

Effect of Dietary Ca and Cd Level of Pregnant Rats on Reproduction and on Dam and Progeny Tissue Mineral Concentrations (38606)

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The toxic effects of Cd on animals are well documented. Most of these effects have been observed in growing animals fed Cd salts (1-8) or in adult males injected with Cd (9-12). Injection of Cd into pregnant rats interrupts pregnancy (13, 14) but preliminary data (15) have failed to show adverse effects on litter size in rats fed diets containing Cd although birth weight of individual pups is depressed.

Dietary Ca level affects the absorption of several trace elements, including Zn (16), F (17) and Pb (18), but no information is available concerning the effect of Ca level on metabolism or retention of Cd in pregnant females or their progeny.

The purposes of the present study were to determine the effect of the addition of Cd to the diet of the pregnant rat on reproductive performance and on tissue concentrations of Cd, to compare the tissue concentrations of Cu, Fe, Zn, Mg and Ca in rats fed diets with or without Cd and to ascertain the effect of dietary Ca on these parameters.

Materials and Methods. Forty female Sprague-Dawley, 160-190 g, 12-wk old rats caged one to one with fertile males were assigned in sequence to one of four experimental diets on the day following mating, resulting in ten females per diet group in a factorial arrangement of treatments as follows: low Ca (0.07%)-low Cd (0 ppm), high Ca (0.96%)-low Cd, low Ca-high Cd (200 ppm), high Ca-high Cd. The composition of each diet is shown in Table I. Animals were caged individually in galvanized wire cages from the day following mating to parturition; feed was available *ad libitum* from porcelain dishes attached to the inside of each cage and water was provided from glass water bottles. Feed intake and body weights were recorded every second day. Postpartum weight of the dam, total litter

birth weight and number of pups were recorded for each litter. Dams which failed to produce litters following mating were discarded. After parturition, dams were anesthetized with ether, their liver and kidneys were removed, blotted free of blood, freeze-dried and ground. A sample of each tissue was used for determination of Cd, Zn, Cu, Mg and Ca by atomic absorption spectrophotometry. Pups were examined grossly for abnormalities, euthanized with ether and all pups in each litter were pooled, freeze-dried, ground in a Wiley mill and a composite sample used for determination of Cd, Zn, Cu, Fe, Mg and Ca by atomic absorption spectrophotometry. Dam serum was also analyzed for Ca by atomic absorption spectrophotometry.

Differences among means for each criterion were tested by analysis of variance.

Results. The addition of 200 ppm Cd (as CdCl₂) to the diet of the dam from the day following mating through parturition significantly ($P < .01$) depressed feed consumption and body weight gain; dietary Ca level (0.07% vs. 0.96%) had no effect on either parameter (Table II). The reduction in feed consumption was apparent throughout pregnancy. Liver and kidney Cd were increased dramatically by high dietary Cd ($P < .01$); the increase was significantly ($P < .01$) greater in the presence of low dietary Ca than with high Ca in both liver and kidney. Liver Zn was significantly ($P < .01$) increased by high dietary Cd but was unaffected by dietary Ca level; kidney Zn was unaffected by diet. Liver Fe was significantly ($P < 0.10$) depressed by high dietary Cd; there was no effect of Cd on kidney Fe. Dietary Ca level had no effect on either liver or kidney Fe concentration. Liver and kidney Cu, Ca and Mg and serum Ca were unaffected by diet.

TABLE I. COMPOSITION OF HIGH- AND LOW-Ca DIETS.

Ingredient	Diet designation	
	Low Ca	High Ca
	%	%
Ground yellow corn	81.00	78.25
Soybean meal	18.00	18.00
Sodium chloride (trace mineralized) ^a	0.50	0.50
Vitamin-Zn supplement ^b	0.50	0.50
Dicalcium phosphate	—	1.50
Calcium carbonate	—	1.25
Total ^c	100.00	100.00

^a Provides the following, mg per kg diet: Co, 0.75; Cu, 1.1; I, 0.35; Fe, 5.85; Mn, 11.25; S, 2.0; Zn, 0.4.

^b Provides the following, units per kg diet: Vitamin A, 2200 U.S.P. units; vitamin D₂, 1320 U.S.P. units; vitamin B₁₂, 11.0 mcg; niacin, 31.25 mg; riboflavin, 4.4 mg; pantothenic acid, 4.95 mg; zinc (as ZnO), 57.2 mg; butylated hydroxytoluene, 22.0 mg. Hopro R, Borden, Inc., Norfolk, VA.

^c The low Ca diet contained 0.07% Ca, 0.33% P and 3452.5 kcal digestible energy per kg; the high Ca diet contained 0.96% Ca, 0.71% P and 3355.4 kcal digestible energy per kg.

