SPECIFICITY OF ESTRADIOL AND TESTOSTERONE PREPARATIONS IN THE DIFFERENTIATION OF SEXUAL FUNCTION IN RATS

by

Harvey H. Feder, B.S.

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[redacted]

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INTRODUCTION

Within adult nonprimate mammals the relationship between feminine behavior of females and the ovarian hormones is relatively specific. Among the substances which the adult ovary is capable of synthesizing are various androgens(87,97,104,105). However, estrogens, acting in synergy with progesterone in some species, are the best substitutes for the endogenous secretions when the ovaries are removed(1,20,28,29, 62,85,89) or are in a quiescent state(76,80). A similar type of specificity exists between the masculine behavior of males and testosterone. Despite the occurrence of estrogens in mammalian males (135), estradiol and estrone are ineffective in restoring male sexual behavior after castration (2). Testosterone,however, restores the behavior of adult castrated males to levels characteristic of the intact condition (54, 108).

The problem of hormonal specificity has been discussed by investigators of genital tract morphology of the adult (25,84,93) as well as those interested in behavior (17,131,132) . For the adult, results of studies on neural tissues mediating reproductive behavior and those of the genital tract do not differ greatly, although consideration must be given to the important limitation for morphology that specificity is dependent upon the particular organ (2,25,93). Those doubting that the relationship between the hormones and reproductive behavior of adults is specific (12,33,72) point to the facts that heterotypic behavior is seen in animals receiving hormones commonly regarded as homologous

(11,15,34) and that homotypic behavior occurs in animals receiving hormones regarded as heterologous (4,5,13,14,15). Closer investigation of these experiments reveals, however, that the responses were weak, incomplete and difficult to elicit (131). Thus, within a sex, considerable specificity is exhibited by behavioral substrates to hormones in adulthood. Earliest workers, with one exception (39), did not take the sex of an animal into account but only the nature of the hormone given in their consideration of the problem of specificity (74,78,82,98,106, 107). Their assumption that ovarian hormones stimulated the display of feminine behavior equally well in adult males and females, and that testicular hormones elicited the masculine response equally well in both sexes, is no longer adequate. It is now apparent that the ease with which feminine copulatory behavior can be elicited by estrogen depends on whether the subject is male or female (45,83). Similarly, males display more complete masculine behavior in response to testosterone than females (90). Thus, the central and peripheral structures mediating mating behavior of the adult differ in responsiveness to hormones in males and females.

The question of what factors influence the differentiation of the relevant structures into male or female type is linked to the question of whether hormonal specificity demonstrable for adults may exist also for fetal and neonatal organisms. This is so because sexual differentiation of central and peripheral tissues regulating mating behavior appears to be a hormone-dependent process. Thus, organization of a male-type organism by testosterone administered to females during a

critical period of fetal life has been demonstrated in guinea pigs using the criteria of suppression of feminine behavior, enhancement of masculine behavior and disruption of ovarian cyclicity in adulthood (26,27,43,90,115). Similar data available for rats indicate that there is a suppression of feminine behavior in females given adequate doses of testosterone during the critical period (9,67,125,129). Moreover, ovarian cyclicity is disrupted in intact female rats given testosterone (7,21,31,63,75,81,96,100,102,110,111) or testicular grafts (88) and in mice given testosterone (6) during this period. The problem of hormonal influence on the differentiation of the ovulatory mechanism has been reviewed recently (19,35,59,113). Among the purposes of the present experiments are: (a) to quantify more precisely the suppression of feminine behavior in rats which is attributable to neonatally administered testosterone and to assess the relationship between genetic sex and age with this suppression; and (b) to determine whether the addition of exogenous testosterone to intact neonatal males might lead to effects inconsistent with conceptualization of testosterone as a factor in organizing a male-type organism.

Estradiol has been said to potentiate development of a female-type organism when given to neonatal male rats; masculine responses are suppressed (60,77,121), feminine responses supposedly enhanced (73,121), and ovulatory capacity increased (60) in adulthood. The last-mentioned claim has been withdrawn recently (59). Paradoxically, more information suggests that genotypic female rats are "masculinized" by the injection of estrogens during early development; feminine behavior (77,122,123,124)

and ovulation (22,40,56,68,86,112,116,117) are suppressed whereas masculine responses may be enhanced; the evidence being scanty for the last-mentioned effect (73,77). Among the other aims of the present experiments are: (a) to quantify more precisely the extent of suppression of homotypic behavior in intact rats of both sexes given estradiol neonatally and to assess the factor of age in relation to severity of suppression; and (b) to determine whether estradiol enhances femininity in genetic males while suppressing it in genetic females.

Stated generally, the purpose was to determine whether testosterone and estradiol act in the neonatal rat as "male" and "female" hormones respectively with as extensive specificity as they do in the adult. Because experiments on intact animals had parallel designs, and because the work was done on a single colony of animals during a relatively short period of time, direct comparisons between the effects of testosterone and estradiol on the neonate become possible for the first time.

