The Effect of Lead and Cadmium on Liver, Kidney, and Brain Levels of Cadmium, Copper, Lead, Manganese, and Zinc, and on Erythrocyte ALA-D Activity in Mice

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Both lead and cadmium are classified as non-essential elements which have no known beneficial effects in living organisms. The absorption of cadmium continues irrespective of the body burden of this element (COTZIAS et al., 1961) and excretion is slow (BURCH AND WALSH 1959, LUCIS et al., 1969). Cadmium is deposited in the liver and kidney and is strongly bound to macromolecules in the intracellular compartment (SHAIKH and LUCIS, 1972). An increase in tissue zinc levels of rat was found after administration of cadmium (SCHROEDER and NASON, 1974). Cadmium can interfere with the metabolism of essential elements like iron, calcium, zinc, manganese and copper. It had also major influence on zinc metabolism after absorption in several tissues (ROBERTS et al., 1973). Lead and cadmium both have been shown to cause hypertension, accelerated atherosclerosis, kidney disease and neoplasia in experimental animals (MORGAN 1972). The increase in the body burden of cadmium accelerates the biosynthesis of cadmium and zinc binding proteins due to defence mechanism against toxic cadmium ions. In the physiological system this binding protein, metallothionein can associate with all three elements cadmium, zinc, and mercury of the II-B sub group of the periodic table as well as copper (HILL et al. 1963).

A positive association between the amount of cigarettes smoked and cadmium burdens of lungs, liver, and kidney has been reported (MARK et al., 1974). Tobacco smoke constitutes a major source for cadmium accumulation in man. Cadmium occurs with lead in nature and both are present as contaminants in food stuffs and beverages. Thus, industrial workers as well as population in general accumulate cadmium and lead simultaneously. In our previous study we found that zinc enhances the toxic effect of lead (SETH et al.). Since zinc and cadmium both are d^{10} block elements and have many similar properties it was felt that cadmium may also alter the toxic effects of lead. The present study was, therefore, undertaken to find the combined

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effect of lead and cadmium for better understanding the effects of exposure of lead under actual conditions.

MATERIALS AND METHODS

Twenty-four male mice, average weight 25 g, of I.T.R.C. animal colony were used in this study. They were fed normal laboratory animal feed and tap water ad lib. The mice were divided into four groups A,B,C, and D. Group A was kept as control, group B was given intraperitoneally (i.p.) 8 mg of lead per kg body weight as lead acetate, group C, 2 mg cadmium (i.p.) per kg body weight as cadmium acetate and group D was given 8 mg lead per kg body weight and 2.0 mg cadmium per kg body weight (i.p.) as acetates simultaneously. The injections were repeated on third and fifth day and ten mice were sacrificed on eleventh day. Blood was collected in heparinized tubes. Liver, kidney, and brain were removed quickly and washed with cold saline to remove blood and then dried and weighed.

Estimation of cadmium, copper, lead, manganese, and zinc.

Digestion of Tissues.

The tissues were homogenised in deionized water. An aliquot representing 0.1 g was taken in a conical flask and 10 ml of concentrated nitric acid was added to it. The flask was placed on a hot plate for complete digestion. After complete digestion, the solution was made up to 10 ml with 1% hydrochloric acid.

Atomic absorption spectrophotometry.

Perkin-Elmer, model - 303 atomic absorption spectrophotometer, equipped with Boling Burner and null read out accessory was used. The metals in the digested solution was estimated using standard conditions (Perkin-Elmer, 1971).

Determination of ALA-D activity.

ALA-D activity in the heparinized blood was estimated immediately after sacrifice by the method of HELEN and SIEGEL (1971).

