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Dietary Cadmium, Iron, and Zinc Interactions in the Growing Rat. (33659)

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The depressing effect of dietary cadmium on blood hemoglobin has been demonstrated in several species (1–3). A mutual antagonism between Cd and Zn has been established (3–7) and a partial amelioration of cadmium toxicity in the chick was noted with Cu fed in conjunction with 100 ppm of Zn (1). With Zn at 25 ppm, both Fe and Cu supplements were shown to ameliorate Cd toxicity.

Cadmium decreases longevity in male mice and possesses innate toxicity (8). These toxic properties of Cd make it desirable to elucidate the factors which will minimize the adverse effects on animals of environmental Cd contamination. The present experiments were undertaken to test the interactions between dietary Cd, Fe, and Zn in the growing rat with respect to effect on body weight gain, blood hemoglobin concentration and tissue levels of trace elements.

To test these interactions, weanling rats were fed diets identical in all respects, within each experiment, except for the level of Cd, Fe, and Zn. These trace elements were added to the basal diet alone and in all possible combinations so that the influence of one on the response to others could be determined. In addition to dietary variables, two experiments involved cage type (galvanized vs stainless steel) as an environmental contribution to Zn ingestion in combination with dietary supplements of Cd and Fe alone and together. Evidence is presented on the protective effect of Fe, in combination with Zn, against Cd toxicity.

Methods. Expt. 1. Forty weanling rats (av wt. 46 ± 7 g) were assigned by weight and sex in a 2 × 2 factorial arrangement of dietary treatments. These were: diet 1, basal (B) (Table I), 100 ppm Fe (as $FeSO_4 \cdot 7H_2O$), O Cd (Table I); diet 2, 100 ppm Cd (as $CdCl_2$); diet 3, 400 ppm Fe, 0 Cd; and diet 4, 400 ppm Fe, 100 ppm Cd. Animals were kept individually in wire cages. Feed was offered *ad libitum* from porcelain feeders and distilled water from glass bottles with stainless steel tubes.

After 4 weeks, rats were weighed, anesthetized with diethyl ether, blood was drawn from the posterior vena cava of each rat for hemoglobin determination and liver was excised from one-half of each group chosen at random. Blood hemoglobin was determined as oxyhemoglobin with 0.1% Na_2CO_3 as a diluent (9). Livers were freeze-dried, ground, ashed, and an aliquot of each was used for spectrographic analysis.[3]

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[3] Analysis was performed in the Spectrographic Laboratory, New York State College of Agriculture, Ithaca, New York 14850.

TABLE I. Composition of Basal Diet (Expts. 1–4).

	Expts. 1 and 4 (%)	Expts. 2 and 3 (%)
Casein[a]	20.0	25.0
Dextrin[b]	46.8	23.0
Glucose monohydrate[c]	24.0	37.2
Vegetable oil[d]	5.0	4.0
Vitamin supplement[e]	2.2	5.0
Salts	2.0[f]	5.8[g]
Total (%)	100.0	100.0

[a] Crude, 30-mesh, National Casein Co., Riverton, N. J.

[b] General Biochemicals, Chagrin Falls, O.

[c] Cerelose, Corn Products Co., Argo, Ill.

[d] Mazola oil, Corn Products Co., Argo, Ill.

[e] NBC Vitamin Diet Fortification Mixture, Nutritional Biochemical Corp., Cleveland, O.

[f] Jones, J. H. and Foster, C., salt mixture, J. Nutr. 24, 245 (1942).

[g] Supplied the following, g/kg of diet: $CaCO_3$, 10.00; $CaHPO_4$, 18.00; KCl, 6.0132; NaCl, 0.8926; Na_2SO_4, 5.3079; $MgCl_2 \cdot 6H_2O$, 7.5753; $CoSO_4$, 0.0003; $MnSO_4 \cdot H_2O$, 0.0308; KI, 0.0001; $CuSO_4 \cdot 5H_2O$. Glucose was used as a carrier to allow addition of the above mixture at 5.8% of the diet.

