Fetal Effects of Cadmium in Pregnant Rats on Normal and Zinc Deficient Diets

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Cadmium has been shown to be intimately involved in various diseases during the human life span (FLICK et al. 1971). While the toxicity of cadmium has been established, the action of cadmium on the unborn has yet to be fully resolved. Cadmium, as either the chloride, acetate, or lactate, was shown to produce placental destruction and fetal mortality when administered subcutaneously to Wistar rats (PARIZEK 1964). Hamsters given cadmium sulfate intravenously produced fetuses with extensive malformations of the face (FERM and CARPENTER 1967). Cadmium chloride injected subcutaneously caused a wide spectrum of fetal malformations when given to rats of the CD strain (CHERNOFF 1973). The incidence and variety of cadmium induced fetal malformations has been shown to vary between two stocks of Wistar rats (BARR 1973).

A maternal dietary zinc deficiency has been shown to produce a high incidence of congenital malformations in the fetal rat. Maternal rats fed a zinc deficient diet throughout gestation produced fetuses with gross congenital malformations in a wide variety of organ systems (HURLEY and SWENERTON 1966). It was not necessary to limit zinc reserves in the maternal rat to induce a zinc deficiency and produce fetal malformations (HURLEY et al. 1971). Transitory periods of dietary zinc deficiency have been shown to produce malformations. Certain of the fetal malformations produced by a transitory maternal zinc dietary deficiency are similar in nature to the malformations produced by administration of cadmium (BARR 1973; HURLEY et al. 1971).

This investigation was conducted to study further the toxic effects of cadmium on fetal development in the Holtzman rat. An interval of maternal dietary zinc deficiency in conjunction with cadmium administration was also studied.

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EXPERIMENTAL

Animals. Timed pregnant Holtzman rats were purchased from a commercial source[1] and were received on either day 2 or 3 of gestation. Matings were confirmed by the commercial source through the presence of sperm in the vaginal smear. The day of finding sperm was considered day 0 of gestation. Following delivery of a shipment of animals, each rat was caged individually in a screen-floored stainless steel cage and given a standard laboratory diet consisting of a commercial laboratory ration[2] and tap water ad libitum.

Cadmium Toxicity in Rats Fed a Standard Diet. Sixty-four rats were randomly assigned to 16 groups of four animals. The rats were administered a 1.0, 1.5, or 2.0-mg/kg dose of cadmium ion, as cadmium acetate, in approximately 0.5 ml of normal saline. Controls received 0.5 ml of normal saline. Injections were given intraperitoneally at 12:00 P.M. on day 8, 10, 12, or 14 of gestation. All animals were maintained on the standard diet.

The animals were sacrificed by ether anesthesia on day 21 of gestation. All fetuses were removed; the number and intrauterine position and the number of resorption sites were noted. The fetuses were weighed, examined for external malformations, and stained with alizarin red for examination of the skeleton (CRARY 1962; STAPLES and SCHNELL 1968). Heads from selected fetuses with externally recognizable malformations as well as from controls were processed for histology and the resultant sections were stained with hematoxylin-eosin (LUNA 1968). The average fetal weight of the litter was calculated.

Cadmium Toxicity in Rats Fed a Zinc Deficient Diet. Forty-eight rats were randomly assigned to eight groups of six animals. Two groups were given a 1.5-mg/kg dose of cadmium ion on day 8 or 12 of gestation and were maintained on a standard diet. One group was administered a 1.5-mg/kg dose on day 8 and was fed a zinc deficient ration and doubly distilled water on days 4 to 8 of gestation, and another group was given a 1.5-mg/kg dose on day 12 and was fed a zinc deficient diet on days 4 to 12 of gestation. Four other groups received 0.5 ml of normal saline in place of the cadmium dose with the remainder of the treatment being identical to the above four groups. Following administration of cadmium or saline solution, the rats on the zinc deficient diet were returned to the standard diet for the remainder of the gestation period.

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[1] Holtzman Co., Madison, Wisc.

[2] Wayne Lab-Blox, Ft. Wayne, Ind.

