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# Effect of Chronic Cadmium Administration on Liver and Kidney Concentrations of Zinc, Copper, Iron, Manganese, and Chromium

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Cadmium toxicity in humans and animals results from its accumulation in almost all of the organs. The widespread effects and interference with the utilization of essential metals, inhibition of protein synthesis, carbohydrate metabolism, drug metabolizing enzymes and cell mediated immune response has been recently well reviewed (Nath et al., 1984). Chronic Cd exposure in animals brings about significant morphological and functional changes in both liver and kidney (Suzuki et al., 1983a). Most studies of cadmium effects on essential metal tissue distribution involve large concentrations of either Cd or essential metals added to the diet. The effect of Cd ingestion on trace metal metabolism of animals consuming usual diets may be marked, as "elements whose physical and chemical properties are enough alike ... will act antagonistically to each other biologically" (Hill and Matrone, 1974). Therefore, the purpose of the present study was to observe the effects of a chronic low dose of Cd added to an otherwise normal diet on the liver and kidney accumulation of zinc, copper, iron, manganese and chromium in the mouse at different times after the cessation of cadmium ingestion.

## MATERIALS AND METHODS

Male Charles River strain CD1 mice, 6 to 7 weeks of age were maintained under controlled environmental conditions (22 to 24°C: 45 to 55% relative humidity: lighting 0800 to 2000 hours). A commercial ration (Purina Mouse Chow #5015, Ralston Purina Co., St. Louis, Missouri) and purified water $\geq$ 17MΩ (Barnstead Purification Systems, Barnstead Co., Boston, MA) were fed ad libitum. There were 30 mice in each of the control and experimental groups. Control mice were fed purified water, while 50 ppm CD (as $CdCl_2$) was added to the water of the treatment group for 3 weeks.

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After the Cd ingestion period and counting from the last day, 10 mice from each group were killed for tissue analyses at 0,3 and 6 weeks. Portions of liver and kidney were removed and prepared for determinations of Cd, Zn, Fe, Cu, Mn and Cr. The remaining liver, kidney and other tissues were retained for immunological determinations. Samples for mineral analysis were freeze-dried for 24 hours, after weighing the whole organ and the aliquot. Tissue samples and ration aliquots were placed in acid washed porcelain crucibles and ashed overnight at 500°C. The ash was dissolved up in 5 mL of 1N HCl (Suprapur$^{R}$, E. Merck, Darmstaat) and after a brief time the resulting solution was made up to 10 ml in acid washed volumetric flasks. Only purified water was used for preparing glassware and in reagent preparation. Standards were prepared from 1000 ug/L stock solutions (Fischer Scientific Co., Fairlawn, NJ). Kidney, liver and ration aliquots were analyzed for Cd, Zn, Cu, Fe, Mn and Cr by atomic absorption spectroscopy (Perkin Elmer, Norwalk, CT). Zinc, Cu, Fe and Mn analyses were carried out by flame using a single slot four inch burner head. Cadmium and Cr analyses were done using a HGA-300 graphite furnace with deuterium arc background correction. All results are reported as ug/g wet weight of organ analyzed as means $\pm$ standard deviations. As there were not enough liver samples for chromium analyses the results for kidney alone are reported in the text.

Statistical procedures were carried out on the Vax computer system at Memorial University of Newfoundland using SPSSx (Nie et al., 1983). Analysis of variance was used to assess differences between treatment and control groups of mice. Duncan's multiple range test was used to assess differences among individual groups. Significant differences were assigned at $P < 0.05$.

## RESULTS AND DISCUSSION

Analyzed levels of trace elements in the stock diet (n=10) were: Cd; 110$\pm$52 ng/g; Zn, 115$\pm$3 ug/g; Cu, 13.1$\pm$1.1 ug/g; Fe, 122$\pm$4 ug/g; Mn, 121$\pm$6 ug/g; Cr, 1.2$\pm$0.2 ug/g. Analyzed levels of trace elements in NBS bovine liver (1577a, n=3) and certified values were: Cd, 0.54+.03 ug/g (0.44+0.06 ug/g); Zn, 119+1 ug/g (123+8 ug/g), Cu, 161+1 ug/g (158+7 ug/g); Fe, 184+1 ug/g (194+20 ug/g); Mn 10+0 ug/g (9.9+0.8 ug/g). Cr values are not reported for bovine liner. Analyzed levels of Cr in NBS Oyster tissue (1566, n=3) and certified values were: Cr 0.97$\pm$0.5 ug/g (0.69$\pm$0.27 ug/g).