Treatment differences in this and subsequent experiments were tested by analysis of variance.

Expt. 2. Forty-eight 4- to 5-week-old rats (av wt. 85 ± 28 g) were assigned by weight and sex in a 2 × 2 × 2 arrangement of dietary treatments involving addition of 100 ppm Cd (as $CdCl_2 \cdot 2H_2O$), 68 ppm Fe (as $FeSO_4 \cdot 7H_2O$), and 200 ppm Zn (as ZnO) to the basal diet (Table I) alone or in all possible combinations. Rats were kept in pairs in the cages used in Expt. 1. Feed and distilled water were offered *ad libitum*. After 3 weeks, rats were weighed, anesthetized, and bled for hemoglobin determination as previously.

Expt. 3. Twenty-four weanling male rats (av wt. 43 ± 3 g) were assigned by weight in a 2 × 2 factorial arrangement of treatments to test the effect of cage (stainless steel or galvanized) and dietary iron level on weight gain and blood hemoglobin in the presence of a high Cd diet (100 ppm). The basal diet was the same as the high Cd–low Zn–low Fe diet of Expt. 2. Feed and water were offered *ad libitum* and after 4 weeks all rats were weighed, anesthetized, and bled for hemoglobin determination.

Expt. 4. Forty-eight weanling male rats (av wt. 45 ± 3 g) were randomly assigned in a 2 × 2 × 2 factorial arrangement of treatments to test the effect of dietary Cd and Fe level and cage (stainless steel or galvanized) on weight gain, blood hemoglobin, and testes and liver mineral composition.

The basal diet (Table I) and the 4 dietary treatments were the same as in Expt. 1. One-half of the animals in each diet group were assigned to individual galvanized and one-half to stainless steel cages as in Expt. 3. Feed and glass-distilled water were offered *ad libitum* and after 30 days all rats were weighed, anesthetized and bled for hemoglobin determination. Testes and liver from each animal were excised, freeze-dried, ground, ashed, and an aliquot of each was used for spectrographic analysis.

Results and Discussion. Expt. 1. Weight gain, hemoglobin, and liver Cu, Fe and Zn concentration are given in Table II. Cd depressed growth at the lower level of Fe, but less so with the high level of Fe; Cd also significantly ($p<0.01$) depressed hemoglobin at the low but not high Fe level. Liver Fe storage was highly variable, but the mean value for the high Cd–low Fe group was lower than that of the other groups. Bunn and Matrone (3) reported an effect of Cd in reducing liver Fe in rats and mice. The depressed level of liver Cu ($p<0.01$) with high Cd suggests an adverse effect of Cd on Cu adsorption and/or storage. In contrast to the effect on liver Cu, the level of liver Zn was significantly increased by Cd. This has been reported previously (2). Liver levels of P, Ca, K, Mg, Na, Mn, B, and Al were not significantly affected by dietary Cd or Fe level and the means are therefore not reported. Since sex failed to affect any of the criteria, the data were combined for analysis and only the combined means are reported.

To eliminate the possibility that the response to dietary Fe level was related to the sulfate provided with Fe added as $FeSO_4$, a separate test was run using Na_2SO_4 to test

the effect on Cd depression of weight gains. There were no differences apparent after 3 weeks, so sulfate was not considered to be of importance in producing the response.

TABLE II. Effect of Dietary Cadmium and Iron Level on Weight Gain, Hemoglobin, and Liver Copper, Iron and Zinc of Weanling Rats (Expt. 1, 10 animals/group).

Cadmium (ppm)	Iron (ppm): 100	400
	Wt. gain (g/day)[a]	
0	4.9	3.4
100	2.8	3.6
	Hemoglobin (g/100 ml)[b]	
0	14.3	16.7
100	6.0	13.4
	Liver copper (ppm)[c]	
0	20.2	17.4
100	8.2	11.0
	Liver iron (ppm)[d]	
0	627	691
100	373	775
	Liver zinc (ppm)[e]	
0	111	108
100	133	134

[a] Significant Cd × Fe interaction ($p < 0.005$); error mean square was 1.09.

