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Structural and Reproductive Modifications in Rats Following a Post-implantational Exposure to Captan

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Captan, a dicarboximide and phthalimide derivative, is a fungicide which is widely and currently used in the protection of agricultural products against fungal diseases, and in household locations (Ware 1978). From a reproductive standpoint, captan was demonstrated to be more toxic in pregnant than in non-pregnant rats, while at the same time producing inflammatory reactions, and inducing lesions in the lung, liver and kidneys (Alnot et al. 1974). When administered to mature female rats for different durations during pregnancy, captan suppressed embryonic development for all doses and at all exposure periods (Zhorzholiani 1970). In another study involving pregnant white rabbits, McLaughlin et al. (1969) indicated that different doses of this fungicide produced only a few malformations among implantations - 12% and 2% at dosages of 75 mg/kg and 37.5 mg/kg respectively. In experiments with pregnant mice treated with captan by three different routes-orally, subcutaneously and by inhalation - reductions in maternal body weights, without the occurrence of any fetal malformations, were observed (Courtney et al. 1978). Untreated female rats and mice, which were mated to males that were treated with five daily doses of captan by intraperitoneal or gavage administrations, responded with an increase in the mean number of early fetal deaths per pregnancy and moderate mutagenicity (Collins 1972).

In association with these different dosages and routes of administration of captan, it was noted that in golden hamsters, a single injection of this compound during organogenesis, was more effective than multiple administrations, in promoting teratogenicity (Robens 1970). Finally, doses of 30 and 60 mg/kg captan caused no adverse effects in the mothers or progeny of beagles (Kennedy et al. 1975), and were nonteratogenic in rabbits, rats and hamsters following gestational administrations (Kennedy et al. 1968).

Decidualized (DCR) pseudopregnant and pregnant rats were employed as experimental animals and as manifestations of reproductive states. These animals were exposed to groups of low level and high level of the test compound in order to further clarify the role of captan as a toxic agent during mammalian reproduction

MATERIALS AND METHODS

The experiment employed female Sprague-Dawley rats which were purchased from the Holtzman Company, WI. These animals ranged from 200 - 260 g at the beginning of each experiment and were maintained under controlled ambient conditions of photoperiodicity (14 hr light: 10 hr darkness) and temperature (20 ± 2°). Purina lab chow and tap water were provided freely. Stages of the estrous cycle were determined microscopically by vaginal lavage, and only those rats which exhibited two consecutive 4 to 5 days estrous cycles were randomly chosen for experimentation. The induction of DCR pseudopregnancy, previously described (Tat-Sing and Spencer 1981), was accomplished by cervicovaginal stimulation during proestrus and estrus. This was followed by uterine scratch trauma on Day 4 of the period of leucocytic infiltration which followed stimulation. Pregnancy was achieved by mating with a fertile, active male, and verified by the microscopic observation of a sperm-positive vaginal smear.

The soluble protein fractions of the supernatant protein concentrations were analyzed by the Folin phenol reagent in accordance with the method of Lowry et al. (1951), using bovine serum albumin as the standard. Glycogen concentrations were determined by the method of Seifter et al. (1950) and modified by Zarrow et al. (1964), using Type III rabbit liver glycogen as the standard. Implantation sites were assessed by a tail-vein injection of Chicago blue dye in association with laparotomy and observation of the uterine horns (Labhsetwar 1971).

Captan, provided by the EPA, Research Triangle Park, NC and obtained from the Chevron Company, was mixed with pulverized Purina chow pellets to obtain the desired concentrations. This compound was orally administered for 5 days during Days 6-10 in both DCR pseudopregnant and pregnant rats. These feeding schedules were applicable after implantation, which in rats normally occurs on Day 4 of pregnancy. The results of this study were generated by DCR pseudopregnant rats which were fed low levels of captan designated 100, 1000 and 10000 ppm, and by both pseudopregnant and pregnant rats whose diet consisted of high levels (2, 3, 5 and 10%) of captan. Statistical evaluations were carried out by analysis of variance (ANOVA) and the Student's t test, with a probability value of at least 0.05 considered significant.

