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# Cadmium Fetotoxicity in Rats Following Prenatal Exposure

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Cadmium administered to pregnant rats, mice, and hamsters has caused increased embryonic resorption and fetal death (CHIQUONINE 1965, FERM 1971, ISHIZU et al. 1973, WOLKOWSKI 1974). The extent of embryonic or fetal death in mice and rats has been shown to be dependent on animal strain, day of cadmium administration and the dose administered (BARR 1973, WOLKOWSKI 1974, PARZYCK et al. 1978). Weight reduction in fetuses exposed prenatally to cadmium has been demonstrated (BARR 1973, CHERNOFF 1973, PARZYCK et al. 1978).

Neuropathological effects of cadmium on the peripheral and central nervous systems of adult and juvenile rats have been demonstrated (GABBIANI 1966, GABBIANI and BAK 1969, GABBIANI et al. 1967, SCHLAEPFER 1971). However, there is a lack of information concerning the neuropathological effects in the fetal rat central nervous system (CNS) following prenatal exposure.

This investigation was designed to observe if neuropathological damage was produced in the embryonic, fetal and neonatal CNS from administration of cadmium to pregnant rats. A dose of 1.0 or 2.0 mg/kg was administered to pregnant rats on day 12, 14, 18, or 20 of gestation. Embryos and fetuses were removed either 2 days later or on day 21 and processed for light microscopic examination. In addition, cadmium induced fetotoxicity was assessed by noting the frequency of intrauterine deaths.

## METHODS AND MATERIALS

Timed pregnant Holtzman rats were received on day 3 of gestation, weighed, and housed in individual stainless steel cages. Tap water and food pellets were allowed ad libitum. The maternal weight gain was monitored throughout the gestational period. A single intraperitoneal injection of 1.0 or 2.0 mg/kg of cadmium, as cadmium acetate, was administered to pregnant rats on day 12, 14, 18 or 20 of gestation (Table 1). Control rats received saline solution. Maternal rats receiving cadmium on day 12 or 14 of gestation (Groups I and II) were sacrificed 2 days later. The majority of maternal rats receiving cadmium on day 18 or 20 (Groups III and IV) were sacrificed on day 21 of gestation while a limited number of Group III and IV maternal animals were allowed parturition on day 21 of gestation and viable neonates sacrificed 2 days later and examined.

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TABLE 1. Experimental Design.

| Cadmium Dose | Group I (Day 12)[a] | Group II (Day 14) | Group III (Day 18) | Group IV (Day 20) |
|---|---|---|---|---|
| 2.0 mg/kg | 8[b] | 10 | 5 + 4[c] | 5 + 5 |
| 1.0 mg/kg | 7 | 10 | 5 + 3 | 6 + 4 |
| 0.0 mg/kg | 7 | 6 | 5 + 3 | 5 + 5 |
| Totals | 22 | 26 | 15 + 10 | 16 + 14 |

[a]Day of cadmium administration.

[b]Number of maternal rats per treatment group.

[c]Second number refers to number of maternal rats allowed parturition on day 21 of gestation.

Embryos or fetuses were removed from the uterus, weighed individually and the data subjected to statistical testing (t test). The number of embryonic resorptions or fetal deaths were noted. Viable embryos from Group I and Group II maternal rats were immersed in 10% neutral buffered formalin and processed for light microscopic (LM) viewing. Fetuses from Group III and IV maternal rats were perfused intracardially with 10% neutral buffered formalin, fetal brains removed, immersed in formalin and processed for LM viewing. Tissue was embedded in Paraplast, serially sectioned, and stained with hematoxylin and eosin (LUNA 1968). Each brain was serially sectioned in either the coronal or longitudinal plane. All tissues were examined for morphological aberrations and/or cellular pyknosis using a binocular microscope. Cellular pyknosis was quantitated using a 1 $mm^2$ ocular micrometer at a magnification of 450X.

## RESULTS AND DISCUSSION

### Gross Morphological Appearances

Maternal weight gains were not influenced by cadmium and maternal animals appeared healthy throughout the gestational period. For the Group I embryos (TABLE 2), no significant weight differences existed among the treatment groups. For the Group II embryos, both the 2.0 and 1.0 mg/kg cadmium treated embryos weighed significantly less than control embryos (t test, $P < .01$). Depressed fetal weights have been demonstrated by other researchers when cadmium was administered to pregnant rats at various gestational periods (BARR 1973, CHERNOFF 1973, PARZYCK et al. 1978). No significant differences in fetal weight were measured between treated and control fetuses from either within or between Group III or Group IV. The average weights of treated

fetuses from Group III or Group IV were 4.6 ± 0.6 g and 4.8 ± 0.5 g, respectively, while control fetuses weighed 4.8 ± 0.7 g.

The incidence of embryonic resorption for Group I and Group II maternal rats was unaffected by cadmium administration. Others have shown that cadmium administered to pregnant rats during days 11-15 of gestation does not necessarily cause embryonic death (FERM 1971, BARR 1973). However, cadmium administered to animals during late gestation may cause increased intrauterine fetal death (CHIQUONINE 1965, CHERNOFF 1973). In the present study, the incidence of fetal death in Group III and Group IV maternal rats appeared to be affected by cadmium (TABLE 3). A 2.0 mg/kg dose resulted in the greatest incidence of fetal death in both the Group III and Group IV rats. However statistical testing revealed no significant differences in fetal death among the cadmium doses within or between Group III or IV because of large variations in fetal death from individual maternal rats.