The standard laboratory ration and the tap water which comprised the standard diet were analyzed for zinc content using atomic absorption spectrophotometry. Samples were also taken of the commercially prepared zinc deficient ration[1] and the doubly distilled water which comprised the zinc deficient diet. Animals on the zinc deficient diet were maintained in cages washed in a 10% nitric acid bath to remove sources of exogenous zinc. The water bottles and drinking spouts were washed in the same manner and the rubber stoppers used in the cadmium toxicity study were replaced with vinyl stoppers to prevent zinc contamination. The zinc concentrations in the standard and the zinc deficient diets are given in Table 1.

TABLE 1

Zinc Concentrations in the Standard and Zinc Deficient Diets.

Dietary Source	Zinc Concentrations, Mean ± S.D., ppm
Standard Diet	
Tap Water	0.50 ± 0.14
Standard Ration	136 ± 38
Zinc Deficient Diet	
Doubly Distilled Water	N.D.[a]
Zinc Deficient Ration	7.2 ± 1.4

[a]Nondetectable by the atomic absorption techniques used, which indicated a zinc concentration less than 0.05 ppm.

The animals were sacrificed on day 21 of gestation in the manner described previously. All fetuses were removed and resorption sites were recorded. Fetuses were examined and processed for histological examination as previously described.

RESULTS AND DISCUSSION

Cadmium Toxicity in Rats Fed a Standard Diet. The average fetal weights of the litters (Table 2) were analyzed for the level of cadmium and day of cadmium administration effects. Fetal weights of the 2.0-mg/kg dose group were significantly ($P < 0.05$)

[1]ICN Biochemicals, Cleveland, Ohio.

TABLE 2

Effect of Cadmium Acetate Given during the Second Trimester of Gestation

Treatment Groups[a]		Average Fetal Weights,[b]		Implantation Sites		
Dose, mg/kg	Day[c]	Mean	S.E., g	Total No.	Resorbed, %	Malformed, %
0	8	5.66	0.19	42	0	0
	10	5.74	0.25	38	0	0
	12	5.65	0.16	32	0	0
	14	5.98	0.09	34	0	0
1.0	8	5.89	0.04	29	0	7.3
	10	5.52	0.08	38	0	15.9
	12	5.89	0.15	40	9.4	18.8
	14	5.83	0.25	41	12.5	0
1.5	8	6.07	0.10	22	0	36.1
	10	5.61	0.22	34	6.2	15.0
	12	5.36	0.19	33	20.0	23.1
	14	5.07	0.27	25	27.5	0
2.0	8	5.15	0.12	37	9.1	42.4
	10	5.34	0.22	37	3.1	17.3
	12	4.96	0.10	44	84.1	1.9
	14	5.17[d]		32	95.0	0

[a]Each treatment group consisted of four rats. [b]At day 21 of gestation. [c]Day of gestation. [d]Only one rat had fetuses.

less than those of the control or the 1.0 or 1.5-mg/kg dose groups, which were not different from each other. The day of cadmium administration produced no significant ($P > 0.05$) effect.

The greatest numbers of fetal resorptions were found among rats administered cadmium on day 12 and 14 of gestation. On these 2 days, there was nearly complete resorption in the 2.0-mg/kg dose group. No resorptions were found in the control group.

The only gross malformation observed in the litters of maternal rats receiving cadmium was a stunting of the fetal body in the cervical and cranial regions. The malformation was characterized by a doming of the calvarium of the fetal head and an attenuation of the neck area. The distance from the fetal eye caudally to an imaginary line drawn perpendicular to the external ear was typically one-third shorter in malformed fetuses than in control fetuses.

Examination of transverse sections of the head taken from selected fetuses with domed and normal skulls revealed that the externally recognized malformation of the fetal head was also evident at the histological level. It was found that the brain of the fetuses with domed skulls was oriented differently within the cranial cavity than the brain of fetal controls. The brain of fetuses with domed skulls was observed to be deeper in the dorsal-ventral direction than the brain from fetal controls. The brain of fetal controls was wider in the transverse direction. A comparable shortening of the brain in the caudal direction was revealed by serial sectioning of the fetal head exhibiting external shortening. The lateral ventricles of the brain from a malformed fetus were one-third longer in the dorsal-ventral direction than those of the fetal controls.

