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Some Observations on the Interaction of Zinc, Copper, and Iron Metabolism in Lead and Cadmium Toxicity

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A brief review of the literature indicates that nutritional deficiencies have been shown to increase the absorption and toxicity of orally ingested lead and cadmium. Results from recent studies indicate that low level oral ingestion of cadmium and lead perturbs the metabolism of zinc, copper, and iron and that these changes may be the earliest manifestation of the toxicity of lead and cadmium. The significance of these findings reveals itself in two ways: namely, that toxicologic investigations of lead and cadmium, whether experimental or clinical, must be based on a definitive consideration of the nutritional status of animals or people, and, secondly, that the preventive role of nutrition, especially that of trace metal intakes, must be taken seriously when establishing measures for reducing, eliminating, or combatting the toxic effects of widespread exposure to lead and cadmium in humans.

A review of literature (*1–12*), summarized in Table 1, indicates that mineral nutrition in general has a role in heavy metal toxicity. This is especially true of the essential micronutrients zinc, copper, and iron with respect to the toxicity of lead and cadmium. The implication of this work is that a dietary insufficiency or deficiency of zinc, copper, or iron will lead to enhanced toxicity of lead or cadmium, while an excessive dietary intake of the essential metals will be protective. The difficulties in evaluating the role of nutrition in the toxicity of lead and cadmium reside in the high dosages which have usually been used, in the routes of administration employed, and in the fact that most research on lead and cadmium has been done with stock diets of unknown composition, most of which have probably been excessive in the essential micronutrients. We have therefore concentrated our efforts on oral administration of low levels of cadmium or lead under carefully controlled dietary regimens. In this paper we shall review this work and that of others who have used a similar experimental design to show that even low oral exposure to cadmium and lead produces demonstrable interaction with zinc, copper, and iron nutriture and metabolism in rats.

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Lead and Zinc Interrelationships

Cerklewski and Forbes (*13*) recently reported that tibia lead and urinary δ-aminolevulinic acid (ALA) were markedly increased when male rats were fed a diet low in zinc and containing 200 ppm of lead. Raising the zinc content of the diet to an optimal and superoptimal level greatly reduced tibia lead and urinary ALA. El-gazzar et al. (*14*) in our laboratory confirmed this protective effect of dietary zinc, showing that both absorption and retention of lead in rats when dietary lead was 100 ppm was reduced when dietary zinc was 50 ppm rather than 5 ppm. These investigators also found that lead seriously disturbed the serum lipoprotein profile when dietary zinc was low, but had much less effect when it was high. Some of their data (*14*) are shown in Table 2.

Finelli et al. (*15*) have found that lead inhibition of δ-aminolevulinic acid dehydrase (ALA-D) was reduced and reversed by zinc *in vitro*. This study suggests that zinc has a protective role in reducing the biological effects of absorbed lead as well as a probable role in altering absorption of lead. The ability of high levels of dietary zinc to play this role has been confirmed by Haeger-Aronsen, Schutz, and Abdulla (*16*).

Table 1. Relationships among lead or cadmium and some essential mineral nutrients.

Mineral nutrient	Lead			Cadmium		
	A[a]	B[a]	Reference	A[a]	B[a]	Reference
Calcium	+	+	(*1,2*)	+	+	(*1,2*)
Zinc	+	+	(*3*)	+	+	(*3, 4*)
Copper	+	+	(*4, 5*)	+	+	(*5, 6*)
Iron	+	+	(*6*)	+	+	(*5–11*)
Manganese	0	0	—	+	?	(*9*)
Selenium	+	+	(*7*)	+	+	(*12*)

[a] A = toxic metal affects metabolism of essential nutrients; B = deficiency of essential nutrients increases toxicity, super-optimal intake of essential nutrients reduces toxicity. (+) Yes; (0) No information.