RESULTS AND DISCUSSION

The levels of lead, cadmium, zinc, manganese, and copper in liver, kidney and brain of mice injected intraperitoneally lead (group B), cadmium (group C), lead and cadmium together (group D), and control

(group A) are shown in Tables 1-3. Lead levels were significantly increased in liver and kidney of group B and group D, but the increase was not significant in group C. However, the levels of lead were not increased significantly in brain in any of the groups. Cadmium levels were found to be increased in liver and kidney of group C ($p<0.005$) and group D ($p<0.005$). Zinc level increases significantly in liver ($p<.05$) and kidney ($p<.01$) of group C. Manganese levels were slightly decreased in liver, kidney and brain of all the groups but the decreases were not significant. Copper levels were decreased significantly in kidney of group B ($p<0.05$), group C ($p<.025$) and group D ($p<.025$). However, no significant alterations in the concentrations of cadmium, zinc, manganese, and copper were observed in brain of all the groups.

Blood delta-aminolevulinic acid dehydratase (ALA-D) activity decreases significantly in group B and group D ($p<0.005$), but the activity remained unaltered in group C (Table 4). This shows that cadmium has no effect on blood ALA-D activity. LAUWERYS et al. 1973 also reported no change in blood ALA-D activity of workers exposed to cadmium.

Table-1.
Concentration of metals in liver (ug/g wet weight)

	ug/g wet weight			
Metals	CONTROL (group A)	Lead Treated (group B)	Cadmium Treated (group C)	Cadmium plus lead (group D)
Pb	2.01±0.2	8.3±0.5 P<.005	2.10±0.27 N.S	8.8±0.47 P<.005
Cd	1.03±0.18	0.95±.08 N.S	5.16±0.12 P<.005	4.98±.28 P<.005
Cu	6.45±0.53	5.98±0.10 N.S	5.2±0.71 N.S	3.92±0.69 N.S
Mn	1.10+0.06	1.0+0.11 N.S	0.93+0.11 N.S	1.07+0.12 N.S
Zn	32.35±3.97	28.36±2.29 N.S	42.0±3.0 P .05	39.4±2.57 N.S

Lead and cadmium both were found to alter the level of copper, zinc and manganese in liver and kidney but did not have any effect on brain levels of these

Table-2.

Concentration of metals in kidney (ug/g) wet weight

Metals	ug/g wet weight CONTROL (group A)	Lead Treated (group B)	Cadmium Treated (group C)	Cadmium plus lead Treated (group D)
Pb	3.0±0.27	8.4±0.37 P<.005	2.8±0.15 N.S	9.2±0.34 P<.005
Cd	0.31±0.02	0.36±0.02 N.S	8.57±0.67 P<.005	8.20±0.35 P<.005
Cu	9.18±1.55	5.52±0.40 P<.05	5.29±0.36 P<.025	5.00±0.32 P<.025
Mn	1.62±0.20	1.35±.07 N.S	1.36±.08 N.S	1.18±.13 N.S
Zn	25.14±2.43	27.75±2.73 N.S	34.32±1.62 P<.01	37.05±3.33 P<.005

Table-3.

Concentration of metals in brains (ug/g wet weight)

Metals	ug/g wet weight CONTROL (group A)	Lead Treated (group B)	Cadmium Treated (group C)	Cadmium plus lead Treated (group D)
Pb	1.2±0.05	2.9±0.10 N.S	1.2±0.04 N.S	3.0±0.11 N.S
Cd	1.21±0.09	1.12±.06 N.S	1.85±0.12 N.S	2.01±0.18 N.S
Cu	3.54±0.38	2.66±0.27 N.S	2.70±0.19 N.S	2.68±0.15 N.S
Mn	0.50±0.06	0.44±0.05 N.S	0.45±0.06 N.S	0.41±0.06 N.S
Zn	36.72±	31.99±3.17 N.S	39.63±1.20 N.S	39.45±2.24 N.S

Table-4.

