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Protective Value of Dietary Copper and Iron against Some Toxic Effects of Lead in Rats

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Both dietary iron and copper were inversely related to lead absorption as indicated by erythrocyte and kidney lead levels, dietary iron having the greatest effect. Kidney copper values were depressed when dietary iron was low, a condition which was worsened by lead. Lead tended to lower heart cytochrome c oxidase especially when dietary copper was low, but also when dietary copper and zinc were high. Lead interfered with hematopoiesis when dietary copper and/or iron were low, the effect being especially severe when both essential nutrients were low. These results show the importance of copper and iron nutriture and metabolism as factors which reduce lead toxicity, and emphasize the necessity of considering nutritional status in evaluating lead toxicity.

Introduction

From many reports it can be inferred that the composition of the diet of an animal or man is intimately related to the toxic effects which may ensue on exposure to lead. Increased lead retention in rats has been observed when dietary calcium is restricted (*1–3*). Vitamin D enhances lead absorption (*4, 5*), presumably in a similar manner as it promotes the uptake of calcium. Certain vitamins may also be protective against the toxic manifestations of lead (*6*).

Recently we reported that the addition of small increments of copper to a semipurified copper-deficient rat diet similar to that previously described (*7*) significantly reduced the adverse effects of lead on growth and hematopoietic parameters (*8*). An inverse relationship between erythrocyte lead and plasma ceruloplasmin levels was also found. About the same time, Six and Goyer reported (*9*) that the level of iron in the diet could significantly alter toxic effects resulting from low-level lead ingestion in rats. It has long been known that an adequate copper intake is essential for normal iron utilization (*10–15*). These factors prompted us to investigate the interrelationships of both dietary copper and iron with respect to the toxicity of orally ingested lead. The addition of excess zinc, a known copper antagonist, to the diet and its effects on the copper–lead interaction was also studied.

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Materials and Methods

Animals, Housing, and Diet

Male weanling Sprague-Dawley rats were housed individually in stainless steel wire cages in an environmentally controlled room (*16*) and given deionized drinking water and the basal diet shown in Table 1. without the iron supplement. The basal diet contained by analysis copper, 0.5μ g/g diet; iron, 6 μg/g; zinc, 20 μg/g; manganese, 55 μg/g; molybdenum, 1.0 μg/g. After a 4-day acclimatization period the animals were randomly assigned to one of the treatment groups shown in Tables 2–7, each consisting of six animals. Iron was added to the feed in the iron-supplemented diets; copper, zinc, and lead were given in the drinking water according to experimental design (Table 2).

Analytical Procedures

At the end of 12 weeks the animals were fasted for 18–20 hr, anesthetized with sodium phenobarbital, and blood was drawn from the heart into

Table 1. Composition of diet.

	Amount, g/kg diet	
	Basal diet	Fe-supplemented diet
Milk, dry fat-free[a]	550.0	550.0
Cornstarch[b]	313.3	313.1
Cellulose[c]	30.0	30.0
Corn oil[d]	90.0	90.0
Choline chloride[e]	1.5	1.5
Fat-soluble vitamin mix[f]	10.0	10.0
Water-soluble vitamin mix[g]	5.0	5.0
Mineral mix[h]	0.165	0.165
$FeSO_4 \cdot 7H_2O$[j]	—	0.174

[a]Kroger commercial instant, nonfat, dry milk solids, Cincinnati, Ohio.
[b]General Biochemicals Inc., Chagrin Falls, Ohio.
[c]Whatman CF11, W. and R. Balston, Ltd., London, England.
[d]Mazola corn oil, Cincinnati, Ohio.
[e]Sigma Chemical Co., St. Louis, Mo.
[f]Corn oil contained in 10 g mixture: ergocalciferol, 0.1 mg; α-tocopherol acetate, 100.0 mg; retinyl palmitate, 30.0 mg.
[g]Cornstarch contained in 5 g mixture: thiamine hydrochloride, 20.0 mg; riboflavin, 20.0 mg; pyridoxine hydrochloride, 10.0 mg; calcium pantothenate, 60.0 mg; niacinamide, 100.0 mg; folic acid, 0.5 mg; i-inositol, 400.0 mg; menadione, 2.0 mg; biotin, 1.0 mg; cyancolbaltamine, 0.02 mg.
[h]Minerals (in mg/kg diet): manganese (as $MnCO_3$), 55; iron (as $FeSO_4 \cdot 7H_2O$), 5.0; chromium [as $CR(C_2H_3O_2)_3 \cdot H_2O$], 5.0; molybdenum as $(NH_4)_6Mo_{24} \cdot 4H_2O$, 0.6.
[j]Iron in Fe-suppl. diet (as $FeSO_4 \cdot 7H_2O$), 35 mg/kg.

heparinized syringes. Whole blood was used for hematocrit and hemoglobin analyses, the latter done by the cyanmethemoglobin method (*17*). The remaining blood was centrifuged and the plasma separated from the erythrocytes.

