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Toxicity, Fertility, Teratogenicity, and Dominant Lethal Tests in Rats Administered Cadmium Subchronically

II. Fertility, Teratogenicity, and Dominant Lethal Tests

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Four groups of Sprague–Dawley rats, each of which consisted of 14 males and 14 females, were administered 0, 0.1, 1.0, and 10.0 mg/kg/day of cadmium (Cd) for 6 weeks. Males and females within each group were mated 6 days a week for 3 weeks, changing partners every week if necessary. Cd was administered during the mating period. Pregnant females were administered Cd during the gestation period and killed on the 20th day of gestation for teratogenicity tests. Males were mated with 2 virgin females per male per week for 6 weeks. Pregnant females were killed on the 13th day of gestation for dominant lethal tests. Numbers of total implants and live fetuses in the 1.0 mg/kg group decreased slightly, but there was no significant difference from the control. Numbers of total implantations and live fetuses decreased significantly in the 10.0 mg/kg group. In this group, the number of resorbed fetuses increased significantly and the number of corpora lutea decreased without showing a significant difference from the control. Fetuses from the 10.0 mg/kg group showed decreased body weight, body length, and tail length and increased placental weight. About one-third of fetuses were subjected to visceral examination, but no specific anomalies considered to be due to Cd toxicity were found. Skeletal examination was performed for the remaining two-thirds of the fetuses. Delayed ossification of the sternebrae and caudal vertebrae was observed. No dominant lethality was found under the conditions used here. Although physiological deterioration caused by 10.0 mg/kg of Cd has an adverse effect on mating performance, mating ratio, the number of total implants, the number of live fetuses, and the ossification of fetuses, Cd induced neither teratogenicity nor dominant lethality.

INTRODUCTION

In order to obtain an overall understanding of the toxicity of cadmium (Cd), a single series of experiments was designed to investigate the toxicity, fertility, teratogenicity, and dominant lethality of Cd. As usual exposure to Cd is chronic and is either oral or by inhalation, these experiments were subchronic with oral administration. The previous report (Sutou *et al.*, 1980) has described the toxicity studies, and here we present the results of fertility, teratogenicity, and dominant lethal tests.

MATERIALS AND METHODS

For details of the materials and methods, see the previous report (Sutou *et al.*, 1980). Statistical analyses were mainly carried out with an IBM 370-138 computer. The *t* test was utilized to determine the significance of differences between average numbers of corpora lutea, total implantations, and live fetuses per female, average

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TABLE 1

THE EFFECT OF CADMIUM ON FERTILITY

Dose (mg/kg/day)	No. of animals	Copulating	Pregnant	Corpora lutea	Total implants	Live fetuses	Macerated	Resorbed
0	14	14	11	15.2 ± 2.0[a]	14.7 ± 2.0	14.2 ± 2.0	0	0.55
0.1	13	13	10	14.2 ± 2.0	13.9 ± 1.7	13.9 ± 1.7	0	0.70
1.0	13	12	12	15.5 ± 1.8	13.6 ± 1.8	13.1 ± 2.3	0	0.50
10	13	5	5	13.6 ± 1.5	10.6 ± 3.1**	7.2 ± 2.4**	0.8	2.6*

[a] Mean ± SD.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

fetal body weight and placental weight per litter, and average length of fetal body and fetal tail per litter for each treatment, compared with the control values. Preimplantation losses were determined by subtracting the number of total implantations from the number of corpora lutea. The preimplantation losses, early deaths, late deaths, and total deaths for each female were transformed to the Freeman–Tukey arc sine form, and then subjected to the t test to compare values for each treatment with the control value. Fertility indices analyzed were as follows: copulating ability (copulating males/total males), impregnating ability (impregnating males/total males), copulated ratio (copulated females/total females), pregnant ratio (pregnant females/total females), and pregnancy efficiency (pregnant females/copulated females). The χ^2 test was used for fertility indices to compare the values for each treatment group with the control value. We tested whether the fertility indices were linealy related to arithmetic or logarithmic dose. The proportion of females with one or more dead implantations in each treatment group was compared with the control value in the same way as for the fertility indices, that is, by χ^2 and Armitage's χ^2 tests. Regression analysis was carried out to determine whether the average number of total implantations per female was related to the arithmetic or logarithmic dose. The rank sum test was employed to compare the number of resorbed fetuses per female and average number of skeletal variations per litter for each treatment group with the control value. An analysis of variance across 6 weeks was made for the average number of total implantations per female, preimplantation losses per female (using the Freeman–Tukey arc sine transformation), total deaths per female (using the arc sine transformation), and the ratio of deaths to total implantations per female (using the arc sine transformation).