[b] Significant Cd × Fe interaction ($p < 0.005$); error mean square was 0.6.

[c] Significant Cd × Fe interaction ($p < 0.005$); error mean square was 9.7.

[d] Significant Cd × Fe interaction ($p < 0.05$); error mean square was 74,522.

[e] Significant Cd effect ($p < 0.005$); error mean square was 155.

Expt. 2. Weight gain and hemoglobin level are given in Table III. Cd depressed weight gain when added to all diets except the one containing both added Zn and Fe. This Cd × Fe × Zn interaction was significant at $p < 0.10$. Hemoglobin was depressed by Cd but there was no apparent interaction as observed for weight gain. The rats used were older than those used in Expt. 1 and probably had greater pretreatment storage of Fe and Cu. Bunn and Matrone (*2*) demonstrated a pretreatment effect with regard to Cu storage which produced differences persisting even through 5 weeks on Cd diets. No sex related differences were observed so the sexes

TABLE IV. Effect of Cage (stainless steel versus galvanized) and Dietary Iron Level on Weight Gain and Hemoglobin of Weanling Rats Fed a High Cadmium Diet (Expt. 3, 6 animals/group).

Cage type	Iron (ppm): 0	68
	Wt. gain (g/day)[a]	
Stainless steel	2.6	2.5
Galvanized	3.0	3.5
	Hemoglobin (g/ml)[b]	
Stainless steel	11.1	14.2
Galvanized	13.0	15.0

[a] Significant treatment differences ($p < 0.01$); error mean square was 0.18.

[b] Significant treatment differences ($p < 0.01$); error mean square was 2.45.

TABLE III. Effect of Dietary Cadmium, Iron and Zinc Level on Weight Gain and Hemoglobin of Weanling Rats (Expt. 2).

Cadmium (ppm)	Iron (ppm):	0		68	
	Zinc (ppm):	0	200	0	200
		Wt. gain (g/day)[a]			
0		1.8 (6)[b]	2.1 (6)	2.6 (4)	1.8 (6)
100		0.5 (6)	0.7 (6)	0.7 (6)	1.8 (6)
		Hemoglobin (g/100 ml)[c]			
0		14.9 (6)	13.6 (4)	15.3 (4)	14.6 (6)
100		12.6 (5)	13.5 (4)	13.4 (5)	12.8 (5)

[a] Significant treatment differences ($p < 0.005$) and Cd × Fe × Zn interaction ($p < 0.10$); error mean square was 0.44.

[b] Number of animals per mean is given in parentheses.

[c] Significant Cd effect ($p < 0.05$); error mean square was 1.77.

TABLE V. Effect of Dietary Cadmium and Iron Level and Cage (stainless steel versus galvanized) on Weight Gain, Hemoglobin, and Liver and Testes Copper, Iron, and Zinc of Weanling Rats (Expt. 4).

	Iron (ppm):	0		300	
Cadmium (ppm)	Cage type:	Stainless steel	Galvanized	Stainless steel	Galvanized
			Wt. gain (g/day)[a]		
0		4.9 (6)	4.9 (6)[b]	4.4 (5)	4.6 (4)
100		3.3 (5)	3.5 (5)	4.3 (5)	4.0 (6)
			Hemoglobin (g/100 ml)[c]		
0		14.9 (4)	14.2 (4)	14.8 (4)	14.3 (3)
100		12.0 (4)	10.8 (5)	14.3 (4)	13.9 (4)
			Liver iron (ppm)[d]		
0		571	490	807	738
100		400	291	720	744
			Liver zinc (ppm)[e]		
0		82	100	87	105
100		107	119	101	132
			Testes iron (ppm)[f]		
0		142.6 (6)	148.0 (6)	167.2 (5)	151.0 (4)
100		103.2 (5)	104.0 (5)	135.2 (5)	147.7 (6)
			Testes zinc (ppm)[g]		
0		149.3 (6)	165.3 (6)	142.0 (5)	159.0 (4)
100		144.0 (5)	142.4 (5)	152.8 (5)	159.3 (6)

