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The Maternal Distribution and Placental Transfer of Cadmium in Zinc Deficient Rats

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INTRODUCTION

The administration of cadmium to rats and hamsters has resulted in arterial hypertension, testicular necrosis, and varying degrees of liver and kidney damage (PARIZEK, 1957; SCHROEDER, 1964; LUCIS et al., 1968). Small doses of cadmium given to pregnant rats and hamsters have produced malformations and death in the developing fetus (CHIQUONINE, 1965; CHERNOFF, 1973; ISHIZU et al., 1973; GALE and FERM, 1974). The fetotoxic effect of cadmium in hamsters was reduced when zinc was administered either prior to or simultaneously with cadmium (FERM and CARPENTER, 1967 and 1968). Conversely, when cadmium was administered to pregnant rats experiencing a transitory zinc deficiency, the extent of fetal resorption and/or fetal malformation was increased (PARZYCK, 1977). A maternal zinc deficiency appeared to enhance or augment the fetotoxic effects of cadmium.

Animals receiving cadmium have been shown to synthesize metallothionein, a protein that specifically binds with cadmium (KARTAR et al., 1974; COLUCCI et al., 1975). Since zinc is an active catalyst for methallothionein production (WEBB, 1972), metallothionein synthesis may be impaired in zinc deficient animals receiving cadmium. Thus, a greater proportion of the cadmium may be free for placental transfer. This investigation was designed to determine the influence of a transitory zinc deficiency on the placental transfer of cadmium in pregnant rats. The distribution of cadmium in zinc normal and zinc deficient maternal animals was examined also.

METHODS AND MATERIALS

A total of 32 timed pregnant Holtzman[a] rats was received on day 2 of gestation, weighed, and housed in individual stainless steel cages. Tap water and food pellets[b] were allowed ad libitum.

[a] Holtzman Co., Madison, Wisconsin.

[b] Wayne Lablox, Allied Mills, Inc., Chicago, Illinois.

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From day 5-13 of gestation, 18 randomly selected rats were given distilled drinking water and a special zinc deficient chow[a] (zinc deficient rats). The remaining 14 rats were maintained on the standard laboratory diet throughout gestation (zinc normal rats).

On day 13 of gestation, all zinc deficient and zinc normal rats received an intraperitoneal injection of cadmium-109[b] labeled cadmium acetate at a dose corresponding to 0.25, 0.50, 0.75, or 1.50 milligrams of cadmium ion per kilogram of body weight. The solutions were prepared by adding sufficient cadmium acetate to distilled water to achieve the proper cadmium ion concentration. The specific activity of the 0.25 and 0.50 mg/kg cadmium solutions was 80 μCi/mg of cadmium, while the 0.75 and 1.50 mg/kg cadmium solutions had specific activities of 53 and 39 μCi/mg of cadmium, respectively. The volume of all injection solutions was approximately 0.5 ml. Control animals received approximately 0.5 ml of distilled water only.

All animals were weighed and sacrificed by decapitation 24 hours after cadmium administration and samples of whole blood, liver, kidney, placenta, and fetus were taken. Fetuses were rinsed twice with saline to remove residual amnionic fluid or blood and then placed in tared scintillation vials. Samples of tissues were weighed in tared scintillation vials, digested at 60°C using perchloric acid and hydrogen peroxide as a bleaching agent, and counted in a liquid scintillation spectrometer[c].

The contribution of non-radioactive tissue to the total background count was determined by counting identical tissue samples from control rats. This background value was subtracted from each sample count to give the true count rate. The minimum detectable activity (MDA) of the counting instrument was also determined and tissue counts below the MDA were considered insignificant. All other samples were counted with a 5% counting error at the 95% confidence level. Using the known specific activity, the data were expressed as nanograms of cadmium per milligram of tissue.

Analysis of variance (ANOVA) testing at the 99% confidence level was conducted on all of the data. The Newman-Keuls Range Test was used where applicable (HICKS, 1973).

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[a] Nutritional Biochemical Laboratories, Cleveland, Ohio.

[b] New England Nuclear, Boston, Massachusetts.

[c] Packard Tri-Carb Model 2002 Liquid Scintillation Spectrometer, Downers Grove, Illinois.

RESULTS AND DISCUSSION

Maternal

The average weight gain for the zinc normal maternal rats was 29.8 g and for the zinc deficient rats was only 16.2 g between day 5 and day 13 of gestation. A decreased maternal weight gain has been shown to indicate a maternal zinc deficiency caused by a lack of dietary zinc (HURLEY, 1969; PARZYCK, 1977).

