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PERSISTENT VAGINAL CORNIFICATION AND POLYCYSTIC OVARIES IN NORMALLY DIFFERENTIATED ADULT FEMALE RATS FOLLOWING TREATMENT WITH TESTOSTERONE PROPIONATE[1]

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Abstract. In normally differentiated adult female rats, a single injection of testosterone propionate (5–10mg) induces persistent vaginal cornification accompanied by polycystic ovaries. Testosterone treatment induces an initial phase of pseudopregnancy followed by persistant vaginal cornification, and then an eventual return to normal estrous cycles. It is proposed that the normal cyclic mechanism controlling ovulation is interfered with in a temporary manner after testosterone treatment in adult female rats in contrast to the permanent persistent vaginal cornification seen after such treatment in neonatal rats.

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Differentiation of the neonatal female rat brain with steroid treatment or the pesticide DDT induces persistent vaginal cornification (PVC) accompanied by anovulation (Barraclough 1961, Gellert *et al* 1974). Elimination of the cyclic mechanism controlling the ovulatory process also may occur in normally differentiated adult rats exposed to constant environmental lighting, hypothalamic lesions, prolonged administration of caffeine or steroids, or as a consequence of aging (Wurtman 1967, Everett 1977, Kawashima and Takasugi 1970, Ward *et al* 1978, Meites *et al* 1978). The present study involves a single subcutaneous injection of testosterone propionate administered to normally differentiated young adult female rats. Such treatment induces pseudopregnancy followed by prolonged persistent vaginal cornification and the development of a polycystic ovarian condition.

MATERIALS AND METHODS

Royalhart Wister rats were maintained under controlled lighting (14 hr light–10 hr dark) and fed water and Purina rat chow *ad lib*. All rats were 3–4 months old at the beginning of experiments. Vaginal smears were taken daily and two 4-day cycles were required before rats were used in an experiment.

Hormones (testosterone propionate, estradiol benzoate, and progesterone) and drugs were administered subcutaneously at 2:00 p.m. unless otherwise indicated (see tables 1 and 3 for doses). Nembutal (sodium pentobarbital, NBTL, 30 mg/kg body weight) was injected on proestrus at 2:00 p.m. Agents utilized for inducing pseudopregnancy were Reserpine, Trilafon (perphenazine), estrogen (estradiol benzoate), and progesterone at doses indicated in table 3.

Selected animals were autopsied and serial ovarian sections were prepared and stained with hematoxylin and eosin to determine if the polycystic ovarian state existed, thus indicating an anovulatory state. In addition, selected animals were tested by the uterine scratch method to see if a positive decidual reaction occurred indicating a true pseudopregnant state (DeFeo 1963).

Statistical methods employed were the χ square with the Yates' correction for continuity and Student's t-test. Where applicable, results are expressed as the mean ± standard error of the mean.

RESULTS

Table 1 summarizes the initial experiments indicating that a single injection of testosterone, with or without Nembutal, induced pseudopregnancy followed by persistent vaginal cornification (PVC) in the normally differentiated adult female rat. Figure 1 depicts selected estrous cycles following treatment with either sesame oil or testosterone propionate (TP–5mg) at proestrus. Following a pseudopregnant phase and a PVC phase, normal estrous cycles resumed in the majority of rats. The PVC stage persisted in 36 rats (exclusive of those

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TABLE 1

Induction of prolonged persistent vaginal cornification (PVC) in adult female rats with testosterone propionate (TP) treatment or testosterone propionate and Nembutal (NBTL) on proestrus.

Treatment	No. Rats	Pseudopreg. No.	(%)	PVC No.	(%)
Sesame Oil	29	0		1	(3.5)
TP-5mg+NBTL	37	37	(100)	24	(65)*
TP-10mg+NBTL	7	7	(100)	4	(57)*
TP-2.5mg	8	8	(100)	0	
TP-5mg	25	25	(100)	15	(60)*
TP-10mg	10	10	(100)	6	(60)*

*$P < .01$ as compared to sesame oil treated controls.

autopsied while still in PVC) for a mean of 13.9 ± 1.28 days following 5–10 mg TP with and without Nembutal treatment. Thirteen animals treated with TP (5–10 mg) were autopsied while in the PVC phase (19.2 ± 1.8 days) and exhibited polycystic ovaries with absence of any recent corpora lutea. No significant differences in the onset of PVC were noted when TP was injected at various stages of the estrous cycle (table 2). The total number of rats entering PVC after treatment with 5–10 mg TP are compared with sesame oil controls ($P < .001$) in figure 2.