Number of pups per litter was not significantly affected by diet, although the mean for high-Cd groups was slightly less than that of low-Cd groups (9.6 vs. 11.1 live pups per litter) (Table III). Whelping rate (% of females producing litters) was 100, 90, 60 and 90 for low Ca-low Cd, high Ca-low Cd, low Ca-high Cd and high Ca-high Cd groups, respectively. Reasons for failure to whelp were not established except for one female in each of the high Cd groups whose uterus contained several partially resorbed and mummified fetuses at term. There was one stillborn pup in one litter from dams fed low Ca-low Cd and high Ca-high Cd diets, one in each of two litters from dams fed low Ca-high Cd and none in litters from dams fed high Ca-low Cd. Average pup birth weight was significantly ($P < 0.01$) less in dams fed high Cd than in those fed low Cd; dietary Ca level had no effect on birth weight. Cd concentration of newborn pup carcasses was significantly ($P < 0.01$) increased by feeding the dam high Cd diets during pregnancy. The increase was approximately 8.6-fold in

TABLE II. EFFECT OF GESTATION DIET Ca AND Cd LEVELS ON BODY WEIGHT, FEED CONSUMPTION, SERUM Ca AND LIVER AND KIDNEY Cd, Zn, Cu, Fe, Ca AND Mg IN RATS.

Criteria	Low Ca ^a Low Cd ^c	High Ca ^b Low Cd	Low Ca High Cd ^d	High Ca High Ca	Standard deviation	Significant effects
Body wt change, g	47.7 ^e	62.4 ^e	9.9 ^f	14.6 ^f	20.80	Cd ($P < 0.01$)
Avg da. feed consumed, g	20.0 ^e	21.4 ^e	17.1 ^f	17.2 ^f	2.05	Cd ($P < 0.01$)
<i>Dam</i>						
liver Cd, ppm D.M.	0.36 ^e	0.28 ^e	51.63 ^f	38.35 ^g	5.06	Cd, Ca ($P < 0.01$)
kidney Cd, ppm D.M.	1.12 ^e	0.89 ^e	103.91 ^f	53.08 ^g	19.80	Cd, Ca ($P < 0.01$)
liver Zn, ppm D.M.	47.2 ^e	45.8 ^e	59.3 ^f	58.2 ^f	10.75	Cd ($P < 0.01$)
kidney Zn, ppm D.M.	51.6	57.4	68.6	57.6	17.4	N.S. ^h
liver Cu, ppm D.M.	5.4	10.5	3.5	5.7	9.1	N.S.
kidney Cu, ppm D.M.	15.9	25.2	21.4	23.1	18.0	N.S.
liver Fe, ppm D.M.	184.6	101.5	78.2	88.0	110.6	Cd ($P < 0.10$)
kidney Fe, ppm D.M.	80.2	74.0	104.9	79.4	54.3	N.S.
liver Ca, ppm D.M.	106.7	117.9	120.3	113.3	36.4	N.S.
kidney Ca, ppm D.M.	1020	1260	1060	990	410	N.S.
liver Mg, ppm D.M.	580	660	670	670	100	N.S.
kidney Mg, ppm D.M.	710	830	750	630	200	N.S.
serum Ca, mg/100 ml	9.72	9.67	9.44	9.89	1.12	N.S.

^a Low Ca = 0.07% Ca, 0.33% P.

^b High Ca = 0.96% Ca, 0.71% P.

^c Low Cd = 0 ppm added Cd.

^d High Cd = 200 ppm Cd added as CdCl₂.

^{e-g} Means in the same row without a common superscript are significantly different from each other.

^h No significant differences.

TABLE III. EFFECT OF GESTATION DIET Ca AND Cd LEVELS ON NUMBER OF PUPS PER LITTER, PUP BIRTH WEIGHT AND CONCENTRATIONS OF Cd, Zn, Cu, Fe, Ca AND Mg IN THE PUP AT BIRTH.

Criteria	Low Ca ^a Low Cd ^e	High Ca ^b Low Cd	Low Ca High Cd ^d	High Ca High Cd	Standard deviation	Significant effects
No. of litters	10	9	6	9		—
Avg no. pups per litter	11.2	10.9	10.0	9.2	2.25	N.S. ^e
Avg pup birth wt, g	5.45 ^f	5.64 ^f	4.43 ^o	4.88 ^o	0.56	Cd ($P < 0.01$)
<i>Pup</i>						
Cd, ppm	0.97 ^f	0.96 ^f	8.67 ^o	3.81 ^h	2.96	Cd ($P < 0.01$) Ca ($P < 0.05$)
Zn, ppm	73.0 ^f	71.8 ^f	67.7 ^o	66.9 ^o	7.3	Cd ($P < 0.05$)
Cu, ppm	14.9 ^f	13.2 ^f	3.7 ^o	6.9 ^o	3.2	Cd ($P < 0.01$)
Fe, ppm	425.8 ^f	316.4 ^f	173.2 ^o	150.2 ^o	126.8	Cd ($P < 0.01$)
Ca, %	3.22 ^f	2.63 ^f	3.43 ^o	3.48 ^o	0.50	Cd ($P < 0.01$)
Mg, %	0.153	0.145	0.150	0.151	0.017	N.S.