RESULTS

Table 1 shows the fertility data. The numbers of copulating and pregnant females decreased when 10.0 mg/kg of Cd was administered. The average numbers of total implantations and live fetuses per female decreased, and the average number of resorbed fetuses increased with significant differences from the control values in the 10.0 mg/kg group. It appears that the higher the dose levels, the smaller the average numbers of total implantations and live fetuses became, although the differences from the control values in the 0.1 and 1.0 mg/kg groups were not significant.

TABLE 2

THE EFFECT OF CADMIUM ON FETUSES

Sex of fetuses	Males				Females			
Dose (mg/kg/day)	0	0.1	1.0	10	0	0.1	1.0	10
No. of fetuses	71	73	73	15	85	59	84	21
Body weight (g)	3.63 ± 0.10[a]	3.58 ± 0.23	3.52 ± 0.27	2.41 ± 0.29**	3.48 ± 0.16	3.40 ± 0.25	3.37 ± 0.23	2.32 ± 0.23**
Body length (mm)	39.1 ± 0.4	39.2 ± 0.9	39.0 ± 1.1	35.2 ± 1.0**	38.5 ± 0.5	39.4 ± 3.4	38.2 ± 0.8	34.3 ± 1.2**
Tail length (mm)	13.8 ± 0.3	13.7 ± 0.3	14.0 ± 0.5	12.3 ± 1.1*	14.1 ± 0.3	14.0 ± 0.5	14.3 ± 0.3	12.5 ± 0.9*
Placenta (mg)	502 ± 25	524 ± 60	530 ± 49	548 ± 46*	479 ± 30	501 ± 78	500 ± 57	554 ± 73*

[a] Mean ± SD.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

Table 2 shows the data on fetuses. Body weight, body length, and tail length of fetuses from the 10.0 mg/kg group showed significant decreases in both males and females, compared with the control values. These fetuses were small and yellowish in color, indicative of anemia and malnutrition. In contrast, the average weight of placentas increased significantly in the 10.0 mg/kg group. Even in the 0.1 and 1.0 mg/kg groups, placental weight was greater than the control value in both males and females. Adaptive hypertrophy would thus be likely.

Table 3 shows the results of visceral examination. No marked changes specific to higher dose groups were detected. Hydronephrosis was observed in one rat in each group. Since the number of fetuses examined in the 10.0 mg/kg group was small, the percentage of this anomaly was high, but no definite conclusion can be drawn from these data.

Table 4 shows the results of skeletal examination of fetuses. As shown in Table 1, fetuses of the 10.0 mg/kg group were small and showed growth retardation, which was reflected in delayed ossification of the sternebrae and the small number of ossified caudal vertebrae.

In dominant lethal tests, five fertility indices (copulating ability and impregnating ability of males and copulated ratio, pregnant ratio, and pregnancy efficiency of females) were statistically analyzed for significant differences from the control values. No difference was observed, indicating that the administration of Cd at oral dose levels of less than 10 mg/kg/day for 9 weeks did not affect the fertility of male rats.

TABLE 3

RESULTS OF VISCERAL EXAMINATION OF FETUSES

Dose (mg/kg/day)	0	0.1	1.0	10
No. of fetuses	53	44	54	12
Hemorrhage in facial area (%)	3 (5.7)	0	4 (7.5)	0
Hemorrhage in adrenal (%)	3 (5.7)	1 (2.2)	0	0
Hemorrhage in abdominal cavity (%)	1 (1.9)	2 (4.5)	0	0
Dilatation of renal pelvis (%)	7 (13.2)	9 (20.0)	7 (13.0)	1 (8.3)
Hydronephrosis (%)	1 (1.9)	1 (2.2)	1 (1.9)	1 (8.3)

