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Behavioral and Biochemical Effects of Low-Level Prenatal Cadmium Exposure in Rats

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In recent years, cadmium (Cd) has aroused concern as an environmental contaminant and several reviews are available (BREMMER 1974, FRIBERG et al. 1971). Much has been learned not only about the disposition of Cd within the body, but also its effects on specific organ systems. Cadmium toxicity is characterized by anemia, proteinuria, poor bone mineralzation, and testicular necrosis. It also adversely affects the respiratory, renal, and reproductive systems (FRIBERG et al. 1971) and may be involved either directly or indirectly in the development of hypertension and arteriosclerosis (SCHROEDER 1967). The teratological effects of cadmium exposure observed during the gestational period have been minor and appear to occur only when extremely high doses of cadmium are given parenterally (CHERNOFF 1973). Although rats fed diets containing high levels of cadmium have pups which weigh less at birth, the number of live or stillborn pups per litter is not affected (POWERS et al. 1973). A high cadmium diet (200 ppm) during gestation also caused a ninefold increase in cadmium in the bodies of new born pups, whereas the body zinc, copper and iron contents were markedly decreased (POND and WALKER 1975).

The potency of Cd as a neurotoxin has been demonstrated in in vitro studies or in experiments employing relatively high level exposure. Gabbiani and coworkers (1966, 1967a) found that parenteral administration of $CdCl_2$ produced acute hemorrhagic lesions in the trigeminal and spinal sensory ganglia of rats. Exposure of neonatal rats to $CdCl_2$ during the first 30 days after parturition resulted in hemorrhagic suffusions accompanied by destruction of fibers and cells in the cerebellar and cerebral regions of the brain (GABBIANI et al. 1967b). It is interesting to note that early exposure to another heavy metal, lead, produces an almost identical effect (PENTSCHEW and GARRO 1966). Cadmium has also been shown to block synaptic transmission at peripheral cholinergic and adrenergic synapses in vitro (COOPER and MANALIS 1974, COOPER and STEINBERG 1977). However, as FRIBERG et al. (1971) point out, there have been no experimental studies of nervous system function during low-dose, long-term cadmium exposure, although certain clinical studies (VOROBJEVA 1957), in which workers exposed to cadmium were examined, have found evidence of nervous system dysfunction. Little or no work has been reported concerning the effect of Cd exposure on behavior; one

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study (RIBAS-OZONAS et al. 1974) reported that locomotor activity increased after Cd injections but gave no data.

While the number of studies dealing with the effects of Cd on the central nervous system is not great, the reported findings do suggest that under certain circumstances, Cd may be a potent neurotoxic agent. In the present study, we examined the effect of prenatal exposure to Cd on trace metal levels, embryotoxicity, wheel running activity, and acquisition of a spatial discrimination task.

METHODS

Two male and four female Sprague-Dawley rats, weighing approximately 100 grams were divided into two equal groups. For the next 90 days, one group was fed Purina Laboratory Chow diet and deionized distilled water. The second group was treated identically except that their drinking water contained 17.2 µg Cd^{++}/ml. After 90 days, breeding trials between control male and females and Cd-exposed male and females were conducted with the Cd exposure continuing through gestation. It is recognized that the number of litters is small so that "litter effects" must be considered when interpreting the data. In extenuation, it should be noted that the procedures reported here have been replicated with essentially the same results obtained the second time. A full-scale study is now in progress.

At birth, pups were separated from their mothers before being nourished and their weights recorded. Four male pups per mother were saved for the behavioral studies. The remaining pups were killed, washed in deionized, distilled water and dried to constant weight in a forced air circulation oven at 120° C. The samples were then dry ashed and digested in concentrated HNO_3. Zinc, copper, cadmium, and iron contents were determined using atomic absorption spectrophotometry.

During the 21 day lactation period, all rats were given deionized, distilled water, regardless of their previous dietary treatments. On day 21, the pups were weaned to Purina Laboratory Chow diet and tap water. Eight male pups from the litters of two Cd-exposed dams and Eight male pups from the litters of two control dams served as subjects in the behavioral tests.

At 35 days of age, both the control and Cd-exposed rats were placed in Wahmann running wheels (LC-34) and observed for the next five weeks. Daily wheel running activity as well as weekly food and water consumption and body weights were recorded. At the completion of the activity test period, the animals were placed in individual home cages.

At approximately 130 days of age, the rats were tested on the acquisition of a cued spatial discrimination task. The apparatus consisted of 3 Coulbourn test cages situated in three

sound attentuated chambers. All programming was controlled by Coulbourn logic modules. The rats were water deprived until 85% of baseline weight was acheived, and then taught to bar press for liquid reinforcement (milk). Two retractable bars were positioned inside the test cages, with the rats receiving equal training on both bars.

The discrimination task began with the onset of the house-light and the presentation of the two bars. The rats had to press the left bar in order to receive reinforcement; incorrect responses were not rewarded. Either a correct or an incorrect response terminated the trial and the bars were withdrawn; an intertrial interval of 20 seconds ensued. The number of correct responses as well as latency to respond were recorded. The animals received 50 trials a day 5 days a week. Once the rats reached criteria (45 out of 50 trials correct) a reversal procedure was initiated, i.e. the right bar became the correct side.