Table 2. Effect of dietary zinc on lead distribution in rat tissue.[a]

Concn in drinking water, μg/ml		Lead content, μg/g fresh weight[b]			
Zn	Pb	Tibia	Brain	Kidney	Spleen
5	0	27.3[c]	0.75[d,e*]	0.27[f]	0.10[g]
5	100	143.1[c, h*]	1.30[d]	1.84[f]	0.50[g, i*]
50	0	25.1[j]	0.62[e*, k]	0.28[m]	0.11[n]
50	100	94.8[h*, j]	1.12[k]	1.95[m]	0.35[i*, n]

[a] Length of experiment 140 days from weaning. Semipurified diet deficient in zinc used. Data taken from report by El-gazzar et al. (*14*).

[b] Matching letters indicate significant differences at $p < 0.01$. Asterisks indicate significant differences at $p < 0.05$.

Lead Interaction with Dietary Copper and Iron

Klauder, Murthy, and Petering (*17*) reported that low dietary copper enhanced the absorption of lead in rats fed a diet containing 5000 ppm, and Six and Goyer (*18*) have reported similar findings with respect to dietary iron. Since copper and iron are metabolically interrelated we have pursued the study of their roles in the toxicity in male rats of dietary lead at 500 ppm. In two studies (*19, 20*), Klauder and Petering found that at low levels of dietary lead increased levels of dietary iron and copper reduce lead absorption, iron being the more important element. When, however, effects on the hematopoietic system of lead were examined, it seemed in this aspect of lead toxicity that copper may be the more important element as shown in Table 3. They also found that only when both copper and iron were at optimal levels in the diet was the effect of lead on iron metabolism completely inhibited.

In unpublished data from the same experiment Klauder (*21*) found that both dietary copper and iron were protective against the growth depression caused by 500 ppm of dietary lead. Furthermore,

Table 3. Influence of iron, copper, and lead on hematopoiesis in rats.[a]

Dietary levels, ppm		Hematocrit, % volume[b]		Hemoglobin, g/100 ml blood[b]	
Fe	Cu	No Pb	500 ppm Pb	No Pb	500 ppm Pb
6	0.5	22.4[c, d]	14.6[d, e]	5.2[c, d]	3.1[d, e]
6	8.5	33.7[c, f]	26.7[d, f]	8.2[c, f]	6.5[d, f]
40	0.5	43.3[g*, h]	34.7[h, i]	12.3[g*, h]	8.8[h*, i]
40	8.5	47.3[g*]	46.9[i]	13.9[g*]	13.1[h]

[a] Semipurified diet containing 20 μg/g zinc. Data taken from Klauder and Petering (*20*).

[b] Arithmetic means. Matched letters indicate significant differences at $p < 0.01$; asterisks denote $p < 0.05$.

these data show that hypertrophy of kidney, brain, and heart was induced by 500 ppm of dietary lead unless both dietary copper and iron were at optimal levels.

Effect of Cadmium on Zinc Metabolism

Using a tissue culture system of mouse L cells, Christian et al. (*22*) found that cadmium cytotoxicity could be prevented in a predictable dose related manner by increasing the Zn/Cd ratio of the culture medium. These findings demonstrate the well-known pattern of antagonism between zinc and cadmium.

In a recent study Lal (*23*) compared the effect of dietary zinc on toxicity in rats of inhaled CdO or $CdCl_2$ given in the drinking water. It was found that pathologic lesions in the liver, lung, heart, and testes caused by cadmium were extensive when dietary zinc was low (5 ppm), but that when zinc was high (40 ppm), only an increase in liver glycogen occurred.