The maternal tissue levels of cadmium increased as the maternal dose of labeled cadmium increased (TABLE 1). The increased levels were evident in all tissues from both the zinc normal and zinc deficient diet groups. ANOVA testing demonstrated that a significant cadmium dose effect was evident in all tissues within each diet group.

TABLE 1. Cadmium concentration in maternal tissues.

Tissue	Cadmium Dose (mg/kg) 0.25	0.50	0.75	1.50
		Zinc Deficient[a]		
Blood	.018 ± .01	.024 ± .01	.037 ± .003	.070 ± .01
Liver	6.64 ± 4.15	11.14 ± 4.84	22.75 ± 4.76	24.76 ± 3.48
Kidney	1.13 ± .625	2.38 ± .58	4.0 ± .33	4.54 ± .53
Placenta	.43 ± .20	.64 ± .23	1.08 ± .15	2.41 ± .08
	N = 4[b]	N = 5	N = 4	N = 5
		Zinc Normal		
Blood	.01 ± .00[c]	.026 ± .003	.029 ± .007	.077 ± .014
Liver	4.14 ± .43	6.97 ± .16	9.49 ± 1.18	21.07 ± 3.93
Kidney	.84 ± .008	1.46 ± .08[c]	1.83 ± .23	4.63 ± .67
Placenta	.23 ± .05	.42 ± .08	.66 ± .18	2.10 ± .39
	N = 3	N = 3	N = 3	N = 3

[a] Mean ng/mg ± S.D.

[b] Maternal rats per dose level.

[c] Mean for two rats.

In all cases for the zinc normal group, the tissues from maternal rats that received the 1.50 mg/kg dose of cadmium contained significantly greater amounts of cadmium than did respective tissues from maternal rats receiving any of the lower doses of cadmium (TABLE 2). The cadmium content of the maternal tissues from the three lower dose levels did not statistically differ from one another. This pattern of cadmium accumulation was apparent in all maternal tissues of the zinc normal diet group.

A different cadmium accumulation pattern was observed in the maternal tissues of the zinc deficient diet group. No significant differences existed between the 0.75 and 1.50 mg/kg cadmium dose levels for liver and kidney tissues. Also, no consistent statistical differences or similarities existed in the cadmium content of the maternal tissues from different cadmium dose levels (TABLE 2). This suggests that the cadmium binding capabilities of the maternal tissues may have been influenced by a transitory zinc dificiency.

TABLE 2. Newman-Keuls Range Test for Dose Effect[a].

Zinc Deficient Dose Levels		Zinc Normal Dose Levels	
Blood:	0.25 0.50 0.75 1.50	Blood:	0.25 0.50 0.75 1.50
Liver:	0.25 0.50 0.75 1.50	Liver:	0.25 0.50 0.75 1.50
Kidney:	0.25 0.50 0.75 1.50	Kidney:	0.25 0.50 0.75 1.50
Placenta:	0.25 0.50 0.75 1.50	Placenta:	0.25 0.50 0.75 1.50
Fetus:	0.25 0.50 0.75 1.50	Fetus:	0.25 0.50 0.75 1.50

[a]Significance test at $\alpha = .05$.
Doses connected by underlines are not significantly different.

Ratios of the absolute amount of cadmium detected in the maternal tissues at each dose level to the absolute amount of cadmium administered to the maternal animals were calculated (TABLE 3). In both diet groups, ANOVA showed that the fraction of administered cadmium that was detected in each maternal tissue remained statistically equal over all the cadmium dose levels studied. The fraction deposited in the tissues was independent of the maternal cadmium dose; increased maternal cadmium doses did not result in a greater proportion of cadmium being detected in each tissue.

ANOVA was used to compare each maternal tissue at each cadmium dose level within the zinc deficient group to the

corresponding tissue and cadmium dose level in the zinc normal diet group. With two exceptions, cadmium in the tissues at any dose level in the zinc deficient group did not statistically differ from the cadmium content of the corresponding maternal tissue and dose level in the zinc normal diet group. In the zinc deficient group, only the liver and kidney tissues from the 0.75 mg/kg dose level contained a significantly greater amount of cadmium than the liver and kidney tissues from the zinc normal diet group.

TABLE 3. Fraction of Maternal Cadmium-109 Dose Detected in Tissue.

