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THE EFFECTS OF LOW DOSE DIETHYLSTILBESTROL ADMINISTRATION IN NEONATAL FEMALE RATS

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SUMMARY

Two groups of ten female rats were injected with 0.5 μg or 1.0 μg diethylstilbestrol (DES) on day 5 or days 5 and 6, respectively. Other groups were injected with 100 μg testosterone propionate (TP) on day 5 or day 6, respectively. One group was kept as uninjected controls. Body weights were the same for all groups even though the treated animals showed precocious vaginal opening. Animals treated on day 5 with TP showed a greater precocity than those treated on day 6. In another experiment, groups of ten rats were treated on day 3 with 30 μg TP, 5.0 μg estradiol benzoate (EB), 0.5 (DES) and sesame oil. All but the oil injected animals showed persistent vaginal estrus. DES and EB treated rats showed an increase in the percentage of persistent estrus with increasing age. The ability of very low doses of DES to sterilize female rats is consistent with observations of a specific estrogen binding protein which is present in the blood of newborn female rats and does not appear to bind non-estrogen steroids, and thus would not block masculinization by DES or TP.

INTRODUCTION

Neonatal administration of androgens [1] and estrogens [2] causes precocious sexual maturation and anovulatory sterility in the female rat [see 3–5 for reviews]. Non-steroidal compounds including clomiphene [6], the plant estrogen coumestrol [7], DDT [8], and diethylstilbestrol (DES) [9], have also demonstrated the same sterilizing effect. It has recently been hypothesized that neonatally administered androgens may cause sterility *via* their conversion to estrogens; it is known that estrogens are produced from androgens *in vitro* by diencephalic and limbic tissues from newborn rats [10]. These findings raise the question of whether low dose peripheral administration of a non-steroidal estrogen which is not bound by circulating estrogen-binding proteins will cause sterility in female rats. In the present studies we used submicrogram quantities of DES to test this hypothesis and to ascertain whether it accelerates the onset of puberty.

MATERIALS AND METHODS

In the first experiment, one day old female Sprague–Dawley-derived rats (Charles River Farms) were pooled into groups of ten and randomly assigned to post-partum dams. At three days of age (counting day of birth as day 1) each member of a group was injected subcutaneously with 30 μg testosterone propionate (TP), 5 μg estradiol benzoate (EB) or 0.5 μg diethylstilbestrol (DES) in a sesame oil diluent (10 μl). Lighting (14 h light and 10 h dark) and temperature (70°F) were controlled. All animals were weaned on day 21. Daily vaginal smears were carried out between day 36 and 46, day 75 and 88, and again from day 107–120. Smears were classified as showing persistent estrus (more than 70% of smears), persistent diestrus (more than 70% of smears diestrus), or vaginal cycles (less than 70% smears estrus, less than 70% of smears diestrus) as previously described [11].

In the second experiment, female Sprague–Dawley-derived (Simonson Albino) rat pups were raised as above. They were divided into four treatment groups of ten animals each: the first two were injected subcutaneously with 0.5 μg and 1.0 μg DES respectively on day 5. The third group received doses of 0.5 μg on day 5 and 6 and the fourth 1.0 μg on days 5 and 6. Two groups were injected with 100 μg TP on day 5 or day 6 for use as androgen sterilized controls. Another group was kept as uninjected controls. The injection vol. for all animals was 25 μl in a sesame oil diluent. Weekly body weight was measured for each animal from weaning to 56 days of age.

RESULTS

In the first experiment, during the three smear periods, 80% of the control animals displayed normal vaginal cycles (Table 1). All of the TP treated animals had persistent estrus cycles during each period. The EB treated animals, however, showed a cyclic pattern

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Table 1. Experiment I—Effects of treatment on day 3 in female Sprague–Dawley-derived rats

Treatment	Normal vaginal cycles (%)	Persistent estrus (%)	Persistent diestrus (%)
	Smear period, 33–47 days		
Control	80	20	0
30.0 μg TP	0**	100**	0
5.0 μg EB	50*	50*	0*
0.5 μg DES	77.8*	0*	22.2*
	Smear period, 76–88 days		
Control	80	20	0
30.0 μg TP	0*	100*	0*
5.0 μg EB	0*	100*	0*
0.5 μg DES	44.5*	55.5*	0*
	Smear period, 107–120 days		
Control	80	20	0
30.0 μg TP	0**	100**	0*
5.0 μg EB	0**	100**	0*
0.5 μg DES	0*	100**	0*