RESULTS AND DISCUSSION

In DCR pseudopregnant animals wherein a regional analysis of structural weights was done with captan at concentrations of 100, 1000 and 10000 ppm, the latter dose caused significant decreases in structural features (Table 1). This trend for reduction was continued with higher concentrations on uterine and body weights (Table 2). There was also a prominent dose response reduction effect in the endometrial/myometrial weight ratios at these concentrations (Fig. 1). However, data on ovarian weights were the

same in control and experimental rats (Table 2). Among the non-reproductive organs, the spleen was prominently affected, whereas the kidney and adrenal were generally unaffected (Table 2).

Table 1. Effects of low levels of Captan in decidualized pseudopregnant rats

Dietary treatment with Captan[b] (ppm)	Final body weight (g) ($\bar{X}$ ± SE)	Wet weight[a] ($\bar{X}$ ± SE) Uterus (mg)	Uterine water (g)	Adrenal (g)
0	236.0 ± 4.30	4240 ± 330	1.99 ± 0.14	0.077 ± 0.008
100	247.6 ± 24.63	4300 ± 730	2.12 ± 0.42	0.078 ± 0.010
1000	219.4 ± 7.24	3460 ± 240	1.63 ± 0.10	0.063 ± 0.009
10000	219.7 ± 1.86 **	3090 ± 230 *	1.39 ± 0.12 *	0.073 ± 0.003

[a]Each reading represents the absolute mean weight of 4-7 rats. [b]Rats were fed Captan from Days 6 through 10, sacrificed on Day 11 of pseudopregnancy.
Student's t test: *$p<0.05$; ** $p<0.01$.

From a biochemical standpoint, low levels of captan significantly affected (ANOVA: $p<0.05$) uterine myometrial glycogen in pseudopregnant rats [control (n=4), $\bar{X}$ = 637.08; 100 ppm (n=5), 322.78; and 1000 ppm (n=4), 358.68 mg/100 g tissue]. Additionally, ovarian protein concentrations were not reduced by high levels of captan treatment ($\bar{X}$ = 50.36, control; 1% = 46.14; 3%=48.72; 5% = 62.28; and 10% = 57.06 per 100 µg/100mg). These data were consistent with the lack of influence of captan on ovarian weights. Equally evident alterations among reproductive (ovary) and non-reproductive (spleen, kidney and adrenal) organs were also observed. The ovarian tissue continued to remain consistently unaffected by captan exposure. Following administration of high level of captan, fetal development as evidenced by the extent of abortions, was drastically affected (Table 4). Moreover, at 5% captan, there was total abortion in 7 out of 7 pregnancies, and in 13 out of 15 pregnancies at 10% captan (unpublished data).

It can generally be concluded then from these results, that captan is capable of a range of reproductive effects which is dependent on the dosage levels. The extent of this toxicity during the reproductive states of DCR pseudopregnancy and pregnancy appears to be non-systemic, since the effects are not equally displayed by all organs. This action may be attributed

Table 2. Organ weights of decidualized pseudopregnant rats fed Captan

Dietary treatment with Captan [b] (% Conc.)	n	Final body weight (g) ($\bar{X}$ ± SE)	Organ wet weight[a] ($\bar{X}$ ± SE) Uterus (mg)	Ovary (mg)	Adrenal (g)	Spleen (g)	Kidney (g)
0	8	237.88 ±5.99	4169±300	75±8	0.079 0.007	0.876± 0.083	1.745± 0.087
1	7	223.86 ±7.47	2941±383*	85±6	0.072± 0.003	0.606± 0.034*	1.667± 0.055
2	7	228.86 ±2.99	2523±469**	100±10	0.079± 0.003	0.600± 0.018*	1.739± 0.056
3	7	230.71 ±4.95	2404±704*	83±4	0.072± 0.002	0.579± 0.033**	1.746± 0.041
5	8	189.38 ±3.78	1180±334**	76±4	0.070± 0.004	0.519± 0.073**	1.523± 0.051
10	8	171.38 ±2.04	830±80**	66±3	0.072± 0.005	0.439± 0.024**	1.478± 0.054*
ANOVA		<0.01	<0.01			<0.01	<0.01

[a]Weight readings represent absolute values. [b]Rats were provided concentrations of Captan from Days 6 through 10, sacrificed on Day 11 of pseudopregnancy.
*Student's t test: $p<0.05$. **Student's t test: $p<0.01$.

