DDT Administered to Neonatal Rats Induces Persistent Estrus Syndrome

Abstract. *The o,p′-isomer of the insecticide DDT when injected into neonatal female rats significantly advanced puberty, induced persistent vaginal estrus after a period of normal estrous cycles, and caused the ovaries to develop follicular cysts and a reduced number of corpora lutea. The uterotropic response to administered estradiol was reduced, and the female pattern of mating behavior was slightly disturbed. Residues of DDT in ovarian, brain, and adipose tissues of the adult animals were the same in both treated and control groups.*

The hypothalamus of developing rats apparently establishes its neural mechanisms for regulating gonadotropin secretion in either a female (cyclic) or male (tonic) pattern during a critical period early in postnatal life (*1*). The direction of this neural development is toward the male pattern if the perinatal gonad secretes male hormone, and toward the female one in the hormone's absence. Little information is available about the critical period of neural development in those species whose offspring are not so immature at birth. In them that period probably occurs earlier, during intrauterine life. Exposure of newborn female rats to various exogenous estrogens or androgens induces permanent sterility with polycystic ovaries, anovulation, persistent vaginal estrus, and absence of female mating behavior (*1*). Clomiphene, 1-[*p*(β-diethylaminoethoxy)phenyl]-1,2-diphenyl-2-chloroethylene, a synthetic agent with weak estrogenic properties, similarly produces permanent sterility (*2*). Uterotropic effects of the insecticide DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane] have recently been described (*3*). The *o,p′*-isomer of DDT, which comprises approximately 20 percent of technical grade DDT, is several times more uterotropic than *p,p′*-DDT. In view of the ubiquity and persistence of chlorinated hydrocarbons and their ability to reach the fetus (*4*), we investigated the effects of *o,p′*-DDT injected into neonatal female rats because of the sensitivity of the neural development to estrogen and the distinctive character of the resultant syndrome.

Sprague-Dawley rats were obtained from timed matings, and birth days were designated as day zero. Female pups were pooled and randomly assigned to a control group or one treated with DDT. The DDT (1 mg) was administered subcutaneously on the 2nd, 3rd, and 4th days of life; control animals received only the propylene glycol and ethanol vehicle. The animals were given free access to Purina Lab Chow and tap water and had a schedule of artificial diurnal lighting with 14 hours of light. All rats were weaned at 21 days of age and caged in pairs. After vaginal opening was noted, daily smears were obtained by vaginal lavage. At 120 days of age, ovariectomy was performed under ether anesthesia, the ovaries were weighed, and one from each animal was fixed for histological examination. The remaining ovaries were pooled and frozen for analysis of DDT. Mating behavior was tested 19 days later after the animals had been primed with a standard alternating schedule of estradiol benzoate and progesterone. Tests were repeated in the following weeks. Responses were scored as lordosis quotients, the ratio of the number of lordoses per ten mounts by a male (*5*). Seventeen days after the second mating test we injected one-half of each group with 10 μg of free estradiol in sesame oil daily for 1 week to evaluate uterine sensitivity to estrogen. The remaining animals received only the oil vehicle. Twenty-four hours after the last injection, the animals were decapitated and the uteri were weighed. The whole brain and samples of suprarenal adipose tissue were frozen and submitted along with the ovaries to analysis of DDT residues by means of gas-liquid chromatography (*6*).

Significant advances in vaginal opening and first estrus were the first indications that DDT treatment during the neonatal period altered the reproductive system (Table 1). Estrous cycles were normal in the treated group (12 animals) until approximately 60 days of age, when the first signs of persistent vaginal estrus appeared (no less than four consecutive days with a cornified vaginal smear). By 100 days of age,

Table 1. Effects of *o,p′*-DDT administered to neonatal rats. All figures are means and standard errors. Numbers of rats are shown in parentheses.