Delta-aminovulinic acid dehydratase (ALA-D) activity in blood

GROUPS	ALA-D activity*
CONTROL (A)	93.09±14.62
Lead Treated (B)	39.28±3.02 P<.005
Cadmium Treated (C)	91.42±4.53 N.S
Cadmium plus lead Treated (D)	34.49±3.41 P<.005

*Expressed as increase in absorbance at 555 nm of 0.100 with 1.0 cm light path, per ml of erythrocytes per hour at 38°C.

metals. Thus it appears that these metals do not accumulate in quantities sufficient to cause the alterations in the levels of these metals in brain, under the conditions of this experiment. MICHELSON et al. (1973) also found no effect of intraperitoneally administrated lead in rat on zinc, copper and manganese levels. Accumulation of cadmium was found to be more in kidney than in liver and brain. Significant increase in the levels of zinc and decrease in the levels of copper and slight decrease in manganese levels in animals poisoned with cadmium and a combination of lead and cadmium suggest that cadmium and lead interact with the metabolism of these metals. FOX 1974 and ROBERTS et al. 1973 also reported that cadmium had a major influence on zinc metabolism after absorption in several tissues and also interferes with the metabolism of other essential elements like manganese and copper.

HILL et al. 1963 were of the opinion that due to chemical similarities that exist between cadmium, zinc and copper, it is possible that cadmium may replace copper and zinc from the active metabolic sites, probably enzymatic, thereby rendering them inactive. DURYAN and VALLEE 1962 have shown that cadmium can replace zinc in liver alcohol dehydrogenase and thus inactivates it. In the present study, it is also found that levels of zinc manganese and copper are altered. Since these metals are cofactors

of several enzymes, it is therefore expected that the decrease or increase in the levels of these metals may be an index of corresponding enzyme activity. Thus alteration in the levels of these metals may be taken as parameters for the study of toxicity of lead and cadmium in laboratory animals. Since cadmium was found to have no effect on blood ALA-D activity, this test may be used as specific test for lead poisoning in workers with suspected lead and cadmium exposure.

Summary

Lead and cadmium were administered intraperitoneally, singly and jointly, to the mice. The levels of cadmium, copper, manganese, lead and zinc were determined in liver, kidney and brain by atomic absorption spectrophotometric technique and delta-aminolevulinic acid dehydratase (ALA-D) activity was determined in erythrocytes. The tissue levels of some of these metals were found significantly altered by cadmium and lead both, but cadmium was found to have no effect on blood on ALA-D activity.

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References

Analytical methods for atomic absorption spectrophotometry (1971). Perkin - Elmer, Norwalk. Connecticut, U.S.A.

BURCH, G.E. and WALSH, J.J.: J. Lab. Clin. Med. 54: 66-72 (1959).

COTZIAS, G.C., BORG, D.C. and SELIECK, B.: Amer. J. Physiol. 201, 927-930 (1961).

DURYAN, R. and VALLEE, B.L.: Federation Proc. 21, 242 (1962).

FOX, M.R.S.: J. Food. Sci. 39, 321-24 (1974).

HELEN, B.B. and SIEGEL, A.L.: Clin. Chem. 17, 1038-41 (1971).

HILL, C.H., MATRONE, G., PAYNE, W.L. and BARBER, P.W. J. Nutr. 80, 227-235 (1963).

LAUWERYS, R.R., BUCHET, J.D. and ROELS, H.A.: Brit. J. Indr. Med. 30, 359 (1973).

LEWIS, G.P., JUSCO, W.J., COUGLIN, L. L. and HARTZ, S. Lancet 1, 291-92 (1972).

LUCIS, O.J., LYNK, M.E. and LUCIS, O. J.: Arch. Environ. Health 18, 307-310 (1969).

MARK, S.S, VOORS, A.W. and GALLAGHER, P.N.: Bull. Environ. Cont. Toxi. 12, 570-576 (1974).

MORGAN, J.M.: Arch. Environ. Health 24, 364-368 (1972).

ROBERT, K.R., MILLER, W.J., STATE, P.E., GEN RY, R.P. and NEATHERY, M.S.: Proc. Soc. Exp. Biol. Med. 144, 906-908 (1973).

SHAIKH, Z.A. and LUCIS, O.J.: Arch. Environ. Health 24, 419-425 (1972).

SCHROEDER, H.A. and NASON, A.P.: J. Nutr. 104, 167-178 (1974).

SETH, T.D., SATIJA, N.K., AGARWAL, L.N. and HASAN, M.Z. (In Press).