Cd treatment had no apparent effect upon the general health of the mice and disappearance of food and water was not different between control and experimental mice over the 3 week period of Cd ingestion. Liver and kidney weights did not differ between

groups at any sampling time but there was a transient significant decrease in the body weight of mice consuming Cd at 3 weeks post-Cd-ingestion period that was not seen in the control mice. By 6 weeks after the end of Cd-ingestion there was no difference in body weights between groups. Cadmium concentration at the cessation of Cd ingestion, in the liver (14±1 ng/g) and kidney (48±2 ng/g) of control mice (n=10) and liver (6±2.1 ug/g) and kidney (8.5±3.4 ug/g) of treatment mice (n=10) were within reported ranges for cadmium and non-cadmium treated animals (Washko and Cousins, 1977; Suzuki et al., 1980). Cadmium concentration in the livers of Cd-treated animals did not differ at any sampling time, but in the kidney, Cd concentrations at 3 weeks post-treatment were significantly greater (17.2±1.0 ug/g) than values found at either 0 weeks (8.5±3.4 ug/g) or 6 weeks (11.2±3.5 ug/g) post-Cd ingestion.

Most researchers examine the effects of Cd administration on tissue concentration of metals immediately after treatment and we are not aware of any data reported on tissue concentrations subsequent to the cessation of treatment. Kidney concentrations of zinc, iron, copper, manganese and chromium in Cd-treated mice at different post-treatment times did not follow the pattern seen for those metals in control mice (Table 1). As well, in the present study, chromium levels in kidney of Cd-treated mice rose significantly between 0 (172±83 ng/g, n = 4) and 3 weeks (446±356 ng/g, n=8) and then showed a trend (P = 0.08) towards a decline by 6 weeks post-treatment (164±120 ng/g, n=7).

Even though Cd treatment had ceased, all metals including Cd increased in the kidneys of affected animals. The reason for this transient increase in Zn, Cu, Mn, Fe and Cr in kidneys of Cd-treated animals is unclear at this time. Toxic effects of Cd may be exacerbated by mineral deficiency or counteracted by supplementing dietary levels of these elements (Weigel et al., 1984) yet the diet in the present study is probably adequate for these trace elements as it supported normal growth in the control mice and is similar in mineral content to diets reported by others (Sugawara, 1984). Interestingly, the weights of animals at the end of Cd treatment (37±3 g) did not differ from that of control mice (37±3 g), but were significantly lower at 3 weeks post-Cd-ingestion (39±3 g, Cd-treated; 46±6 g, control). By six weeks post Cd-ingestion body weights were not statistically different (49±3 g, Cd-treated, 46±6 g, control). We did not measure food intake after the treatment period and do not know if a reduced intake led to lower weight gain compared to controls which coincided with the increase in kidney metal concentrations. This decreased weight gain suggests possible tissue breakdown allowing a buildup of metals in the kidney perhaps bound to metallothionein which is induced by Cd administration (Margoshes and Vallie, 1957). Metallothioneins which bind metals to sulfur in cysteinyl residues are known to have an affinity for these metals (Pulido et al., 1966; Suzuki

TABLE 1. Zinc, copper, iron and manganese concentrations in liver and kidney of control and cadmium treated mice at 0, 3 and 6 weeks post-Cd-ingestion (ug/g, wet weight)[1].

