grew more slowly, the mean latency period was about twice as long (7-8 weeks as compared to 4 weeks), and about 41-60% of the S-D rats developed palpable tumors as compared to 100% in Fischer rats. Neither estradiol nor PMS and HCG altered tumor incidence in the S-D rats. The MtT.F_4 readily transplanted from S-D to S-D rats. As in Fischer rats, large amounts of prolactin, growth hormone and ACTH were secreted by the MtT.F_4 in S-D rats, as indicated by intense mammary stimulation; enlargement of the liver, kidneys and spleen; and a 4-6-fold increase in adrenal size. Unlike the Fischer rat, stomach ulcers were found in most Sprague-Dawley rats with successful tumor transplants.

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Sexual Receptivity and Fertility of Female Rats that are in Androgen Induced Persistent Vaginal Estrus. (31059)

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It has been amply demonstrated that neonatal androgen treatment of female rats will cause persistent vaginal estrus*(1). Limited evidence is also available to show that, depending upon the dose, some androgen-treated female rats will copulate but with unreliable frequency. Furthermore, these females which do mate continue to exhibit persistent vaginal cornification, and autopsy shows they have not ovulated(2). Daily cervical stimulation does not induce pseudopregnancy in androgen-sterilized rats(3), even though, as Everett(4) reported, persistent vaginal cornification is interrupted and ovulation takes place in spontaneous persistent estrous rats which copulate.

The work reported here was undertaken to establish if a small dose of androgen given to neonatal rats would produce sterile females with nymphomania.

Materials and methods. One hundred and fifty female Spartan (Sprague-Dawley strain) rats were injected s.c. with 10 μg of testosterone propionate (TP) in 0.05 ml of peanut oil on the 5th day after birth (day of birth called day 1). These females were weaned at 21 days, placed 5 to a cage and maintained at 72° ± 2°F with 14 hours of daily artificial light. Untreated virgin females 70 to 120 days old constituted the control group. Rats taken from these two groups were used in 4 experiments.

Experiment I. Vaginal smears were taken by lavage from 15 TP-treated rats when they were 62 days old and continued daily for 11 days. The smears were examined for types of cells and for the cell type which was present in the largest quantity. Four females, which had cornified cells for the first 6 successive days, were then placed overnight with adult males. Exposed females were autopsied 10 days post-exposure and number of concepti counted.

II. In 25 additional TP-treated rats, vaginal smears were taken for 14 days beginning on day 87. Vaginal smears were handled as described in Exp. I. Starting on the evening of the 100th day, each female was caged overnight with a different male for each of 10

* Persistent vaginal estrus is a condition characterized by the continuous presence of a predominance of cornified cells in the vagina.

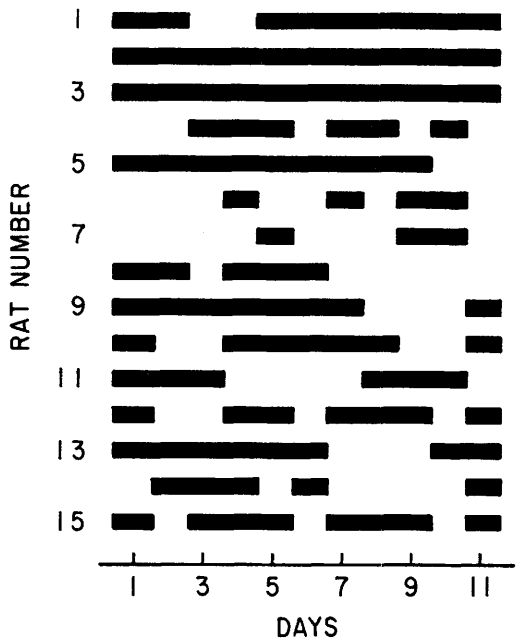

FIG. 1. Measurement of vaginal cornification as an indicator of persistent estrus in androgen-treated rats starting when 62 days old (day 1 in Fig.). Solid bars = predominantly cornified cells.

consecutive nights. The males were divided into 3 groups of 25 animals and each group of males was used every 3rd night. Females were examined each morning for vaginal plugs, presence of sperm in the vagina, and types of vaginal cells. Those which became pregnant were either autopsied later in pregnancy or allowed to go to term. Females with young were checked for persistent vaginal estrus after weaning. The remaining females were checked for vaginal cornification 29 days after their last exposure to males.

