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Reproductive Responses to Rotenone During Decidualized Pseudogestation and Gestation in Rats

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Rotenone is mainly applied as a piscicide (CHEMICAL WEEK PESTICIDE REGISTER 1977). A toxic role for rotenone is believed to be carried out through an inhibition of the mitochondrial system involving NAD (ERNSTER et al. 1961). At present, there is heightened interest in utilizing rotenone and other rotenoids as selective insecticides (MEINWALD et al. 1978) since these compounds are implicated to have low residual effects in the environment. Degradation of rotenone in the aquatic environment is rapid. A solution of rotenone (20 mg/L of 50% rotenone) becomes nontoxic during a 7-day period at 20°C (MEADOW 1973). Moreover, rotenone is photodegradable (CHENG et al. 1969).

A study conducted in pregnant mice has demonstrated that the hemoendothelial placenta effectively prevents rotenone from reaching the fetuses (BOWMAN 1978). The teratogenic potential of rotenone has been displayed in chicken embryos (RAO & CHAUHAN 1971). However, the effects of rotenone on the reproductive system of maternal mammals have not been determined. Therefore, this study was conducted to assess some of the reproductive responses to rotenone by using decidualized pseudopregnant and pregnant rats.

METHODS AND MATERIALS

Adult female Sprague-Dawley rats (ranging from 200-300 g) from the Holtzman Company, WI, were individually housed, with free access to food and water. The induction of decidualized pseudopregnancy (DCR) and the attainment of pregnancy in female rats have been previously described (TAT SING & SPENCER 1981). The experimental animals received either a stock chow diet (control diet, Purina Laboratory chow) or the stock diet containing rotenone in different dietary concentrations. Decidualized pseudopregnant rats were given the rotenone-containing diet from Days 6 to 10 (5 days) of decidualized pseudopregnancy; whereas, pregnant rats were fed from Days 6 to 15 of gestation (10 days). Uterine tissues from Day 10 DCR rats and placental tissues from Day 16 pregnant animals were analyzed for protein (LOWRY et al. 1951) and glycogen (SEIFTER et al. 1950). In

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pregnancy studies, implantation sites were counted on Day 6 using the method described by LABHSETWAR (1971). The number of viable embryos was recorded on Day 12 during laparotomy. At birth, the number of live fetuses was noted.

One-way analysis of variance was used to determine control versus treatment effects. Student's t-test was employed to depict inter-group differences. The analyses were conducted on an IBM computer equipped with SAS program (SAS INSTITUTE 1970).

RESULTS

Table 1 shows the significant reductions ($p < 0.05$) in body and uterine wet weights of decidualized pseudopregnant (DCR) rats fed 10 to 1000 ppm of rotenone. Visible signs of toxicity exhibited by these animals, especially those exposed to 750 and 1000 ppm, were lethargy and ataxia (loss of muscular coordination), and a rough, unkempt fur. Significant decreases in dietary consumption accompanied these weight changes. Biochemical parameters like uterine protein and glycogen concentrations (Fig. 1, 2) were diminished in dosage-dependent manners. Post-mortem examination of these DCR animals revealed that the fatty tissues adjacent to uteri were greatly reduced in rats exposed to 750 and 1000 ppm of rotenone.

In pregnant studies, losses in body weight from Days 6 to 15 of gestation, were demonstrated by maternal organisms treated with up to 800 ppm rotenone (Table 2). At doses of 600 and 800 ppm, dams displayed the external toxic signs of lethargy and ataxia. Biochemically, there were significant decreases ($p < 0.05$) in ovarian protein, and in placental protein and glycogen contents at doses up to 600 ppm (Table 3). The reductions in these biochemical profiles were unaffected by dosages.