^a Low Ca = 0.07% Ca, 0.33% P.

^b High Ca = 0.96% Ca, 0.71% P.

^c Low Cd = 0 ppm added Cd.

^d High Cd = 200 ppm Cd added as CdCl₂.

^e N.S. = no significant differences.

^{f, h} Means in the same row without a common superscript are significantly different from each other.

pups from dams fed the low Ca diet compared with 3.8-fold in pups from dams fed the high Ca diet. This effect of dam dietary Ca level on newborn pup Cd content was significant ($P < 0.05$). High Cd diets fed to the dam resulted in a significant ($P < 0.01$) decrease in concentrations of Zn, Cu and Fe and a significant increase in Ca in newborn pups; there was no effect of dam diet Cd level on pup Mg concentration. Dietary Ca level had no effect on pup Zn, Cu, Fe, Ca or Mg concentration.

Discussion. The observed reduction in weight gain and feed consumption of dams fed Cd diets and the smaller birth weight of their pups is in agreement with preliminary results from this laboratory (15). The accumulation of Cd in liver and kidney of pregnant females fed high Cd diets would be expected in view of the similar response consistently reported in growing animals (4, 19, 20). The partial protection by high dietary Ca against accumulation of Cd in liver or kidney may be related to decreased absorption of Cd from the gastrointestinal tract or to increased excretion of Cd or both; the mechanism of this protective action needs study. No explanation is offered for the increase in liver Zn in the presence of high Cd in the diet although this increase has been previously observed in growing

rats and mice fed Cd (2). It is clear that interactions between these two elements exist in that supplemental Zn partially protects against Cd toxicity (6). The reduction in liver Fe in Cd-fed rats in the present study has been observed previously in Cd-fed Japanese quail (4) and may be related to reduced absorption of Fe from the gastrointestinal tract in Cd-fed animals. Iron deficiency anemia partially preventable by orally or parenterally administered Fe in Cd-fed rats (21) and pigs (22) is a major manifestation of Cd toxicity (1-6).

Of particular significance is the large increase in Cd concentration of pups from Cd-fed dams, showing the occurrence of placental transfer of Cd in the rat. Furthermore, the higher Cd concentration in newborn of dams fed low Ca diets than in those of dams fed high Ca diets suggests a protective effect of high dietary Ca not only against accumulation of Cd in liver of pregnant females fed high Cd-diets but also against accumulation of Cd in their newborn progeny.

The effect of dam dietary Cd intake on content of other elements in the body of the newborn may be of biological importance. Although dam diet was adequate in all trace elements, pup Zn, Cu and Fe contents

were decreased significantly by maternal Cd ingestion and pup Ca was increased by Cd irrespective of Ca content of the diet. These effects of Cd on fetal uptake of Zn, Cu, Fe and Ca suggest important changes in transfer of mineral elements across the placenta in response to Cd accumulation in the tissues of the dam. Whether these changes are associated with altered concentrations of the respective elements in the plasma of the dam or with altered permeability of the placenta is not known.

Summary. Pregnant Sprague-Dawley rats were used to determine the effects of the addition of 200 ppm of Cd (as CdCl₂) to the diet factorially with two levels of dietary Ca (0.07% and 0.96%) on reproductive performance, concentrations of Cd, Cu, Fe, Zn, Ca and Mg in dam liver and kidney and in newborn progeny. High Cd significantly increased liver and kidney Cd, Zn and Ca and decreased liver Fe. High dietary Ca partially protected against accumulation of Cd in liver and kidney but had no effect on concentration of other elements. Number of live or stillborn pups per litter was not significantly affected by diet but high Cd significantly reduced pup birth weight. No grossly abnormal pups were noted. Concentration of Cd in bodies of newborn pups was increased approximately 8.6-fold by high Cd in the diet of dams fed the 0.07% Ca-diet and 3.8-fold by high-Cd in the diet of dams fed the 0.96% Ca diet. Pup, Zn, Cu and Fe contents were significantly decreased and Ca was significantly increased by high-Cd in the maternal diet whereas pup Mg content was unchanged. Maternal Ca intake had no effect on concentration of Zn, Cu, Fe or Ca in newborn pups. The biological importance of the alteration in maternal and fetal tissue concentrations of Zn, Cu

and Fe by high-Cd maternal diets is unknown.

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