Treatment	Age (days)		Weight (mg/100 g body weight)			Lordosis quotient	
	Vaginal opening	First estrus	Ovarian	Uterine			
				Oil	Estradiol	Test 1	Test 2
Control	33.9 ± 0.6 (13)	34.9 ± 0.9 (13)	31.1 ± 2.0 (8)	34.6 ± 1.8 (4)	141.4 ± 5.1 (4)	69 ± 13 (8)	65 ± 12 (8)
DDT	30.9 ± 0.9* (12)	31.5 ± 1.0† (12)	25.2 ± 1.8‡ (12)	39.5 ± 3.1 (6)	110.4 ± 5.9* (6)	51 ± 14 (12)	50 ± 12 (12)

* Different from controls ($P < .01$). † $P < .02$. ‡ $P < .05$.

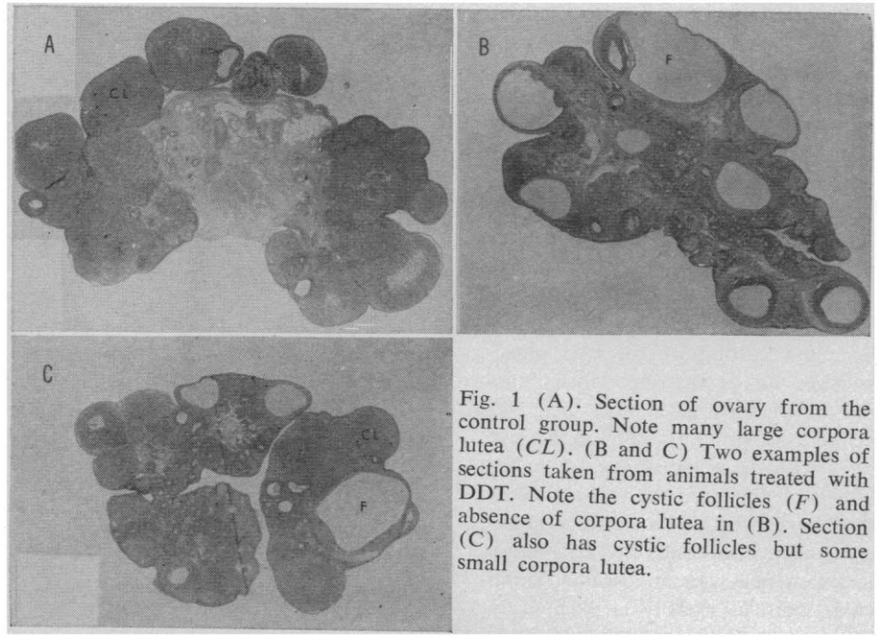

Fig. 1 (A). Section of ovary from the control group. Note many large corpora lutea (*CL*). (B and C) Two examples of sections taken from animals treated with DDT. Note the cystic follicles (*F*) and absence of corpora lutea in (B). Section (C) also has cystic follicles but some small corpora lutea.

Table 2. DDT residues in tissues of rats treated with *o,p'*-DDT as neonates. Unless data are given, concentrations of *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD in the tissues were below the level of sensitivity (0.01 ppm) of the technique. Each value represents a single determination from a pool size shown in parentheses.

Residue	Tissue	Amount (ppm)	
		Control	DDT
p,p'-DDT	ovary	0.03 (4)	0.03 (7)
p,p'-DDT	brain	0.01 (4)	0.01 (6)
p,p'-DDT	adipose	0.30	0.34
p,p'-DDE	adipose	0.18 (4)	0.18 (6)

all of the treated rats showed the first signs of persistent estrus, and by 120 days, when ovariectomy was performed, persistent cornification was established in all rats.

Ovaries of the treated group contained large cystic follicles and a marked reduction in fresh corpora lutea (Fig. 1). These histologic findings were reflected in the differences in ovarian weight, which were of borderline statistical significance (Table 1).

When the persistent estrus syndrome is marked by a delay in the onset of anovulation it is termed the delayed anovulatory syndrome. The incidence of anovulation in animals treated with testosterone as neonates increases with time (*7*). Since the high incidence of cornified vaginal smears was occasionally interrupted by 1 or 2 days of diestrus and the differences in ovarian weight of the groups had only a borderline statistical significance, it is likely that our animals were in the early stages of the development of this syndrome. Had the observation period in this experiment been extended, a longer absence of ovulation and greater regression of existing corpora lutea would likely have yielded a more dramatic diminution in ovarian weight.