At laparotomy on Day 12 of pregnancy, no resorption of implantation sites was observed in rotenone-exposed rats (Table 4). Abortifacent effects were non-existent. A slight reduction in embryonic size was visualized but was not quantified. The fetal survival rate in maternal organisms given rotenone was reduced (Table 4), but was dose-independent. The weights of the fetuses delivered from dams fed rotenone were not affected.

DISCUSSION

Results from decidualized pseudopregnant rats demonstrate that a degeneration in biochemical entities of the uterus stems from an outcome of rotenone's toxicity. These detrimental effects on the uterine environment

TABLE 1

UTERINE WEIGHT AND BODY WEIGHT OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN ROTENONE.

Dietary treatment with rotenone[a] (ppm)	N	Daily chow intake (g/day/rat) $\bar{X}\pm SE$	Daily intake of rotenone (mg/kg/day) $\bar{X}\pm SE$	Body weight of rats (g) $\bar{X}\pm SE$	Uterine wet weight (g/100g body weight) $\bar{X}\pm SE$
0	10	17.7±0.6	----	257± 6	1.46±0.40
10	10	18.5±0.5	0.74±0.02	248± 4	1.39±0.01
100	10	18.4±0.5	7.08±0.17	262± 3	1.24±0.10
200	10	17.6±0.6	14.08±0.45	248± 6	1.26±0.09
250	10	15.9±0.7	15.90±0.70	249± 6	1.05±0.10
500	10	12.5±1.1	26.03±2.33	239±10	1.13±0.11
750	10	10.5±0.8	32.82±2.60	240± 6	1.10±0.10
1000	10	9.0±0.6	40.91±1.49	218±12	1.03±0.06
ANOVA		<0.05	<0.05	<0.05	<0.05

[a]Rats were given rotenone in the respective dietary concentrations from Days 6 through 9 of decidualized pseudopregnancy.

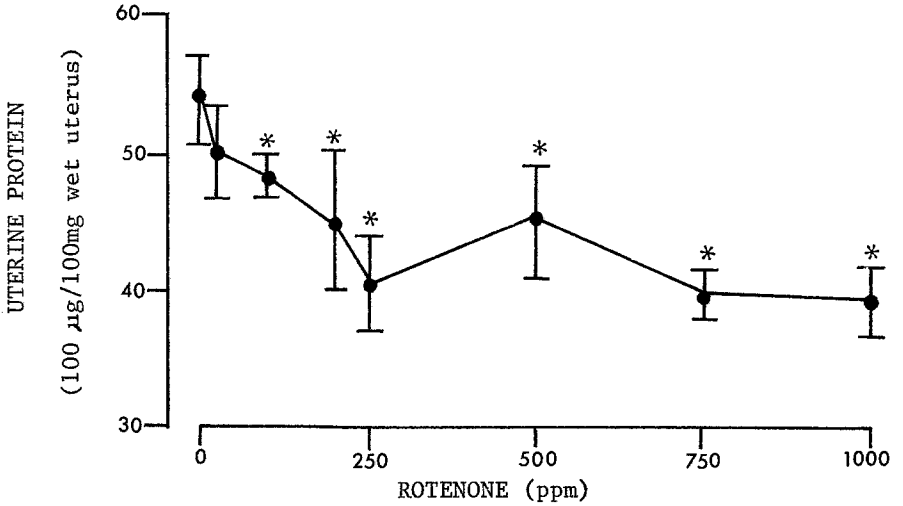

Fig. 1. Uterine protein concentration of Day 10 decidualized pseudopregnant rats fed different concentrations (ppm) of a diet containing rotenone. Each point represents the mean of 10 rats. Each bar indicates the SE. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