TABLE 4

RESULTS OF SKELETAL EXAMINATION OF FETUSES

Dose (mg/kg/day)	0	0.1	1.0	10.0
No. of fetuses	103	88	104	24
Delayed ossification of				
Os parietale (%)	0	6 (6.8)	0	0
Os interparietale (%)	3 (2.9)	13 (14.8)	3 (2.8)	2 (8.3)
Os sphenoides (%)	5 (4.9)	12 (13.6)	2 (1.9)	4 (16.7)
Cervical vertebrae (%)	0	0	0	2 (8.3)
Sternebra				
1st (%)	0	1 (1.1)	0	2 (8.3)
2nd (%)	0	1 (1.1)	2 (1.9)	6 (25.0)
3rd (%)	0	1 (1.1)	4 (3.8)	0
4th (%)	1 (1.0)	3 (3.4)	5 (4.8)	7 (28.0)
5th (%)	33 (32.0)	37 (42.0)	38 (36.5)	21 (87.5)**
6th (%)	26 (25.2)	28 (31.8)	22 (21.2)	24 (100)**
Cervical rib (%)	0	0	0	3 (12.5)
14th rib (%)	11 (10.7)	14 (15.9)	1 (1.0)	0
No. of caudal vertebrae	4.21 ± 0.40	3.87 ± 0.33*	4.09 ± 0.47	2.13 ± 1.18**

* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

Table 5 shows the results of dominant lethal tests. The number of impregnated females, and the average numbers of corpora lutea, total implantations, preimplantation losses, live fetuses, late deaths, and total deaths per female did not show any significant differences from the control values. The analysis of variance across 6 weeks for total implantations, preimplantation losses, total deaths, and the ratio of deaths to total implantations also showed no differences compared with the control values. The proportions of females with one or more dead implantations in all treatment groups were similar to each other. Thus, no dominant lethality attributable to Cd was detected under our experimental conditions.

DISCUSSION

Toxic signs were apparent in male and female rats administered Cd at a dose of 10.0 mg/kg/day, as described in the previous report (Sutou *et al.*, 1980). Poisoned rats showed reduced fertility, but neither teratogenicity nor dominant lethality was observed. As we did not carry out fertility studies by mating between Cd nontreated males and Cd-treated females, it was not clear which sex was more affected with regard to fertility. Dominant lethal tests, however, carried out by crossing between Cd-treated males and nontreated females suggested that Cd had little effect on the fertility of male rats. Consequently, it is likely that females were more strongly affected.

The effects seen in the fertility studies were delayed copulation, a decreased pregnant ratio, decreased average numbers of total implantations and live fetuses, an increased number of resorbed fetuses, and growth retardation of fetuses. These findings can perhaps be explained mainly in terms of two factors: anemia and hormonal imbalance.