RESULTS AND DISCUSSION

Results of pup body weights and body metal levels are presented in Table I. Birth weights of pups exposed prenatally

TABLE I

Effect on Neonates of Dietary Cadmium (17.2 µg/ml) Given to Rat Mothers

Neonatal Parameter	Group Control Mean ± S.E.M.	Cadmium Treated Mean ± S.E.M.
No. of pups/litter	11	12
Pup birth weights (g)	6.8 ± 0.1	6.0 ± 0.2*
Weight at weaning (g)	58.0 ± 0.6	59.0 ± 0.6
Whole pup body		
Zinc, (µg/g)	119.0 ± 5.0	125.0 ± 5.0
Copper, (µg/g)	11.0 ± 0.8	12.0 ± 0.9
Iron, (µg/g)	327.0 ± 24.0	207.0 ± 16.0*
Cadmium, (µg/100g)	16.0 ± 2.2	17.0 ± 2.4

* $p<0.01$

to Cd were significantly depressed ($t = 4.04$, $df = 45$; $p<0.01$), whereas their weight gain during lactation was comparable to that of control pups. Whole body metal analysis at birth indicated that control pups in general had 59% more iron in their body than the cadmium pups, while whole body levels of Zn, Cu, and Cd were unchanged.

The weekly spontaneous locomotor activity levels of control and experimental animals are shown in Figure 1. It can be seen

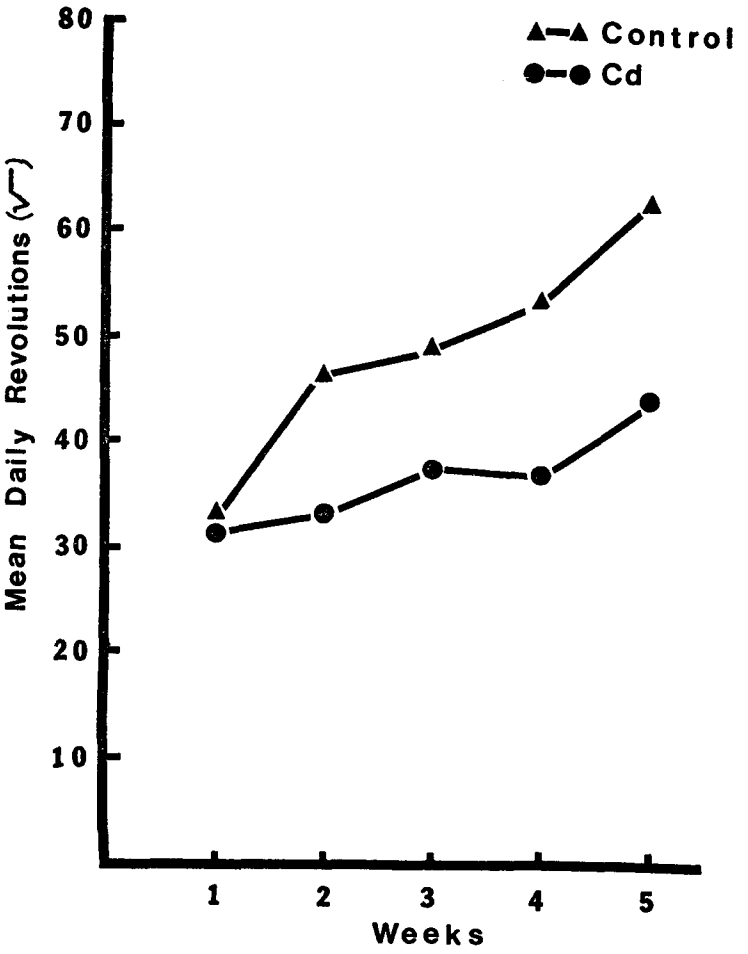

Figure 1. Running wheel activity of rats beginning at 5 weeks of age. The rats' mothers received either 17.2 µg Cd^{++}/ml of drinking water, or deionized water only, for a period of 111 days prior to parturition.

that the overall activity level of the experimental group was significantly depressed in comparison with the controls ($F(1,14) = 4.79$, $p<0.05$). Although food and water consumption as well as body weight was slightly less for the experimental group, the differences were not significant. Thus, rats which had been prenatally exposed to cadmium showed a reduction in spontaneous locomotor activity, but were normal in growth and food and water consumption.

There were no significant differences between the two groups in the number of days to criterion on either acquisition or reversal of the spatial discrimination task. Nor did the mean response latencies differ either during acquisition or reversal.

These results show that low-level cadmium exposure before and during the gestational period reduces body weight and lowers whole body Fe concentration of newborn rats. Although the pups born to Cd-treated nothers rapidly attain normal weight and growth, and appear normal to casual observation, the striking depression of spontaneous locomotor activity in these animals is clearly suggestive of some residual defect. At present, the exact nature of the mechanism(s) responsible for this alteration in behavior is unknown, but several different possibilities exist. For instance, the present of Cd in the maternal water supply may cause a reduction in birth weight by directly altering some metabolic pathway in either dam or pups. Simple malnutrition probably was not involved since both control and Cd-exposed females show equivalent weight gains during the 90 days exposure as well as the gestational period. In either case, reduced birth weight must be taken into account since reduction in birth weight has previously been shown to affect locomotor behavior later in life (FRANKOVA and BARNES 1968). Since the whole body iron content has also been shown to affect activity levels (GLOVER and JACOBS 1972) the role iron plays in suppression of activity must be further investigated. The interaction of these factors preclude the development of any precise statements on the relationship between prenatal Cd exposure and activity.

The failure to find any significant differences between control and Cd-exposed rats on either acquisition of reversal of a discrimination task could be interpreted as evidence that Cd has no effect on CNS function. Such a conclusion would be premature since a) the task was very easily acquired and may not have been sensitive enough to detect any subtle differences, or b) Cd may have produced a deficit which could not be effectively assessed by the discrimination training. In any event, much additional work is needed to clarify the relationship between early Cd exposure and its effect on behavior.

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