguchi N, Toyokawa K (1973) Increase of sulfhydryl group in proteins from kidney of mice administered with various heavy metals. Ind Health 11: 73-74

Petering HG (1978) Some observations on the interaction of zinc, copper and iron metabolism in lead and cadmium toxicity. Environ Health Perspect 25: 141-145

Shearer DA, Cloutier RO, Hidiroglou M (1977) Chelate extraction and flame atomic absorption spectrometric determination of nanogram amount of manganese in blood and animal tissue. J Assoc Off Analyt Chem 60: 155-159

Six KM, Goyer RA (1972) The influence of iron deficiency on tissue content and toxicity of ingested lead in the rat. J Lab Clin Med 79: 128-136

Slater EC, Bonner WD (1952) The effect of fluoride on the succinoxidase system. Biochem J 52: 185-195

Tandon SK, Khandelwal S (1982) Chelation in Metal Intoxication X. Influence of different polyaminocarboxylic acids and thiol chelating agents on the excretion and tissue distribution of ^{54}Mn in rat. Res Commun Chem Pathol Pharmacol 36: 337-340

Washko PW, Cousins RJ (1976) Metabolism of ^{109}Cd in rats fed normal and low calcium diet. J Toxicol Environ Health 1: 1055-1066

Webb M (1972) Protection by zinc against cadmium toxicity. Biochem Pharmacol 21: 2767-2771

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