* No significant difference between controls and treated groups.
** $P < 0.001$—significance of control versus treated group [20].

which changed with increasing age. At the lower age 50% of the animals had normal cycles and 50% had persistent estrus cycles, but by the time they were 75 days old all of the EB animals had stopped having normal cycles and showed persistently cornified vaginal epithelia. The pattern of cycles in the DES treated rats also changed with age. When first smeared, 77.8% of the DES treated animals were cycling normally and 22.2% had persistent diestrus cycles. No leukocytes appeared in the cornified smears of the EB and DES treated group. When these animals were older, days 75 through 88, only 44.5% exhibited normal cycles and the other 55.5% had persistent estrus cycles. During the final smear period, 107–120 days, 100% of all treated rats showed persistent estrus (Table 1).

In the second experiment, all animals treated with DES exhibited "pinhole" vaginal patency by day 15 (Table 2). All control animals with opened vaginas by day 47 attained puberty at a significantly greater age ($P < 0.001$) than the DES treated group. The TP treated rats also showed precocious patency, indicating a decreasing response with increasing age at injection (Table 2). The body weight curves of all groups were equivalent during this period of development.

DISCUSSION

The vaginal cycles which developed in the DES and EB treated animals differed from cycles described in some previous reports in animals treated with low doses of EB [12] and the non-steroidal compound clomiphene citrate [6]. Gorski[12] described prolonged but regular cycles in EB treated rats. However, the finding that 50% of the EB treated rats in this experiment had persistent estrus when first smeared at 36 days of age and that 100% displayed persistent estrus by 75 days of age is consistent with several other reports [9]. The frequency of cycles in DES treated animals also diminished with age. The changing pattern of vaginal cycles with increasing age is similar to that described by Gorski[12] after treatment with low doses of TP and by Heinrichs *et al.*[8] after treatment with *o,p'*-DDT. Unlike the clomiphene treated rats described by Gellert[6], neither the EB nor the DES treated animals in this experiment had leukocytes present in their cornified smears.

It is apparent that a very low dose of DES is effective in causing anovulatory sterility in the adult female rat. The presence of specific fetal and neonatal steroid binding proteins may explain this phenomenon [13]. Since neonatal administration of exogenous estrogens causes sterility in female rats, some

Table 2. Experiment II—Vaginal Patency

Treatment	First day 100% open	Significance vs. control	Dose response	Time response
Control	47			
100 μg TP day 5	36	$P < 0.02$		$P < 0.001$
100 μg TP day 6	42			
0.5 μg DES day 6	15	$P < 0.001$	NS	NS
1.0 μg DES day 6	14			
0.5 μg DES days 5 and 6	13	$P < 0.001$	NS	NS
1.0 μg DES days 5 and 6	13			

N.S.—Not significant [20].

protection of sensitive tissue from maternal estrogen is probably necessary [10]. The presence of specific estrogen binders in rat amniotic fluid [14], prepubertal plasma [15] and fetal and neonatal brain cytosol [13] could provide such protection. These binding proteins, which disappear early in life, do not appear to bind testosterone or diethylstilbestrol and thus would not interfere with normal masculinization of males nor would they block sterilization of females by DES or testosterone. Further, this apparently high potency ratio of DES to EB or TP under these circumstances is consistent with the hypothesis that testosterone sterilizes through aromatization to estrogen [10].

The earlier sexual maturation of animals treated with TP on day 5 or DES on days 5 and 6 as compared to that in rats treated only on day 6 (Table 1) provides additional evidence for the possibility of a perinatal "critical period" during which the CNS may be organized by androgens and/or estrogens for control of pubertal timing [16]. Exogenous non-steroidal estrogens may also influence this period. A similarly hypothesized "critical period" regulating the growth rate of developing rats has been demonstrated to be under dose-dependent androgen control [17]. In the present study, the observation that high doses of TP administered after this "critical period" cause sterility without affecting growth is consistent with the hypothesized sequence of neonatal "critical periods" of the CNS imprinting or organization which affects growth [17], sexual behaviour [18], and fertility [19].

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