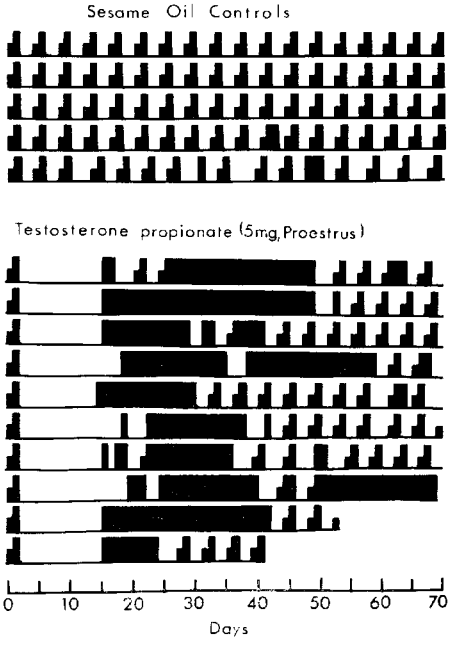

FIGURE 1. Vaginal smear record following injection of testosterone propionate (TP, 5mg) or sesame oil on proestrus of 4-day cyclic rat. Solid bars represent vaginal cornification (estrus); intermediate bars represent proestrus; baseline represents diestrus.

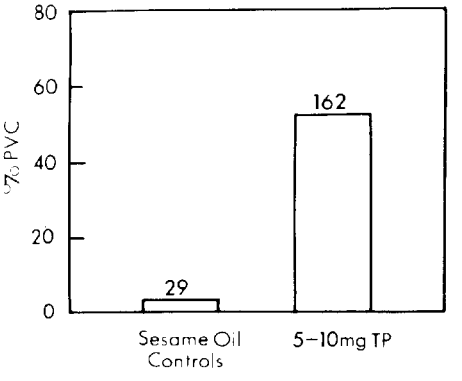

FIGURE 2. Percent animals entering persistent vaginal cornification (PVC) following treatment with single injection of testosterone propionate (with or without Nembutal) or sesame oil ($P < .001$).

Data showing that other known chemical inducers of pseudopregnancy fail to elicit persistent vaginal cornification seen after testosterone propionate treatment are shown in table 3. Five rats treated with 5 mg TP at 2:00 p.m. on proestrus received uterine traumatization, and decidual reactions were observed indicating active progesterone secretion during TP —induced pseudopregnancies.

Pseudopregnancies following 5mgTP, 10mgTP and 5mgTP+NBTL were 15.1 ± 0.66, 17.5 ± 1.45, and 16.3 ± 0.48 days,

TABLE 2

Influence of injection of testosterone propionate (5mg) in adult female rat at various times in estrous cycle.

Day of Cycle	Treatment Time	No. Rats	Pseudopreg. No.	(%)	PVC No.	(%)
Estrus	2:00 PM	14	14	(100)	2	(14)
Diestrus 1	2:00 PM	10	10	(100)	6	(60)*
Diestrus 2	2:00 PM	10	10	(100)	4	(40)**
Proestrus	11:00 AM	10	10	(100)	4	(40)**
Proestrus	2:00 PM	29	29	(100)	13	(45)*
Proestrus	6:00 PM	10	10	(100)	6	(60)*

*$P < .01$ compared to sesame oil treated controls (table 1).
**$P < .05$ compared to sesame oil treated controls (table 1).

respectively (table 4). Comparison of pseudopregnancy duration following 5 or 10 mg of TP revealed a significant difference ($P < .05$). All above treatments were significantly different ($P < .01$) from the length of pseudopregnancy induced by reserpine ($11.4 + 0.29$), progesterone (10.5 ± 0.34), and estrogen (11.3 ± 0.53). Trilafon induced pseudopregnancies averaged 14.7 ± 0.44 days. Statistical tests revealed significant differences in length of pseudopregnancy from rats treated with 5mg of TP at diestrous day 2 as well as reserpine, progesterone, or estrogen, but animals treated with 5 mg TP+ Nembutal did not show any significant difference.

DISCUSSION

Persistent vaginal cornification and polycystic ovaries resulted from testosterone propionate (TP) treatment in adult female rats (Quinn 1974). A typical sequence of pseudopregnancy, persistant vaginal cornification (PVC) followed by return to normal estrous cycles follows a single injection of TP. Anovulation during PVC was confirmed by histological analysis of ovaries. My studies can be compared with those involving prolonged treatment of immature rats (27 days) and young adult rats (2–3 months) with dehydroepiandrosterone (DHA). With DHA treatment, there was an induction of PVC and develop-

TABLE 3

Failure to induce persistent vaginal cornification in normal adult female Wistar rat following various procedures known to induce pseudopregnancy.