| Time (weeks) | Liver | | Kidney | |
|---|---|---|---|---|
| | Control | Cd-treated | Control | Cd-treated |
| | | Zinc | | |
| 0 | 42.4±3.2$^{a}$ (10) | 41.7±4.1$^{a}$ (10) | 3.20±4.7$^{b}$ (10) | 27.8±2.5$^{c}$ (10) |
| 3 | 37.6±2.8$^{b}$ (10) | 42.6±5.1$^{a}$ (10) | 27.3±2.5$^{c}$ (10) | 54.2±3.8$^{a}$ (10) |
| 6 | 39.5±3.2$^{b}$ (10) | 38.7±4.7$^{b}$ (10) | 26.7±1.9$^{c}$ (10) | 30.0±7.6$^{b}$ (10) |
| | | Copper | | |
| 0 | 22.82±6.92$^{a}$ (8) | 8.75±1.5$^{b}$ (7) | 13.91±2.29$^{bcd}$ (8) | 11.69±3.39$^{abc}$ (7) |
| 3 | 25.55±2.78$^{a}$ (10) | 9.84±1.55$^{b}$ (10) | 11.6±1.83$^{abc}$ (10) | 16.80±6.17$^{cd}$ (10) |
| 6 | 24.4±3.92$^{a}$ | 8.12±1.94$^{b}$ | 9.06±1.74$^{abc}$ | 9.54±1.61$^{abc}$ |
| | | Iron | | |
| 0 | 277±78$^{c}$ (8) | 141±48$^{a}$ (7) | 101±14$^{a}$ (8) | 107±329$^{a}$ (10) |
| 3 | 169±28$^{ab}$ (10) | 199±19$^{bc}$ (10) | 95±23$^{a}$ (8) | 162±75$^{b}$ (10) |
| 6 | 169±39$^{ab}$ (9) | 180±420$^{abc}$ (10) | 131±22$^{ab}$ (8) | 109±20$^{a}$ (10) |
| | | Manganese | | |
| 0 | 1.63±0.34$^{abc}$ (6) | 1.98±0.51$^{bc}$ (4) | 3.48±0.77$^{ab}$ (5) | 2.77±0.86$^{a}$ (6) |
| 3 | 1.34±0.25$^{abc}$ (9) | 1.69±0.74$^{bc}$ (10) | 2.96±0.58$^{a}$ (10) | 4.31±2.01$^{b}$ (10) |
| 6 | 1.24±0.21$^{ab}$ (10) | 1.24±0.28$^{ab}$ (9) | 3.04±0.41$^{a}$ (10) | 2.84±0.57$^{a}$ (9) |

[1]Results are means ± S.D., where n is the number in parentheses; within the same metal and organ, values not sharing the same superscript letter are significantly different according to Duncan's Multiple Range test.

et al., 1983b; Held and Hoekstra, 1984). In the present study there was not enough tissue to analyze for this protein. Alternatively kidney, damaged by cadmium may require extra metals for tissue reconstruction. Certainly zinc is known to be required for tissue repair (Underwood, 1977) and these animals were still showing altered kidney morphology 6 weeks post-Cd treatment (Borgman, Au, Chandra, unpublished results).

In the liver, Zn concentration did not differ but there were decreases in Cu and Fe concentrations with Cd ingestion as has been reported previously (Suzuki et al, 1983b; Bunn and Matrone, 1966). Cadmium is known to be antagonistic to Cu and Fe absorption (Hill and Matrone, 1974, Weigel et al., 1984). We found no difference in liver Mn concentrations in either group contrary to reports of increased (Schroeder and Nason, 1974) or decreased (Doyle and Pfander, 1975) liver Mn after Cd-ingestion. These latter studies fed Cd for considerably longer periods of time (>200 days) than was done in the present study.

Schroeder and Nason (1974) report that the mechanism of the effect of supplemental Cd on trace element concentrations in rat organs is obscure. The transient rise in kidney trace elements in the present study are not explained by normal metabolism as very little of these elements are excreted in urine (Underwood, 1977). This generalized transient increase did not occur in liver where there was a unique response for each metal to Cd toxicity. It is noteworthy that except for Cu in the liver, after 6 weeks post Cd-ingestion the liver and kidney appear to gain homestatic control of these trace elements suggesting that trace element metabolism may recover from short term Cd-ingestion.

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