At autopsy a 5% homogenate was made of the left ventricle of each heart in a 0.1*M* sodium phosphate buffer, pH 7.2. Each homogenate was centrifuged at 1000*G* for 10 min in a refrigerated Sorvall. The resulting supernatant was used for the analysis of cytochrome c oxidase activity by spectrophotometrically monitoring the rate of oxidation of reduced cytochrome c, as described by Smith (*18*). The reaction system contained 2.5 ml of 0.1*M* phosphate buffer at pH 7.2, 0.5 ml of a 90μ*M* solution of reduced cytochrome c in the same buffer, and 20 μl of heart supernatant.

Tissue metal contents were determined by a method similar to that described by Petering, Yeager, and Witherup for hair analysis (*19*). The biological materials of interest, erythrocytes (wet weight) and kidney (dry weight), were wet ashed in concentrated nitric acid. The clear residues were diluted to 10.0 ml with 10% nitric, and the metal analyses of each prepared sample were done using atomic absorption spectroscopy.

Statistical Analysis

Statistical analyses were performed on all data using analysis of variance techniques. Each measurement was analyzed separately to determine the effects of each dietary treatment in the presence or absence of lead. To stabilize variances within dietary groups, in those cases when it was necessary, the data were transformed to logarithms before statistical tests were performed. Geometric means are given where transformations were necessary, otherwise arithmetic means are shown.

Results

As shown in Table 2, the inverse relationship between dietary copper levels and the accumulation of lead on red blood cells previously reported (*8*) was confirmed. It was interesting that this copper effect was only expressed when dietary iron levels were optimal. The protective effect of copper in the presence of iron was not significant, however, because of the far greater influence of iron on erythrocyte lead. An iron-deficient diet containing 500 ppm lead resulted in a threefold elevation of RBC lead. Thus there is an additive protective effect by having optimal dietary levels of both copper and iron. Extra dietary zinc did not affect these relationships.

Although we have additional data showing alterations in tissue metal contents in response to controlled manipulations of copper, iron, zinc, and lead in the diet, kidney lead and kidney copper values, being somewhat representative of changes found in other organs, are given in Tables 3 and 4, respectively. The kidney lead results were not unlike those reported for erythrocyte lead. Small increases in kidney lead retention secondary to copper deficiency were observed whereas markedly significant elevations were produced by iron deficiency. In fact, a tenfold elevation in kidney lead levels was observed when dietary iron was lowered to 6 ppm.

Table 2. Erythrocyte lead.

Zinc, ppm	Iron ppm	Copper, ppm	Erythrocyte lead, μg/100 g wet weight[a]	
			Lead = 0 ppm	Lead = 500 ppm
20	6	0.5	16.0[b]	355[b,g]
20	6	8.5	29.0[c,d*]	412[c,e]
20	40	0.5	16.6[f]	155[g,f]
20	40	8.5	13.0[h,d*]	125[e,h]
140	40	0.5	17.1[i]	158[i]
140	40	8.5	18.6[j]	115[j]

[a]Geometric means given. Matched superscript letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.05$.

Table 3. Kidney lead.

Zinc, ppm	Iron, ppm	Copper, ppm	Lead, ppm	Kidney lead, μg/g dry weight[a]
20	6	0.5	500	1910[b]
20	6	8.5	500	1490[c]
20	40	0.5	500	126[b]
20	40	8.5	500	111[c]
140	40	0.5	500	112
140	40	8.5	500	90

[a]Geometric means given. Matched superscript letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.05$. All control values (no Pb added) were 1–2 μg/g.

Table 4. Kidney copper.

Zinc, ppm	Iron, ppm	Copper, ppm	Kidney copper, μg/g dry weight[a] Lead = 0 ppm	Lead = 500 ppm
20	6	0.5	10.4[b,c]	6.8[c,d]
20	6	8.5	34.1[b,e,f*]	17.6[d,e,g,]
20	40	0.5	12.0[h]	9.1[i]
20	40	8.5	49.8[h,f*]	38.6[g,i]
140	40	0.5	9.64[j]	7.23[k]
140	40	8.5	38.6[j]	46.6[k]

[a]Geometric means given. Matched subscript letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.05$.

Once again both copper and iron enter into the picture when assessing the lead effects on kidney copper levels (Table 4). A significant increase in kidney copper, $p < 0.01$, was found in all cases in which copper was added to the basal diets. The significant lead-induced depressions of kidney copper observed at both levels of copper when dietary iron was deficient were, interestingly, prevented by iron supplementation. Even in the absence of lead there were definite effects of iron on kidney copper.