Indirect evidence suggests that the anovulation is due to damage to hypothalamic mechanisms regulating cyclic secretion of luteotropic hormone (LH). Anovulatory animals produced by neonatal treatment with testosterone ovulate after administration of LH, electrical stimulation of the preoptic area of the hypothalamus (*8*), or administration of LH releasing factor (*9*). Furthermore, the syndrome is prevented by simultaneous administration of depressive agents of the central nervous system such as chlorpromazine and barbiturates (*10*). The advancement of puberty may be due to an altered hypothalamic sensitivity regulating gonadotropin secretion; however the mechanism is unknown. The presence of a persistently cornified vaginal smear without the usual cyclic diestrous appearance of the leukocytes is probably due to the tonic secretion of estrogen as well as to the absence of progesterone which normally attends ovulation.

Female rats treated during the neonatal period with sex steroids often fail to show lordosis and female mating behavior (*5*). In two such mating tests, the treated animals scored lower than controls, but the differences were not statistically significant (Table 1). Neonatal neural mechanisms are more sensitive to the induction of the persistent estrus syndrome than are those controlling behavior (*11*). The high dose of estrogen increased uterine weight in both the control and treated groups (Table 1); however the increment in the latter group was significantly reduced. We presume that the injection schedule for estrogen and progesterone used in tests for mating behavior increased the uterine weights of control and treated rats; however the increments could not be measured. Nevertheless, the similarity in initial weights of both groups injected with oil indicates that the degree of uterine regression was similar in both groups after cessation of that treatment. Harris (*12*) also noted reduced uterine responses to estrogen in animals treated with sex steroids during the neonatal period. The reduced sensitivity may be due to decreased estradiol receptor (*13*), but the mechanism whereby the receptor is permanently reduced is unknown. Preliminary data obtained by sucrose density gradient centrifugation suggest that the concentration of estradiol receptor in the uterine cytosol from the treated animals is less than controls (*14*).

It is surprising that the amounts of DDT residues in ovary, brain, and adipose tissue (Table 2) were not affected by treatment of neonates with DDT. Purina Lab Chow contained 0.01 part of *p,p'*-DDT per million which probably accounts for the residues found in all the animals. The similar amounts in the treated and control groups indicate that the injected DDT had been cleared before the autopsy. Therefore, the alteration of uterine response in vivo and in vitro cannot be accounted for by residual DDT, although the amounts in uterine tissue were not measured. The *o,p'*-DDT isomer was not detected in any of the tissues, probably because it is converted to the *p,p'*-isomer in living tissues (*15*).

These data show that DDT, in addition to sex steroids, can induce the constant estrus syndrome with permanent sterility. The similarity between this syndrome in rats and the polycystic ovary syndrome found in women has been described (*16*). The oligo-ovulation and relative sterility in the human syndrome could be related to the presence of DDT in the fetal environment (*4*), but the relationship remains to be established.

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6. We thank Dr. J. Allard and A. L. Robbins of the Washington State Division of Health for analyzing the DDT residues in our samples. The technique, a modification of that reported by H. F. Enos, F. J. Biros, D. T. Gardner, and J. P. Wood [*Abstr. Amer. Chem. Soc. Meet. 154th* (1967)], has a sensitivity of 0.01 ppm for the compounds named in Table 2.
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17. We thank Dr. Mary Sarff for measuring the concentration of the uterine receptor; Dr. Harvey Feder, Department of Biology, Rutgers University for aid in the sexual receptivity studies; and Susan Robinson, Jane Bennett, and G. Comito for technical assistance. Supported by American Cancer Society, Inc., grant T-543 and PHS grants HD-03825 and AM-05638-09. Portions of this study were presented at the 1971 Annual Meeting of the Western Society for Clinical Research, Carmel, Calif.; they have been published in abstract form [W. L. Heinrichs, R. J. Gellert, J. L. Bakke, N. L. Lawrence, *Clin. Res.* **19**, 171 (1971)].

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