TABLE 5

RESULTS OF DOMINANT LETHALITY TESTS OF CADMIUM

Week	Doses (mg/kg/day)	No. of animals	Corpora lutea	Total implants	Preimplantation loss[a]	Live fetuses	Early death[a]	Late death[a]	Total deaths[a]
1	Control	23	15.1 ± 2.6	13.3 ± 2.1	0.62 ± 0.42	12.8 ± 2.0	0.45 ± 0.24	0.27 ± 0.03	0.45 ± 0.24
	0.1	17	14.6 ± 2.2	12.9 ± 2.2	0.59 ± 0.47	12.5 ± 2.4	0.40 ± 0.25	0.28 ± 0.03	0.40 ± 0.25
	1	26	15.4 ± 1.9	14.4 ± 1.4[b]	0.51 ± 0.26	13.7 ± 2.0	0.41 ± 0.30	0.30 ± 0.12	0.46 ± 0.30
	10	19	16.4 ± 3.3	13.2 ± 4.3	0.73 ± 0.70	12.7 ± 4.2	0.45 ± 0.27	0.31 ± 0.12	0.47 ± 0.27
2	Control	18	14.2 ± 2.3	13.8 ± 2.0	0.37 ± 0.21	13.0 ± 2.1	0.47 ± 0.25	0.31 ± 0.16	0.51 ± 0.27
	0.1	22	14.4 ± 1.7	12.5 ± 3.3	0.64 ± 0.53[b]	12.1 ± 3.6	0.45 ± 0.27	0.28 ± 0.05	0.45 ± 0.27
	1	21	15.8 ± 2.7[b]	14.2 ± 2.1	0.59 ± 0.39[b]	13.5 ± 2.1	0.46 ± 0.24	0.28 ± 0.08	0.48 ± 0.24
	10	20	14.4 ± 1.4	12.8 ± 3.8	0.51 ± 0.42	12.0 ± 3.9	0.61 ± 0.49	0.30 ± 0.12	0.61 ± 0.49
3	Control	23	15.3 ± 1.9	14.6 ± 2.0	0.46 ± 0.23	13.7 ± 2.1	0.51 ± 0.27	0.26 ± 0.02	0.51 ± 0.27
	0.1	22	15.3 ± 1.2	14.1 ± 2.2	0.54 ± 0.37	13.4 ± 2.3	0.54 ± 0.21	0.26 ± 0.03	0.54 ± 0.21
	1	24	15.8 ± 2.4	13.6 ± 4.1	0.68 ± 0.58	12.8 ± 3.9	0.52 ± 0.21	0.31 ± 0.14	0.54 ± 0.24
	10	22	15.9 ± 1.4	14.0 ± 3.8	0.62 ± 0.55	13.2 ± 3.8	0.54 ± 0.32	0.31 ± 0.13	0.56 ± 0.32
4	Control	19	15.2 ± 1.7	14.7 ± 1.7	0.41 ± 0.22	13.8 ± 1.8	0.50 ± 0.22	0.30 ± 0.12	0.53 ± 0.23
	0.1	21	14.7 ± 1.7	13.2 ± 3.1	0.56 ± 0.49	12.4 ± 2.9	0.50 ± 0.23	0.31 ± 0.11	0.53 ± 0.24
	1	19	14.4 ± 1.3	13.5 ± 2.2	0.49 ± 0.35	12.9 ± 2.6	0.47 ± 0.29	0.29 ± 0.09	0.49 ± 0.28
	10	23	14.8 ± 1.7	14.2 ± 2.0	0.44 ± 0.26	13.5 ± 2.3	0.48 ± 0.25	0.28 ± 0.09	0.49 ± 0.26
5	Control	25	15.0 ± 1.5	13.8 ± 2.5	0.51 ± 0.44	12.9 ± 2.7	0.50 ± 0.28	0.31 ± 0.12	0.53 ± 0.30
	0.1	25	15.4 ± 1.8	13.7 ± 4.0	0.62 ± 0.56	13.3 ± 3.9	0.41 ± 0.20	0.30 ± 0.11	0.42 ± 0.20
	1	23	15.1 ± 1.6	14.6 ± 1.8	0.43 ± 0.21	13.5 ± 2.0	0.58 ± 0.26	0.27 ± 0.08	0.59 ± 0.25
	10	26	15.4 ± 1.6	14.5 ± 2.0	0.41 ± 0.38	13.7 ± 2.0	0.49 ± 0.23	0.29 ± 0.09	0.51 ± 0.24
6	Control	27	15.2 ± 1.6	13.8 ± 3.3	0.49 ± 0.31	13.2 ± 3.4	0.50 ± 0.44	0.30 ± 0.13	0.51 ± 0.44
	0.1	26	14.5 ± 3.1	12.7 ± 4.2	0.70 ± 0.54	12.2 ± 4.1	0.50 ± 0.25	0.30 ± 0.12	0.50 ± 0.25
	1	27	15.1 ± 2.1	13.1 ± 3.5	0.69 ± 0.53	12.4 ± 3.3	0.51 ± 0.23	0.31 ± 0.13	0.53 ± 0.23
	10	25	15.1 ± 1.6	14.5 ± 2.0	0.41 ± 0.29	14.0 ± 2.0	0.39 ± 0.23	0.27 ± 0.07	0.39 ± 0.25

[a] The t-test was applied after Freeman–Tukey transformation.
[b] $P < 0.05$.

As discussed in the previous report (Sutou *et al.*, 1980), anemia may arise due to inhibition of resorption of nutrients, including iron cations. Anemia was directly observed as a pale pinkish color of the dams' eyes, compared with the pinkish-red color of normal albino rats, and in the yellowish brown color of fetuses, reminiscent of jaundice. The increased placental weight (Table 2) seemed to be due to adaptive hypertrophy, compensating for the anemia, and the inhibition by Cd of the synthesis of placental hormones.

Dencker (1975) showed that Cd accumulated mainly in the liver, kidneys, adrenal cortex, pituitary, and placenta in pregnant hamsters and mice. Kar and Das (1959) showed that Cd has strong toxicity toward steroid-producing organs. Disturbances in hormone production in the ovaries, placentas, and other organs may well interfere with ovulation and conception.

Only a small amount of Cd is accumulated in the testes compared with the liver and kidneys (Matsubara-Kahn and Machida, 1975). This seems to be the main reason for the absence of dominant lethality after subchronic administration of Cd. Acute poisoning by Cd can induce sterility in male animals, which must be distinguished and negligible chronic effect on the testes.

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