In another experiment, El-gazzar, Boyle, and Petering (*24*) examined the metalloprotein elution profiles of the liver cytosol chromatographed on G-75 Sephadex from rats fed a stock diet and given $CdCl_2$ in the drinking water at 0, 4.3, 8.6, and 34.4 μg/ml for 200 days. Their data for female rats, shown in Figure 1, indicated that before there was any significant induction of metallothionein (MT) there was a profound alteration of the zinc metalloprotein elution profile. Their results also suggest that prolonged oral ingestion of cadmium probably proceeds in a stepwise fashion with the early formation of three distinct peaks of cadmium-binding proteins of about equal cadmium contents, i.e., peaks (or regions) I, V, and VI as shown in the 4.3 μg/ml profile. In this same cytosol we found the original three zinc-binding protein regions of the control cytosol had been increased to four peaks

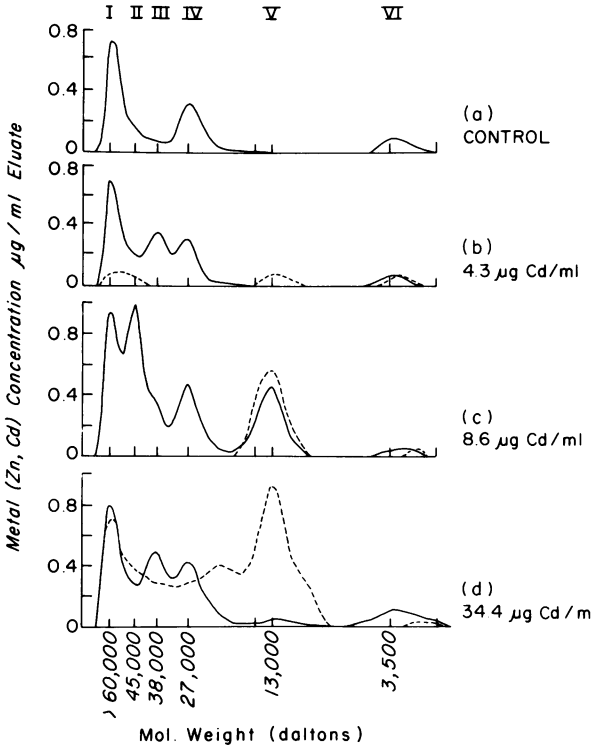

FIGURE 1. Zinc and cadmium metalloprotein profiles of liver cytosol from female rats exposed orally to different concentrations of $CdCl_2$ in drinking water (amounts = μg/ml): (--) Cd; (—) Zn. Data taken from El-gazzar et al. (*24*).

with the appearance of peak III estimated to have a molecular weight of 38,000 daltons.

When the amount of cadmium was increased to 8.6 μg/ml, there appeared in the elution profiles a marked increase in the high molecular weight zinc-binding proteins and there was definite appearance of the cadmium-thionein peak V (MT) which not only contained cadmium but also zinc in the ratio of Cd/Zn of 2/1. When dietary cadmium was raised to 34.4 μg/ml, there was only a small amount of zinc in peak V (MT) and at least half of the cadmium of the cytosol was present in high molecular weight regions where zinc-binding proteins were diminished almost to the level found in the 4.3 μg elution profile.

These data suggest that oral ingestion of cadmium by rats produces a dose-related effect in the cytosol zinc-binding proteins in addition to the induction of metallothionein synthesis.

Effect of Absorbed Cadmium on Copper and Iron Metabolism

Recently Petering et al. (*7*) published data which suggest that male rats receiving 17.2 μg Cd/ml in the drinking water of male rats fed a semipurified diet adversely affected copper and iron metabolism, some of the effects being minimized by additional amounts of dietary copper.

In the same experiment it was found that decreases in hematocrit and hemoglobin values caused by cadmium could only be prevented or minimized by high dietary levels of both zinc and copper.