Copper and iron are both essential to the activity of cytochrome c oxidase, the terminal respiratory cytochrome responsible for the reduction of O_2 to H_2O. In other experiments we have found that heart tissue is very sensitive to dietary copper; thus this organ was selected for the study of the response of cytochrome oxidase to our dietary conditions. As shown in Table 5, this enzyme did reflect the state of copper nutriture in our experimental animals. Lead statistically aggrevated the copper deficient condition with respect to heart cytochrome oxidase activity only in rats simultaneously deficient in iron, $p < 0.05$. A lead-induced depression in enzyme activity, although not significant, was also seen at the highest level of each metal (Zn, 140; Cu, 8.5; Fe, 40). The adverse zinc effect observed in this same group of animals, $p < 0.05$, indicates that lead and zinc are acting additively to depress this important copper enzyme. Dietary iron alone had no effect on heart cytochrome oxidase activity.

Table 5. Heart cytochrome oxidase.

Zinc, ppm	Iron, ppm	Copper, ppm	Heart cytochrome oxidase k x 10^4 mg protein[a] Lead = 0 ppm	Lead = 500 ppm
20	6	0.5	12.9[b,c*]	8.8[d,c*]
20	6	8.5	39.1[b]	41.8[d]
20	40	0.5	9.9[e]	7.3[f]
20	40	8.5	39.0[e]	38.5[f,g*]
140	40	0.5	11.1[h]	9.3[i]
140	40	8.5	33.2[h]	24.9[i,g*]

[a]Geometric means given. Matched superscript letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.05$.

Table 6. Hematocrit.

Zinc, ppm	Iron, ppm	Copper, ppm	Hematocrit, %[a] Lead = 0 ppm	Lead = 500 ppm
20	6	0.5	22.4[b,c,d]	14.6[c,e,f]
20	6	8.5	33.7[b,g,h]	26.7[e,g,i]
20	40	0.5	43.3[d,j,k*]	34.7[f,j,l,m]
20	40	8.5	47.3[h,k*]	46.9[i,l]
140	40	0.5	44.5[n]	29.6[m,n,p]
140	40	8.5	45.9	44.9[p]

[a]Arithmetic means given. Matched letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.01$.

Table 7. Hemoglobin.

Zinc. ppm	Iron, ppm	Copper, ppm	Hemoglobin, g/100 ml blood[a] Lead = 0 ppm	Lead = 500 ppm
20	6	0.5	5.2[b,c,d]	3.1[c,e,f]
20	6	8.5	8.2[b,g,h]	6.5[e,g,i]
20	40	0.5	12.3[d,k,j*]	8.8[f,k,l,m]
20	40	8.5	13.9[h,j*]	13.2[i,l]
140	40	0.5	11.9[n,p]	7.1[m,p,q]
140	40	8.5	13.6[n]	13.4[q]

[a]Arithmetic means given. Matched letters indicate significance at $p < 0.01$; asterisk shows significance at $p < 0.05$.

Both copper and iron deficiency produced significant reductions in hematocrit and hemoglobin levels (Tables 6 and 7). Of great interest was the finding that the lead-induced anemia was completely prevented when optimal levels of copper and iron were supplemented in the diet. Neither copper nor iron prevented the anemia due to lead in the presence of a deficiency state of the other metal. These results are in agreement with those reported by Six and Goyer (*9*) with animals receiving adequate copper in their diets. Extra zinc further magnified the lead effect when dietary copper was low, perhaps because of its antagonistic effect on copper metabolism.

Discussion and Conclusions

Rat hematocrit and hemoglobin levels are depressed by lead, at 500 ppm in the diet, if either or both copper and iron are low. We have additional evidence to suggest that this effect results from a direct lead—copper antagonism and that the observed iron influence is secondary to the changes in copper metabolism.

The marked increase in erythrocyte lead when dietary copper and iron are low is an important finding, as this parameter is universely used as the index of lead absorption. When rats are given 500 ppm lead in this deficient diet they actually have an internal exposure which is three times that of rats ingesting the same dose of lead given in a copper- and iron-fortified diet. Since most stock diets are excessive in both copper and iron, it may well be that the resistance of rats to lead toxicity is due in large measure to the protective effects of these elements.

It was found that kidney lead, which was related to erythrocyte lead, was also inversely affected by iron and to a lesser extent by copper. The fact that kidney lead levels were magnified more than ten times in rats fed the copper and iron deficient diet containing lead compared to the lead-fed controls, whereas erythrocyte lead showed only a threefold increase in the same animals, suggests that the lead–copper–iron interactions are not occurring only at the site of intestinal absorption.

Alterations in copper distribution and utilization were also observed following an oral lead exposure. Kidney copper, depressed by lead, was returned toward control by copper if dietary iron was adequate. Heart cytochrome c oxidase was adversely affected by lead and zinc but not by lead alone. This suggests that exposure to lead and other environmental agents which inhibit copper metabolism may disturb the integrity of the cellular respiratory system of the heart or other organs.

Results presented here indicate that the toxic effects of lead are greatly minimized when dietary copper, iron, and zinc are adequate. These data suggest a complex interaction of lead with these three essential trace minerals which could be simply diagramed as shown in Figure 1.

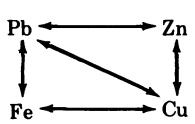

FIGURE 1.

The importance of these findings with regard to lead toxicity in humans needs to be explored.

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