The dosages used were, when the work began, lower than those in previous investigations dealing with postnatal hormone treatment and reproductive behavior.[1]

[1] Sections of the thesis were published or are in press.

METHODS

General Procedures

Subjects and Rearing Conditions

Holtzman albino rats were used in all experiments. They were bred at the Endocrine Laboratory at the University of Kansas and at the Oregon Regional Primate Research Center and were raised under a partially reversed light cycle (12 hours light, 12 hours darkness) and under moderately well controlled conditions of temperature (70-76°).

Hormones and Injections

Name	Abbreviation
(a) Testosterone Propionate[2]	TP
(b) Estradiol Dipropionate[2]	ED
(c) Progesterone	P

All injections were made subcutaneously. The vehicle for (a) and (b) was sesame oil and for (c) peanut oil.

Gonadectomies

Castrations of neonatal males were carried out by making two ventral incisions and drawing out the testes and adnexa with fine forceps while the animal was anesthetized by cooling with ice. The incisions were closed by a drop of collodion.

Gonadectomies of adults were carried out while animals were under ether anesthesia. Incisions were closed with wound clips which were removed prior to testing.

[2] Perandren and Ovocylin; supplied by courtesy of Ciba Inc., Summit, N. J. The Ovocylin was a form estradiol-17β.

Histology

Gonads were fixed in Bouin's solution or 10% formalin, cut at 10 μ and stained with hematoxylin and eosin.

Statistical Procedures

A two-tailed Mann-Whitney U-test was used to test for statistical significance unless otherwise noted. Probability values less than or equal to .05 were considered significant.

Behavioral Terms

The three components of male sexual behavior which were studied are defined as follows:

(a) Mounting with pelvic thrust -- male mounts female from rear and then performs one or more pelvic thrusts with palpation of the flanks. There is no penile insertion.

(b) Intromission -- same pattern as (a) except that penile insertion is achieved and dismounting is characterized by a rapid backward lunge.

(c) Ejaculation -- male deposits a seminal plug (usually, but not always, within the vagina) and dismounts slowly, with forepaws raised.

Feminine receptivity was assessed by using the lordosis quotient. This is the number of lordosis responses divided by the number of mounts (when number of mounts > 1) made by the stimulus males. This ratio is then multiplied by 100 and expressed in terms of per cent. No distinction was made between "normal" and "forced" lordoses (123).

The lordosis response was also elicited manually by lightly placing the tip of the thumb between the scapulae while vigorously stroking the region at the base of the tail with the second and third fingers (45).

Experiment 1 -- Treatment of Intact Males with TP and ED -- Effects on Masculine Behavior.

Fifty intact male rats were divided into seven groups. Animals in groups I, II and III were given a single injection of 1.25 mg. TP. Group I was injected at 2 days (N = 5), group II at 5 days (N = 7) and group III at 20 days (N = 7). Animals in groups IV, V and VI were given a single injection of 100 μg. ED. Group IV was injected at 2 days (N = 7), group V at 5 days (N = 8) and group VI at 20 days (N = 7). Group VII consisted of untreated males (N = 9). Animals were weaned at 27 days of age and placed in colony cages. Behavioral tests were started at 95 to 125 days of age.

Each test consisted of placing a male in a chamber having a one-way mirror at its front surface. The male was allowed 3 minutes in the chamber alone, then an intact, estrous female was introduced. A test lasted 10 minutes or until ejaculation occurred. Sexual activity was assessed by recording the rates of display of mounting and intromission patterns. For example, if a male ejaculated at the end of 5 minutes, and had performed 6 mounts up to that time, his mounting rate for a 10 minute interval would be 12. The percent of these tests in which the ejaculatory response occurred was also recorded. Seven tests were given to each animal on seven consecutive nights.

When testing was completed representative animals from each group (except group III) were castrated and the testes prepared for histological study. Macroscopic observations of penile structure were made just prior to castration.

Experiment 2 -- Treatment of Intact Females with TP and ED -- Effects on Feminine Behavior.

Fifty-three intact female rats were divided into seven groups. Animals in groups I, II and III were given a single injection of 1.25 mg. TP. Group I was injected at 2 days (N = 8), group II at 5 days (N = 8) and group III at 20 days (N = 9). Animals in groups IV, V and VI were given a single injection of 100 µg. ED. Group IV was injected at 2 days (N = 6), group V at 5 days (N = 6) and group VI at 20 days (N = 8). Group VII consisted of uninjected females (N = 8). All animals were weaned at 25 days of age, checked for rupture of the vaginal membrane (except group VI) and then placed in colony cages.

Tests for feminine behavior began at 94 - 131 days and lasted for 15 consecutive nights. Each test consisted of placing a female with a stimulus male for 10 minutes (or until ejaculation occurred) in a chamber having a one-way mirror at its front surface. The stimulus males used were the most sexually vigorous available in the colony at the time of testing. They were sexually aroused by placing an estrous female in the chamber prior to actual testing. A mean lordosis quotient was calculated for experimental females by taking the scores from tests in which lordosis occurred. Those animals which never showed lordosis were assigned a score of 0. The mean lordosis quotients for each

animal were then added and a group mean calculated. Data on cyclicity and incidence of behavior commonly associated with estrus were also collected.