III. Twenty-five TP-treated females were selected from the stock group when 101 days old because each had a cornified vaginal smear. They were placed individually with mature males overnight once weekly for 4 weeks. To establish if the females had copulated, they were checked for vaginal plugs and sperm in the vagina.

IV. Varying numbers of virgin post-puberal untreated rats were caged overnight with males once weekly for 11 weeks. These females were caged 1 or 2 per male without determining the stages of their estrous cycles. Verification of mating was determined as described above.

Results. Fig. 1 shows that most of the TP-treated females were not in persistent vaginal estrus from the 62nd to the 72nd days of life. The rats in prolonged vaginal estrus had smears which contained a mixture of cell types, of which the cornified cells predominated. Of the 4 females exposed to males (rat Nos. 3, 5, 9 and 13, Fig. 1) after having cornified cells for 6 days, female No. 5 mated and upon autopsy had 16 implantation sites.

The frequency in which cornified cells predominated vaginal smears from 25 TP-treated females starting on day 87 is given in Fig. 2. For the 14-day period in which vaginal smears were taken, 12 of the TP-treated females had only cornified cells in their smears. Seven additional females also had cornified cells each day but on a few days they had nucleated epithelial cells or leukocytes mixed with the cornified cells. The remaining female rats had predominantly cornified cells but there were days when only leukocytes or nucleated epithelial cells were present. As an example, one female had 9 days of vaginal smears which contained only cornified cells and then 5 days of primarily leukocytic cells (rat No. 40, Fig. 2).

When TP-treated females were 100 days of age (the 14th day of vaginal smearing), 22 of the 25 were in vaginal estrus as determined by the presence of cornified cells. The remaining 3 rats had vaginal smears containing only leukocytic cells. All 25 females were placed individually with adult males on the evening of this day; 22 of the females mated, of which 21 had cornified cells in the vagina. The act of mating was apparently sufficient stimulus to change the vaginal mucosa as the mated females had mostly leukocytes in their smears by 2 days post-mating. Ten females mated more than once during the 10-day period with 1 female mating 6 and another mating 7 times (Fig. 2). The presence of cornified cells was not necessary for mating as the female that copulated 7 out of 10 nights had vaginal smears which contained mostly leukocytes. Six females (rat Nos. 17, 19, 25, 30, 35 and 36, Fig. 2) that mated on the 1st night of exposure and later had bloody vagi-