[a] Significant treatment differences ($p < 0.005$); error mean square was 0.387.
[b] Number of animals per mean is given in parentheses.
[c] Significant treatment differences ($p < 0.005$); error mean square was 1.56.
[d] Significant treatment differences ($p < 0.005$); error mean square was 32,102.
[e] Significant treatment differences ($p < 0.005$); error mean square was 396.
[f] Significant treatment differences ($p < 0.005$); error mean square was 401.6.
[g] No significant treatment differences; error mean square was 313.1.

were combined in the final statistical analysis of the data.

Expt. 3. Weight gain and hemoglobin level are given in Table IV. There were significant treatment differences ($p<0.01$) for both weight gain and hemoglobin. The galvanized cages apparently provided sufficient additional Zn to the animal to offset the effect of 100 ppm Cd in the diet. There was a tendency for a Fe $\times$ Zn interaction as in Expt. 2 such that weight gain and hemoglobin were further improved with added Fe.

Brinkman and Miller (10) noted the importance of type of cage as related to Zn contamination in affecting the response to dietary molybdenum.

Expt. 4. Weight gain, hemoglobin, and liver and testes Fe and Zn levels are given in Table V. Cu was not detected in the testes of rats in any group and in only about one-half of the livers. The Cu concentration of livers with detectable amounts averaged 4.5 ppm (range 1–10 ppm). The level did not seem to be related to treatment. Liver and testes levels of P, Ca, K, Mg, Na, and Mn were not related to treatment so the means are not reported. B and Al were not detected in either tissue. There were significant differences among treatments ($p<0.005$) in weight gain, hemoglobin, liver Fe and Zn and testes Fe concentration. Cd depressed weight gain, hemoglobin, liver and testes Fe level, increased liver Zn but had no effect on testes Zn level. Fe almost completely alleviated the effect of Cd on the parameters measured. In this experiment, galvanized cages increased liver Zn

concentration as previously, but galvanized cages failed to have an effect on weight gain of rats fed high Cd diets and tended to decrease hemoglobin at both the high and low Cd levels. There was no effect of cage on testes Fe or Zn level.

The failure of cage to increase the growth of rats fed high dietary Cd in this experiment may be related to the difference in Fe content or to other differences in composition of the basal diet in this experiment as compared to Expt. 3 or to a difference in the amount of Zn contamination from the cage surface in the two experiments.

These data demonstrate that levels of dietary Fe which exceed the normal requirement offer almost complete protection against Cd toxicity in the growing rat and that the level of dietary Zn may be important in this response. It seems probable that the observed amelioration of Cd toxicity produced by supplemented Fe and Zn is highly dependent on some interaction between these two elements in a similar manner to that indicated with regard to Zn and Cu (1). Such interactions among trace elements offer a possible means of counteracting the adverse effects on biological systems of environmental cadmium contamination.

Summary. Weanling rats were used in 4 experiments to determine the effect of Zn and Fe added to the diet alone and in combination in preventing Cd toxicity. Cd added to the diet at a level of 100 ppm depressed weight gain and blood hemoglobin in all 4 experiments. Supplemental Fe at 300 ppm in the diet offset the effect of Cd on weight gain and hemoglobin. Liver Cu was reduced and liver Zn was increased by Cd. Liver Fe was reduced by Cd at 100 ppm dietary Fe but not at 400 ppm. Depression of weight gain by Cd in one experiment was prevented by 68 ppm of Fe and 200 ppm of Zn added to a low Fe–low Zn diet, but not when either was added singly. Weight gain and hemoglobin of rats fed Cd were increased when rats were kept in galvanized cages as compared to stainless steel cages in one of two experiments. A Cd × Fe × Zn interaction appears to exist in the growing rat and environmental contamination with Zn is an important factor in determining the response to variable dietary Cd and Fe levels.

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