A final example of the effect of cadmium on copper and iron metabolism involves its effect on fetuses and neonates of rat dams fed stock diets and given 17.2 μg Cd/ml of drinking water only during the gestation period. The data taken from those of Choudhury et al. (*25*) and shown in Table 4 indicate that this level of dietary cadmium given to the dams produced marked biochemical and physiological changes in their fetuses and neonates, while not producing any overt teratogenic changes. Cadmium reduced birth weights and weight gains of pups during the first 21 days of life. In addition, there were significant reductions in the newborn whole body concentrations of Zn, Cu, and Fe, while there was no increase in cadmium. These data coupled with those previously mentioned as shown in Figure 1 show that cadmium can cause profound changes in essential metal metabolism even when its accumulation is very low.

Those neonates whose mothers were exposed to oral cadmium during gestation only and which were allowed to live grew at a slower rate than control neonates. In addition, it was found that the pups of the exposed dams exhibited significantly less spontaneous activity than did the control pups, even though we could find no evidence of gross deficiencies of iron, copper, or zinc metabolism or evidence of health defects at the time of neurobehavioral testing.

Discussion and Conclusions

Several important concepts, which are illustrated by the experimental data presented here, need to become an integral part of our understanding of heavy metal toxicology. First, we need to be more concerned about the nutritional factors which may play a role in the host response to ingestion of heavy metals and for other toxic agents, for they probably are extremely important both in experimental and clinical evaulations of the toxicity of cadmium, lead, and other toxic metals.

We have shown that the chronic oral toxicity of cadmium and lead may be due as much to alterations of zinc, copper, and iron nutrition and metabolism as it is to direct cellular inhibitory action of these metals themselves. This is an important con-

Table 4. Effect of maternal cadmium (17.2 μg/ml given during gestation on body weights and body trace metals of neonates.[a]

Experimental group	Body weight, g	Lactation wt. gain, g	Body trace metal, μg/g pup dry weight[b]			
			Zinc	Copper	Iron	Cadmium
Control	6.8 ± 0.1(21)	52 ± 1.0(13)	116 ± 4(8)	9.4 ± 0.4(8)	330 ± 6(8)	0.46 ± 0.05(8)
Cadmium	6.4 ± 0.1[c](22)	44 ± 1.6(8)	10.4 ± 3[c](14)	5.7 ± 0.4[d](14)	267 ± 17[d](14)	0.48 ± 0.04(14)

[a] $CdCl_2$ given in drinking water. Data of Choudbury et al. (*25*).
[b] Data expressed as means ± SEM. Statistically significant differences by student test. Values in parentheses denote number of neonates.
[c] $p < 0.05$.
[d] $p < 0.01$.

cept, since it brings into focus the interplay of level of exposure and body or organ burden on the one hand with nutritional status as a host defense mechanism on the other. It also indicates a possible important preventive measure which has been overlooked clinically until now, and one which probably needs to be studied and used more since oral cadmium and lead exposure will be with us for a long time even if we had no more industrial use of these metals.

Then too, we also need to remember that age and length of exposure are important factors in determining the interaction of cadmium and lead with essential nutrient metabolism. In a recent report (*26*), Cerklewski and Forbes indicated that increasing dietary copper to optimal and even higher levels for rats caused an increase in lead toxicity over that found when dietary copper was minimal as indicated by several parameters. These authors, however, fed the lead for only 4 weeks post-weaning, a totally inadequate period to evaluate either the effect of 200 ppm of lead or the effects of copper. Furthermore, the differences in kidney lead, the only important measure of lead absorption and retention with a significant change, was minimal and may not have had any biological significance. In addition, their data which showed no effect on lead toxicity due to copper insufficiency was for the postweaning period in which stores of copper are high and dietary intake probably is not critical. On the other hand, experiments by Klauder (*21*) showed very definitely that the normal metabolism of copper in the rat changes markedly from the 8th week post-weaning to the 17th week, and other results showed that there was a significant difference between the effects of lead after 8 weeks and after 12 weeks of exposure to lead.

We conclude that essential metal nutrients such as zinc, copper, and iron can protect against the oral toxicity of cadmium and lead and that more attention to their preventive action of increasing host defenses against these heavy metals is urgently needed.

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