Table 3. Organ weights of pregnant rats administered Captan

Dietary treatment with Captan [b] (% Conc.)	n	Final body weight (g) ($\bar{X}$ ± SE)	Organ wet weight [a] ($\bar{X}$±SE) Ovary (mg)	Adrenal (g)	Spleen (g)	Kidney (g)
0	11	304.82 ±7.28	144±11	0.079± 0.004	0.748± 0.043	1.952± 0.075
2	5	300.60 ±9.55	143±8	0.079± 0.007	0.884± 0.085	1.970± 0.077
5	8	228.63* ±7.76	107±9	0.070± 0.004	0.371±* 0.044	1.654±* 0.053
10	8	206.00* ±5.81	107±6	0.083± 0.004	0.343±* 0.031	1.629±* 0.044

[a]Weight results represent absolute values. [b]Rats were fed Captan for Days 6 through 10 of pregnancy and sacrificed on Day 16.
*Student's t test: $p<0.01$.

Table 4. Influence of oral administration of Captan on fetal development in rats

Dietary treatment with Captan [b] (% Conc.)	n	Body weight at birth (g) ($\bar{X}$ ± SE)	Implantation sites at Day 10 ($\bar{X}$ ± SE)	Live fetuses at birth ($\bar{X}$ ± SE)	Percentage of fetal survival rate
0	12	292.00±5.92	13.17±0.59	8.67±1.02	65.81
2	7	255.86±7.21	12.00±0.79	6.71±1.36	56.00
10	8	299.80±14.00	13.88±0.64	2.38±1.69*	17.11

[a]Rats were fed Captan for Days 6 through 10 of gestation.

*Student's t test: $p < 0.05$.

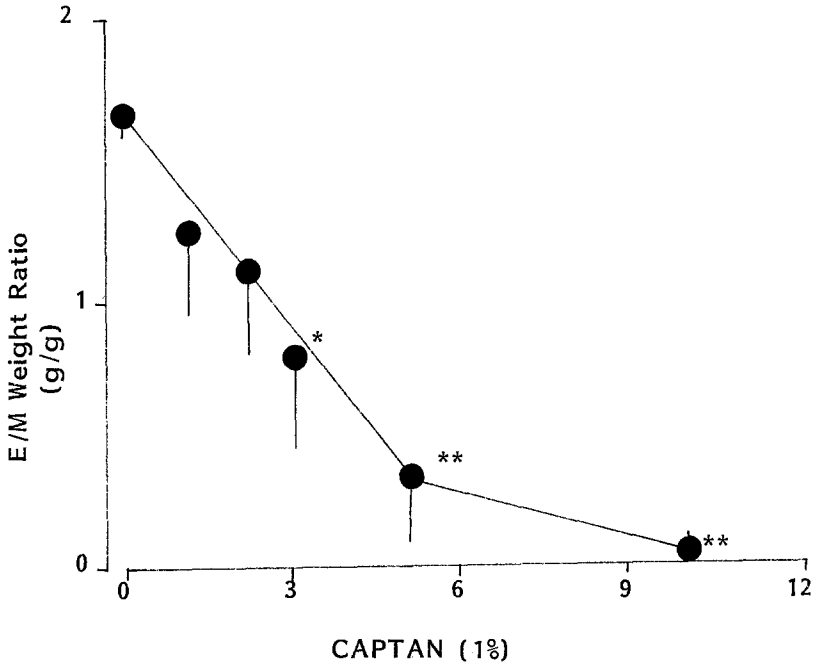

Fig. 1. Effect of Captan on endometrial/myometrial (E/M) weights of decidualized pseudopregnant rats. Each point represents the mean of 7 rats. Student's t test: *$p<0.01$; **$p<0.001$.

to the rapid metabolism and excretion of orally administered captan (Debaun et al. 1974) which may affect its selective uptake by these anatomical sites. In this regard, the gravid uterus appears to be especially vulnerable to the toxic insults emanating from captan.

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