Treatment Cycle Day	Treatment Time	No. Rats	% Continuing to Cycle	% Pseudopreg.	% Resuming Cycles after Pseudopreg.
Reserpine (0.5mg/kg) at Estrus	2:00 PM	11	100	—	—
Reserpine (0.5mg/kg) at Diestrus Day 1	2:00 PM	11	18	82	100
Trilafon (5mg/kg) at Diestrus Day 1,2,3,4,5*	2:00 PM	9	—	100	100
Estrogen (82.25μg) at Proestrus	2:00 PM	10	—	100	100
Progesterone	10:00 AM	11	82	18	100
(10mg)	2:00 PM	10	80	20	100
at Estrus	2:00 PM	9	78	22	100

*A single injection was given each of the first 5 days of diestrus.

TABLE 4

Length of pseudopregnancy following testosterone propionate (TP), estradiol benzoate, progesterone, Trilafon and reserpine.

Cycle Day Treatment Time	Treatment	No. Rats	Days of Pseudopreg. ± SE
Proestrus 11:00 AM	TP–5mg	10	13.8±0.68**
Proestrus 2:00 PM	TP–2.5mg	8	12.6±1.07
	TP–5mg	25	15.1±0.66**
	TP–5mg	29	15.1±0.53**
	TP–5mg+NBTL†	37	16.3±0.48**
	TP–10mg	10	17.5±1.45**
	TP–10mg+NBTL	7	16.9±2.20**
Proestrus 6:00 PM	TP–5mg	10	15.4±1.07**
Estrus 2:00 PM	TP–5mg	14	13.4±0.48**
Diestrous Day 1 2:00 PM	TP–5mg	10	15.6±0.78**
Diestrous Day 2 2:00 PM	TP–5mg	10	12.5±0.65*
Diestrous Day 1 2:00 PM	Reserpine	9	11.4±0.29*
Diestrous Days 1,2,3,4,&5 2:00 PM	Trilafon	9	14.7±0.44**
Proestrus 2:00 PM	Estradiol benzoate	10	11.3±0.53*
Estrus 2:00 PM	Progosterone	6	10.5±0.34

*$P < .01$ compared to rats treated with Trilafon.
**$P < .01$ compared to rats treated with Reserpine.
†NBTL = Nembutal.

ment of polycystic ovaries during treatment periods with resumption of normal estrous cycles following cessation of the treatment (Knudsen *et al* 1975, Ward *et al* 1978). The effects of a single injection of androgen in the adult female rat were comparable with the PVC and polycystic ovaries that appeared in normally differentiated adult female rats following anterior deafferentation, preoptic area lesions, or exposure to constant light (Gorski *et al* 1975). Each of these manipulations probably interfered with the cyclic release of luteinizing hormone from the pituitary gland.

Androgen treatment in immature and adult rats contrasts significantly with similar treatments in neonatal rats. In the latter case, permanent sterility or delayed anovulation occurs and permanent changes are hypothesized to occur in the preoptic-medial basal hypothalamus region of the brain, while pituitary and ovary tended to remain normal (Barraclough 1961, Barraclough 1973, Quinn 1966). In the present study, brain mechanisms may have been altered temporarily by a single injection of testosterone propionate because there was a return to normal estrous cycles following the PVC phase.

The duration of pseudopregnancy in the rat is approximately 12–14 days (Everett 1961). In my studies, the lengths of pseudopregnancy following 5 mg or 10 mg of TP fell within this range, taking into account variations seen within groups (table 4). Moreover, these pseudopregnancies are comparable to those resulting from cervical stimula-

tion (14.3 ± 0.4 days; 13.5 ± 0.3 days) and sterile mating (14.5 ± 0.3 days) (DeGreef and Zeilmaker 1974, Kisch 1971). Reserpine treatment (0.5mg/kg) induced pseudopregnancies with average lengths of 12.6 days (Kisch 1971). That true pseudopregnancies were induced in the rat by the testosterone propionate treatment was confirmed by the ability to induce positive decidual reactions in these animals. These studies clearly indicate that abnormal levels of testosterone propionate administered to normally differentiated young adult female rats induce a temporary condition of persistent vaginal cornification and polycystic ovaries with a phase of true pseudopregnancy preceding the onset of persistent vaginal cornification.

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