Fetal malformations of the head followed administration of cadmium on either day 8, 10, or 12 of gestation. The 2.0-mg/kg dose of cadmium given on day 8 of gestation produced the greatest number. No malformations were observed in the litters of rats given saline or among rats given either 1.0, 1.5, or 2.0-mg/kg doses of cadmium on day 14 of gestation. Examination of the intact stained fetal skeletons revealed that the stunting of the fetal head was not accompanied by any gross malformation of any other part of the fetal skeleton.

It is not known whether cadmium acts directly on the maternal system, whether it affects the placenta and placental transfer, or whether it has a direct effect on fetal tissue. Although the mechanism behind the deleterious action of cadmium is not known, it is of value to compare the data with those obtained from other studies of cadmium toxicity in the rat.

A 1.8-mg/kg dose of cadmium given intraperitoneally to Wistar rats on either day 9, 10, or 11 of gestation produced a reduction in fetal weight which was dependent on the day of

cadmium administration (BARR 1973). The 2.0-mg/kg dose in the present study produced a significant reduction in day 21 fetal weights in Holtzman rats given cadmium on day 8, 10, 12, or 14 of gestation. The day of cadmium administration, however, was not found to have a significant effect on the reduction in fetal weight.

A 1.8-mg/kg dose of cadmium produced a significant number of fetal resorptions in Wistar rats after intraperitoneal administration on either day 9, 10, or 11 of gestation (BARR 1973). No fetal resorptions were found when the 1.8-mg/kg dose was given on either day 6, 7, 8, or 12 of gestation. In the present study, fetal resorption was pronounced in Holtzman rats given a 2.0-mg/kg dose of cadmium on either day 12 or 14 of gestation. Administration of either a 1.5 or 2.0-mg/kg dose on either day 8 or 10 did not produce large numbers of fetal resorptions in Holtzman rats. Day 10 of gestation was critical in the production of fetal resorptions among Wistar rats while both day 12 and 14 were critical days of administration for the Holtzman rat.

A malformation of the head was the only fetal malformation seen in the present study. Wistar rats given a 1.8-mg/kg dose on either day 9, 10, or 11 of gestation produced fetuses with many malformations including abnormalities of the forelimbs, the diaphragm, and the tail (BARR 1973). In addition, malformations of the head including anophthalmia, microphthalmia, and hydrocephaly were seen. These malformations of the fetal head were most frequent following intraperitoneal administration of cadmium on day 9 of gestation. The hydrocephaly was not seen after administration of 1.8 mg/kg on day 10. In the present study, Holtzman rats administered either a 1.5 or 2.0-mg/kg dose of cadmium on day 8 of gestation produced the greatest percentage of fetuses with a malformation of the head.

The above comparison of the results for the present study with the results for the Wistar rat indicates that the type and incidence of cadmium induced fetal effects are a function of the strain of rat used and time of gestation. The greater variety of malformations in Wistar rats suggests that this strain is more sensitive to cadmium than the Holtzman strain.

Cadmium Toxicity in Rats Fed a Zinc Deficient Diet. As was the case in the above study, there were no resorptions in rats on the standard diet when given a 1.5-mg/kg dose of cadmium on day 8 (Table 3). When animals were maintained on the standard diet and were administered cadmium on day 12, 13.0% of the fetuses were reabsorbed (20.0% in the above study). The zinc deficient diet alone did not induce any resorptions when cadmium was not given. With cadmium administration on day 8 and day 12, there were 4.5% and 75.2% resorptions, respectively, in rats fed the zinc deficient diet. The extensive resorption on day 12 was undoubtedly due to the combined effects of the longer period of zinc deficiency and the later time, during gestation, at which cadmium

was given. These results show that the zinc deficiency, although not producing resorption itself, substantially increased the resorption rate observed when cadmium was given in conjunction with the zinc deficient diet.