TABLE 2. Group I and II Embryonic Weights.

| | Cadmium Doses (mg/kg)[b] | | |
|---|---|---|---|
| Treatment Group[a] | 2.0 | 1.0 | 0.0 |
| Group I | 79 ± 15[c] | 86 ± 16 | 87 ± 10 |
| Group II | 286 ± 31 | 297 ± 25 | 321 ± 30 |

[a]Cadmium administered on day 12 (Group I) or day 14 (Group II) of gestation and sacrificed 2 days later.

[b]At least 6 embryos were weighed from each maternal animal.

[c]Mean values ± standard deviations, mg.

TABLE 3. Incidence of Fetal Death in Group III and IV Maternal Rats.

| | Cadmium Doses (mg/kg) | | | |
|---|---|---|---|---|
| Treatment Group[a] | 2.0 | 1.0 | 0.5 | 0.0 |
| Group III | 14 ± 16[b] | 0 | 5 ± 10 | 0 |
| Group IV | 33 ± 34 | 7 ± 15 | 0 | 0 |

[a]Cadmium administered on day 18 (Group III) or Day 20 (Group IV) of gestation and sacrificed on day 21.

[b]Percent of total number of fetuses per litter that were not viable. Mean ± standard deviation.

A number of the Group III and IV maternal rats were not sacrificed on day 21 of gestation, but were allowed to give birth and viable neonates sacrificed and examined 2 days later. The number of neonates per litter was not affected by cadmium administration. Of the 5 maternal rats that received 2.0 mg/kg on day 20 of gestation (Group IV), 4 rats experienced 100% fetal mortality. All fetuses from these 4 rats were either stillborn or died shortly after parturition. The fetuses from the remaining maternal rat were all viable. A 2.0 mg/kg cadmium dose administered to 4 pregnant rats on day 18 of gestation (Group III) resulted in 100% fetal mortality in only 1 of the rats that were allowed to give birth on day 21 of gestation. The remaining Group III maternal rats gave birth to normal appearing offspring on day 21 of gestation. Group III and Group IV maternal rats serving as controls or receiving 1.0 mg/kg experienced newborn mortalities of 0-14%.

Other researchers have demonstrated that continuous cadmium ingestion of 100 ppm (POWERS et al. 1973), 200 ppm (POND & WALKER 1975) and 17.2 µg/mL of water (HASTINGS et al. 1978) in pregnant rats throughout gestation caused no fetal mortality when parturition was allowed. Cadmium administration had no effect on the number of neonates per litter and all neonates produced were viable. However, POND & WALKER (1975) found that 40% of maternal rats that received 200 ppm cadmium failed to deliver. In the present study, all treated rats produced litters, but 4 of 5 maternal rats that received 2.0 mg/kg of cadmium on day 20 of gestation produced litters that were stillborn or had died after parturition. A reason for this high incidence of fetal mortality was not established although it occurred only when 2.0 mg/kg was administered on day 20 of gestation.

## Light Microscopic Results

Light microscopic (LM) examination of hematoxylin and eosin stained tissue revealed no histological alterations or lesions in the CNS of embryos from Group I and II maternal rats or in brains of fetuses from Group III and IV. Quantitation of cellular pyknosis in the embryonic, fetal, and neonatal CNS demonstrated that no significant cadmium-induced pyknosis occurred in treated tissue, regardless of dose or day of administration.

Cadmium is known to cause damage to the peripheral and central nervous system when administered directly to adult and newborn animals (GABBIANI et al. 1967, SCHLAEPFER 1971). Cadmium-induced lesions observed in the cerebral and cerebellar cortex at the LM level included hemorrhage, neuronal cell pyknosis, nerve fiber degeneration and capillary deterioration. In the present study, when the fetus was exposed indirectly to cadmium through the maternal rat and placenta, the embryonic and fetal CNS tissue appeared to be unaffected. No neuronal or vascular damage was observed. Apparently a direct cadmium insult is capable of producing pathological changes in the CNS while an indirect insult, via the maternal animal and placenta, had no observable effect on the fetal CNS when examined with the light microscope.

In the present investigation, lack of cadmium-induced lesions in the fetal CNS may have resulted from insufficient quantities of cadmium transferring across the placenta. Although placental transfer of cadmium does occur (ISHIZU et al. 1973, WOLKOWSKI 1974, DENCKER 1975), only minute quantities transfer and are deposited in the fetal tissues (FERM et al. 1972, LUCIS et al. 1972). ROHRER et al. (1978) demonstrated that the fraction of a maternal cadmium dose detected in the fetus was 4.0-7.0 x $10^{-9}$, resulting in fetal cadmium burdens of only 2.7-21.8 x $10^{-4}$ ng/µg of tissue. This fetal cadmium burden was shown to be dependent on the amount of cadmium administered to the maternal animal. Perhaps a minimum amount or threshold value must be available to the fetus before observable neuropathological injury can occur and, in the present investigation, this threshold value was not attained. Alternatively, perhaps cadmium induced neuropathy did occur in the embryonic and fetal CNS but was not detected by the techniques used in the present investigation.

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