Tissue	Cadmium Dose 0.25	0.50	0.75	1.50
		Zinc Deficient[a]		
Blood	2.95 ± .16	1.98 ± .77	1.99 ± .15	1.83 ± .02
Liver	1063 ± 665	978 ± 386	1211 ± 251	660 93
Kidney	181 ± 101	190 ± 47	214 ± 17	121 ± 14
Placenta	69.3 ± 32	50.9 ± 18.10	57.8 ± 8.30	64.2 ± 2.20
Fetus	.04 ± .03	.04 ± .04	.04 ± .01	.06 ± .01
	N = 4[b]	N = 5	N = 4	N = 5
		Zinc Normal		
Blood	1.62 ± .07	2.06 ± .21	1.54 ± .37	2.04 ± .04
Liver	662 ± 67	648 ± 167	506 ± 63	562 ± 105
Kidney	134 ± 12	117 ± 7[c]	97 ± 12	124 ± 21
Placenta	41.4 ± 7	33.6 ± 6.80	35.2 ± 9.80	56.4 ± 10
Fetus	.04 ± .03	.07 ± .02	.04 ± .02	.06 ± .02
	N = 3	N = 3	N = 3	N = 3

[a] True mean ± S.D. values obtained by multiplying 10^{-7}.

[b] Rats per dose level.

[c] Mean for two rats.

Fetal

FERM et al. (1969), LUCIS et al. (1972) and WOLKOWSKI (1974) have demonstrated the placental transfer of cadmium. In the present investigation, cadmium transferred across the placenta of both zinc deficient and zinc normal pregnant rats. In general, the cadmium content in fetuses from both diet groups tended to increase as the maternal dose increased (TABLE 4). This tendency was apparent in the zinc normal fetuses, but in the zinc deficient fetuses no increased cadmium content was observed between the 0.50 and 0.75 mg/kg dose levels. ANOVA showed a significant dose effect

TABLE 4. Cadmium concentration in fetal tissue.

Tissue	Cadmium Dose 0.25	0.50	0.75	1.50
		Zinc Deficient[a]		
Fetus	2.36 ± 1.7 (24)[b]	5.46 ± 5.07 (40)	6.59 ± .84 (40)	21.79 ± 4.22 (41)
	N = 4[c]	N = 5	n N = 4	N = 5
		Zinc Normal		
Fetus	2.65 ± 1.97 (14)	8.28 ± 2.82 (14)	7.71 ± 3.38 (19)	21.79 ± 4.79 (48)
	N = 3	N = 3	N = 3	N = 5

[a]Ture mean ng/kg ± S. D. values obtained by multiplying by 10^{-4}.

[b]Total fetuses sampled.

[c]Maternal rats per dose level.

in both diet groups and subsequent Newman-Keuls analysis demonstrated that fetuses from the 1.50 mg/kg dose level contained significantly more cadmium than fetuses from any of the three respective lower cadmium dose levels (TABLE 2). In both diet groups, the cadmium content of fetuses from the three lower doses did not significantly differ from one another. A much greater absolute quantity of cadmium was administered at the 1.50 mg/kg dose level in relation to lower doses, and thus much more cadmium was available for placental transfer. Within each diet group, the fraction of administered dose that transferred across the placenta remained statistically equal over all the cadmium dose levels.

ISHIZU et al. (1973) and CHERNOFF (1973) found that as the dose of cadmium administered to pregnant mice and rats was increased, the resulting fetal malformation and fetal death rate increased proportionally. In the present investigation, the amount of cadmium that crossed the placenta and localized in the fetus appeared to be proportional to the dose of cadmium administered to the maternal animal. However, ISHIZU et al. (1973) demonstrated that the cadmium content in the fetus was not necessarily related to the presence of external malformations.

Increased fetotoxicity of cadmium in zinc deficient rats (PARZYCK et al., 1977) apparently was not the result of increased placental transfer. In the present study ANOVA showed that the cadmium contents of fetuses from the zinc deficient rats and the zinc normal rats were statistically equal. A transitory maternal zinc deficiency did not affect the transfer of cadmium across the placenta. Furthermore, according to FERM et al. (1969), the placental transfer of cadmium was not affected even when excess zinc was administered along with cadmium in pregnent animals (FERM et al., 1969). Neither a zinc deficiency nor a zinc excess appeared to influence the transfer of cadmium across the placenta.

SUMMARY

Although a transitory maternal zinc deficiency has been shown to result in an increased cadmium-induced fetotoxicity, the results of the present investigation indicated that a maternal zinc deficiency apparently did not affect the placental transfer of cadmium. However, a zinc deficiency did alter the maternal distribution of cadmium. The increased cadmium fetotoxicity associated with a maternal zinc deficiency may be caused by a maternal alteration rather than a direct effect on the fetus. Further study is necessary prior to any definitive statement concerning the effects of a maternal zinc deficiency on cadmium. fetotoxicity.

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