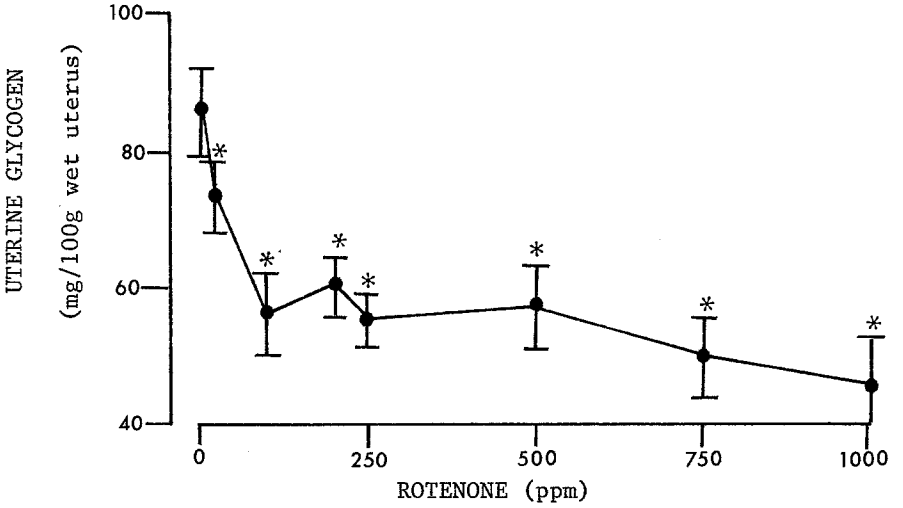

Fig. 2. Uterine glycogen concentration of Day 10 decidualized pseudopregnant rats fed different concentrations (ppm) of a diet containing rotenone. Each point represents the mean of 10 rats. Each bar indicates the SE. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

TABLE 2

BODY WEIGHT OF PREGNANT RATS GIVEN ROTENONE.

Dietary[a] treatment with rotenone (ppm)	Daily chow[b] intake (g/day/rat) $\bar{X}\pm SE$	Daily intake[b] of rotenone (mg/kg/day) $\bar{X}\pm SE$	Maternal[b] body weight at Day 6 (g) $\bar{X}\pm SE$	Maternal[b] body weight at Day 12 (g) $\bar{X}\pm SE$	Maternal[b] body weight at Day 16 (g) $\bar{X}\pm SE$	Cumulative[b] weight gain Days 6-16 (g)
0	18.6±0.4	----	232± 8	247±12	265±12	+ 33
10	19.8±0.9	0.77±0.03	221± 6	236± 7	258± 6	+ 37
100	19.2±0.5	8.10±0.02	226± 6	228± 7	244± 9	+ 18
200	17.3±0.3	12.80±0.23	254± 7	259± 9	272± 8	+ 18
400	8.5±0.5	16.50±0.95	239±11	221±11	205±10	- 34
600	6.2±0.3	19.20±0.81	230±15	206±12	196±14	- 34
800	5.4±0.2	22.80±0.89	262±12	226± 9	195±11	- 67
ANOVA	<0.05	<0.05	<0.05	<0.05	<0.05	---

[a]Rats were given rotenone in the respective dietary concentrations from Days 6 through 15 of pregnancy.

[b]Each value represents the results for 7 rats.

TABLE 3

OVARIAN PROTEIN, PLACENTAL PROTEIN, AND PLACENTAL GLYCOGEN CONCENTRATIONS OF DAY 16 PREGNANT RATS GIVEN ROTENONE.

Dietary[a] treatment with rotenone (ppm)	Daily chow[b] intake (g/day/rat) $\bar{X}\pm SE$	Daily intake[b] of rotenone (mg/kg/day) $\bar{X}\pm SE$	Ovarian protein[b] (100 µg/100mg ovary) $\bar{X}\pm SE$	t-test	Placental protein[b] (100 µg/100mg placenta) $\bar{X}\pm SE$	t-test	Placental glycogen[b] (mg/100g placenta) $\bar{X}\pm SE$	t-test
0	19.7±1.3	----	47.9±3.0	----	54.4±4.6	----	482±23	----
100	19.6±1.5	9.8±0.8	46.0±3.4	NS	57.1±2.6	NS	495±19	NS
200	17.1±0.3	16.7±0.3	36.4±6.2	<0.05	41.6±6.1	<0.05	462±62	NS
400	11.1±0.7	25.3±1.7	23.9±2.5	<0.05	39.6±7.3	<0.05	351±29	<0.05
600	8.1±0.7	28.5±2.3	29.4±2.2	<0.05	38.0±2.1	<0.05	343±15	<0.05
ANOVA	<0.05	<0.05	<0.05		<0.05		<0.05	