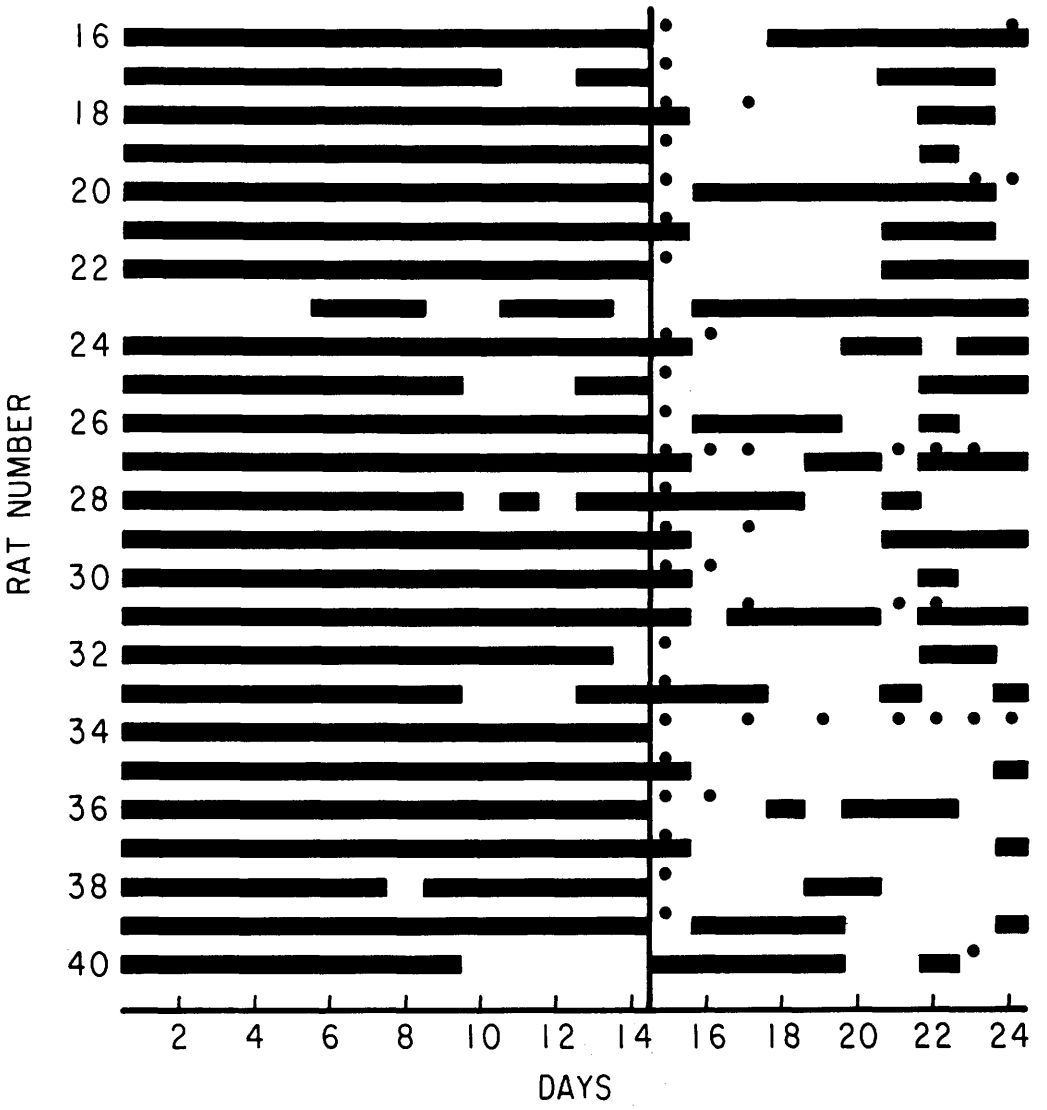

FIG. 2. Persistent vaginal cornification and sexual receptivity of TP-treated rats. Vaginal smears began on day 87 (day 1 in Fig.) of life and daily exposure to males 14 days later. Solid bars = predominantly cornified cells in smear. Circles above bar = days which females mated. Vertical line = beginning of exposure of females to males.

nal smears were autopsied for presence of concepti 17 days post-mating. Five of the 6 were pregnant with an average of 5.6 implantation sites; however, fetuses from 4 of the 5 females would not have gone to term. All placentae were present but the fetuses had undergone resorption(5). The remaining pregnant female had 2 implantation sites, one of which looked normal. An additional 5 females (Nos. 22, 24, 26, 28 and 33, Fig. 2) that did not have bloody smears were found to be pregnant and delivered at term. These females had an average of 8.8 young born, all of which were alive and normal appearing. The mothers were not in persistent vaginal estrus at weaning but all had regained this condition 20 days later. Interestingly, none of the TP-treated females which became pregnant mated after the 2nd exposure. Thirteen of the 14 non-pregnant females had predominantly cornified cells in the vaginal smears 29 days after the last exposure to males.

TABLE I. Sexual Receptivity of TP-Treated Female Rats Exposed to Adult Males Once Weekly for 4 Weeks.

Rat No.	Weeks 1	2	3	4
41	+	—	—	—*
	+	—	—	—*
43	+	+	+	+
	—	+	+	—
45	—	—	+	—
	+	—	—	—*
47	—	+	—	—
	+	—	—	—
49	—	—	+	—
	—	—	—	—
51	—	—	—	—
	+	—	—	—
53	+	—	—	—*
	—	+	—	—*
55	—	—	—	+
	—	—	+	—
57	—	—	+	—
	+	—	—	—
59	+	—	—	—
	—	+	+	+
61	—	+	—	—*
	+	—	—	—
63	—	+	+	—
	+	—	—	—*
65	+	+	—	+

+ Mated. — Did not mate. * Pregnant.

The number of TP-treated females which mated decreased after the 1st week of a 4-week, once weekly exposure to adult male rats (Table I). Twelve of the 25 females were sexually receptive to the males the 1st week while only 4 out of the same 25 mated the 4th week. It should be noted that 7 of the rats were definitely pregnant at the end of the 3rd week. All but 2 of the females mated at least once, with one mating 4 times and 2 females mating 3 times.