TABLE 3

Effect of Dietary Zinc Deficiency on the Toxicity of Cadmium Acetate

Treatment Groups[a]		Implantation Sites		
Dose, mg/kg	Day[b]	Total No.	Resorbed, %	Malformed, %
		Standard Diet Only		
0	8	41	0	0
1.5	8	58	0	30.6
		Zinc Deficient Diet, Day 4-8		
0	8	56	0	22.0
1.5	8	61	4.5	48.0
		Standard Diet Only		
0	12	55	0	0
1.5	12	58	13.0	27.6
		Zinc Deficient Diet, Day 4-12		
0	12	62	0	20.1
1.5	12	59	75.2	7.9[c]

[a]Each treatment group consisted of six rats. [b]Day of gestation on which cadmium was administered. [c]Only four rats had fetuses.

The only fetal malformation observed in any of the litters was the stunting of the cranial-cervical region of the fetal body. This malformation was seen externally and examination of transverse sections taken from selected fetuses confirmed the condition at the histological level. Staining with alizarin red revealed no other skeletal malformation.

No malformations were seen in rats on the standard diet when cadmium was not given. With cadmium, the percentages of malformations were 30.6 and 27.6% on days 8 and 12, respectively. These percentages are about the same as the 36.1 and 23.1% on days 8 and 12, respectively, seen in the previous study when the dose given was 1.5 mg/kg.

For the zinc deficient diet, and when no cadmium was given, the percentages of malformations were similar, 22.0 and 20.1%, for the different periods of zinc deficiency, days 4 to 8 and days 4 to 12, respectively. However, when the zinc deficiency extended from days 4 to 8, and when 1.5 mg/kg of cadmium was given on day 8, the percentage was 48.0%. This value is almost equal to the sum of the values for cadmium alone (30.6%) and for zinc deficiency alone (22.0%). The additive effect suggests that each treatment, cadmium administration and zinc deficiency, exerts its effect independently of the other and that one does not potentiate the action of the other. This contrasts with the effect on resorption; zinc deficiency was not effective by itself but did potentiate the action of cadmium.

For the group which received cadmium on day 12 and was given the zinc deficient diet from days 4 to 12, the percentage of malformations was small, only 7.9%. This low value can be attributed to the high percentage of fetal resorptions, 75.2%.

Administration of cadmium at a dose of 1.5 mg/kg on day 8 of gestation after a 4-day interval of dietary zinc deficiency resulted in approximately the same incidence of fetal malformation (48.0%) as when a 2.0-mg/kg dose was given on day 8 of gestation to rats fed a standard diet (42.4%). Also, cadmium at a dose of 1.5 mg/kg on day 12 after an 8-day interval of zinc deficiency resulted in approximately the same incidence of resorption (75.2%) as when a 2.0-mg/kg dose was given on day 12 to rats fed a standard diet (84.1%). The production of the same type of fetal malformation, the additive effect displayed by the fetal malformation data, and the comparison of the 1.5-mg/kg data with data at the 2.0-mg/kg dose level suggest an interrelationship of effect. The full extent of this interrelationship is not known.

Female Sprague Dawley rats given a zinc deficient ration from day 4 to 12 of gestation had dead or resorbed fetuses at 12% of the implantation sites (HURLEY et al. 1971). The Holtzman rats in the present study had no fetal resorptions after either the 4 or 8-day interval of dietary deficiency.

A wide variety of fetal malformations were found among the litters of Sprague Dawley rats fed a zinc deficient ration for short and transitory periods during gestation (HURLEY et al. 1971). Malformations of the fetal head, spine, appendages, and viscera were observed upon examination of the fetuses. In the present study, the malformed fetuses taken from Holtzman rats exhibited a stunting in the area from the forelimbs to the crown of the fetal head. No gross abnormalities of either the spine, appendages, or viscera were observed.

SUMMARY

This investigation has shown that not only the extent of fetal resorption and malformation but also the types of malformation seen in rats depend upon the strain used and day of gestation. Furthermore, the effects of zinc deficiency and cadmium administration on the fetus can be at least additive, as was seen for malformations. For fetal resorption, zinc deficiency potentiated the action of cadmium.

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