[a]Rats were given rotenone in the respective dietary concentrations from Days 6 through 15 of pregnancy.

[b]Each value represents the results for 7 rats.

TABLE 4

THE OUTCOME OF PREGNANCY IN PREGNANT RATS GIVEN ROTENONE.

Dietary[a] treatment with rotenone (ppm)	Number of litter (N)	Number of implantation sites per dam at Day 6 $\bar{X}\pm SE$	Number of conceptuses per dam at Day 12 $\bar{X}\pm SE$	Percentage of fetal survival rate per litter at birth		Average fetal weight per litter at birth (g)
				$\bar{X}\pm SE$	t-test	$\bar{X}\pm SE$
0	7	12.3±1.2	12.3±1.2	67.5± 8.1	----	6.94±0.13
10	7	12.3±0.4	12.3±0.4	60.8± 8.8	NS	7.53±0.32
100	7	12.6±0.5	12.6±0.5	32.7±11.5	<0.05	6.66±0.22
200	7	11.0±0.7	11.0±0.7	23.5± 7.2	<0.05	7.00±0.12
400	7	12.6±0.5	12.6±0.5	23.3±11.5	<0.05	7.02±0.34
600	7	11.0±0.7	11.0±0.7	22.4±12.6	<0.05	6.49±0.41
800	7	11.4±0.4	11.4±0.4	15.8± 4.6	<0.05	7.05±0.15
				<0.05[b]		NS[c]

[a]Rats were given rotenone in the respective dietary concentrations from Days 6 through 15 of pregnancy.

[b]Effects of treatment groups vs. control, using one-way analysis of variance.

[c]Effects of treatment groups vs. control, using Mann-Whitney U Test.

(Table 1, Figure 1) allude to a uterotoxic role on the part of rotenone. Over and above this, the toxicant also had a deleterious systemic effect on pregnant as well as on pseudopregnant rats. Significantly reduced body weights, which were prominent in both gestational types, exemplify this feature. These data are consistent with other studies reporting similar systemic effects of rotenone in rats (SANTI et al. 1963); HAUSEN et al. 1965).

Regional analysis of tissue protein and glycogen in pregnant animals revealed another aspect of rotenone's toxicity. Now although there was no diminution of implantation sites during embryogenesis, resorptive effects of fetuses were very much evident after Day 12 of gestation. The reason for this dichotomy cannot be explained. Albiet, the placenta is fully functional during this period of embryogenesis (gestation Day 12 to parturition). Hence, it would be reasonable to assume that the placenta could constitute an access route for rotenone to the fetus. However, BOWMAN et al. (1971) have presented evidence demonstrating the inaccessibility of the placenta to rotenone in mice.

A possible, and perhaps only plausible mechanism then for rotenone's fetotoxic potential might be in association with an indirect route. In view of the reductive effects on maternal weights and uterine biochemistry during decidualized pseudogestation, and because of decreases in maternal weights and placental biochemistry in pregnant rats, we are postulating that the embryotoxic role of rotenone in rats may be reflective of such changes. Hence, this hostility on the fetus may be accomplished via indirect, systemic pathways. Demonstrated here is an harmonious juxtaposition of the decidualized pseudopregnancy data along and coincident with reproductive end-points and character during pregnancy. This provides reinforcement for a previously established claim made with respect to the two gestational types and their responses to toxicants in rats (TAT SING & SPENCER 1981).

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