The expected percentage of virgin untreated female rats that will mate when placed once overnight with adult males was determined by putting an average of 186 females with males each week for an 11-week period. These females were in all stages of the estrous cycle. Out of the 2,042 females exposed to males, 367 or 18.0 per cent mated.

Discussion. Vaginal smears initiated at 62 days of age to TP-treated females showed that during the period through the 72nd day, these females had a modified vaginal cycle. It appeared that these females were undergoing several modified cycles prior to establishing a more constant vaginal estrous pattern. A trend in this direction is substantiated by noting that these females tended to have cornified cells in their smears for much longer durations than normal rats with a 4- to 5-day cycle length.

By day 100 a persistent vaginal estrus had been established in most of the TP-treated rats. Barraclough and Gorski(2) state that persistent estrus is indicative of sterility, and 10 μg of TP was the minimal effective dose which produced sterility in 70% of their animals. These authors also report bizarre patterns of mating behavior in the androgen-treated females which remained anovulatory and continued to exhibit vaginal cornification. Our work confirms the bizarre patterns of mating behavior, but is in disagreement with their findings on the continuation of vaginal estrus and sterility of the mated females. We found that the act of mating provided sufficient stimulus to change the persistent cornification pattern and bring about a somewhat modified estrous cycle. As to sterility, our data show that approximately one-half (46%) of the females (Exp. II) conceived upon mating, but of those that became pregnant about one-third would not have gone to term. Females which mated but did not become pregnant also had an interruption of their persistent cornification, although this condition returned upon separation from the males. These data suggest that the act of mating may trigger the release of factors from the hypothalamus which may or may not cause ovulation but does bring about a change in the vaginal mucosa. Previous work has implied that androgen treatment to prepuberal female rats brings about changes in the hypothalamus(6). Other workers report that small doses of TP do not prevent mating and pregnancy early in the post-puberal phase but later the rats become anovulatory while sexual receptivity is maintained(7).

Approximately 1 out of 5 (18.0%) of the untreated normal female rats mated when placed overnight with males during an 11-week period. There were over 2,000 females used in unknown stages of their estrous cycles, but the number which mated is the expected ratio for rats with a 4- or 5-day

cycle(8). In contrast to this, the TP-treated females which were exposed once weekly to males for a 4-week period had a much higher mating percentage for the first 3 weeks. The fact that some of the females became pregnant lowered the number which were sexually receptive during the last week. Pregnancy and unreliable mating behavior are two deterrents against the use of TP-treated females for mating on a once weekly schedule.

Summary. Female rats treated with 10 μg of testosterone propionate (TP) on the 5th day of life had modified estrous cycles through day 72. Alteration of the cycle was predominantly shown by an increased frequency of cornified cells. By day 100 over 80% of the TP-treated females were in persistent vaginal estrus. Placing these females with males for 10 consecutive nights resulted in a sporadic pattern of sexual receptivity. Copulation caused a temporary break in the persistent vaginal cornification. Some of the rats became pregnant, but a high percentage of the pregnancies were terminated by resorption of fetuses. Those females which went to term gave birth to normal appearing young; none of the pregnant females mated after the second exposure. It is concluded that 10 μg of TP given on day 5 will not provide a reliable source of sterile female rats with nymphomania between the ages of 70 and 130 days.

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Iron and Transferrin Distribution and Turnover in Iron-Overloaded Rabbits.* (31060)

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The plasma concentration of transferrin as measured by the total iron binding capacity (TIBC) is found to change in many conditions in man and laboratory animals. In man it rises during pregnancy and in iron deficiency, and it commonly falls in diseases associated with iron overload or with decreased plasma albumin levels. A decrease has also been found in iron-overloaded rabbits(1). Such results, plus the demonstration that transferrin may be bound to the surface of erythropoietic cells(3), have suggested that such binding may occur at the surface of iron-loaded cells of the reticuloendothelial system, and may hence lead to the decreased plasma concentration.

The aim of the present work was to investigate this possibility using iron-overloaded rabbits and radioiodine-labeled purified rabbit transferrin. At the same time the opportunity was taken of determining the effects of iron loading on plasma iron turnover and distribution to the organs of the rabbit, fecal excretion of the injected radioiron, and the presence of any histological evidence of tissue damage due to the excess iron.

Materials and methods. Six female New Zealand rabbits were used. Three of them

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