Observations on the time of vaginal opening were made. When behavioral testing was completed, representative animals from each group were ovariectomized and the ovaries prepared for histological examination.

Experiment 3 -- Treatment of Intact and Castrated Males with ED -- Effects on Feminine Behavior.

Thirty-six male rats were divided into four groups. Group I consisted of 8 rats castrated 16 to 32 hours after birth; group II of 9 rats castrated 16 to 30 hours after birth and given 100 µg. ED on day 5; group III of 9 rats sham-operated 16 to 30 hours after birth and given 100 µg. ED on day 5; and group IV of 8 rats sham-operated 16 to 30 hours after birth. Animals in groups III and IV were castrated when 60 days old.

Animals were weaned at 30 days of age, placed in colony cages until 60 days of age, and then placed in single-unit cages. Tests were begun when males were 80 - 95 days old. To induce estrous behavior each rat was injected with 4 µg. ED followed 40 hours later with 0.5 mg. P. One hour after P injection, each animal was "fingered" for the lordosis response and then placed with three stimulus males in their home cage for 3 minutes or until it had been mounted 10 times. These sessions with males were repeated every 2 hours until 13 hours had elapsed from the time of P injection. This entire procedure constituted one test.

Animals underwent three such tests at 2-week intervals.

Lordosis quotients were calculated for each test and for each animal by taking the mean scores attained in bi-hourly sessions in which lordosis occurred. The mean for the three tests is computed using 0 as the score when no lordosis was recorded for a test. The group mean was then calculated. Latency to lordosis from the time of P injection was calculated using 13 hours as the latency when no lordosis occurred. Data on responsiveness to fingering and incidence of ear-quivering and crouching activities were also collected.

Experiment 4 -- Treatment of Castrated Males with TP --
Effects on Feminine Behavior.

Ten male rats were castrated 22 - 27 hours after birth and given no hormone treatment (Group I). 12 males castrated at this time received injections of 0.25 mg. TP every other day for the first 19 days of life (Group II). Additionally, a group of males was castrated at 20 days of age and given no hormone treatment (N = 7, Group III).

Animals were weaned at 30 days, placed in colony cages until 60 days of age, and then placed in single-unit cages. Tests for feminine behavior began when animals were 84 to 113 days old. Each subject was injected with 20 µg. ED followed 40 hours later by 0.5 mg. P. Results of experiment 3 indicated that for castrated untreated males the latency to the strongest lordosis responses was 9 hours on the average. Accordingly, tests for lordosis in the present experiment were limited to the corresponding time period. Nine hours after P injection the

subjects were "fingered" to elicit the lordosis response and then placed with three males in their home cage and observed for 5 minutes. Lordosis quotients and incidence of other estrous behavior were recorded for the four tests given at weekly intervals.

RESULTS

Experiment 1.

Behavior

An age-dependent difference in the effects of testosterone and estradiol on male sexual behavior was found. Administration of 1.25 mg. TP at 2, 5 or 20 days of age did not significantly reduce the capacity of intact male rats to display any of the components of masculine behavior measured during adulthood (Table 1). In contrast, 100 μg. ED at 2 days produced significant decrements in all three behavioral components, and at 5 days caused significant deficits in intromission and ejaculation patterns in adulthood. Though not tabulated, the males given ED at 2 or 5 days frequently mounted the stimulus females from the front or side, whereas males in other groups did not display poorly oriented responses. No behavioral deficiencies were observed in adult males given the ED at 20 days (Table 1).

Morphology

Data on spermatogenesis and penile condition corresponded to the behavioral findings. Spermatogenesis appeared normal in the TP-treated animals examined. In contrast, ED treatment at 2 or 5 days caused varying degrees of suppression of spermatogenesis whereas treatment at

20 days had no effect (Figs. 1-4). Testicular descent occurred in untreated and 20-day ED-treated and all TP-treated males by the time of weaning (27 days) but was delayed until 38 to 69 days of age or more in males given ED at day 2 or 5. Abnormally small penes were noted only in males given ED at these ages and hypospadias occurred frequently in the former group (Table 2).

At the dosages used, the injection of TP into intact males had no deleterious effects on masculine behavior, spermatogenesis or penile size which would have been incompatible with conceptualization of TP as a "male hormone." On the other hand, ED given to intact males at 2 or 5 days "feminizes" the external genitalia and is incompatible with the complete performance of the male pattern.

Experiment 2.

Behavior

Unlike neonatal males, females were affected by both TP and ED administered at 2 or 5 days of age. Females given either hormone at these ages displayed the lordosis response only infrequently in adulthood and the results suggest that ED caused an even more severe inhibition of the capacity for the display of lordosis than TP, the difference being more noticeable in animals treated at 5 days than at 2 days (Table 3). Qualitatively, the lordoses exhibited by all of the above-mentioned females were usually weak, incomplete and of short duration. As in the case of males, females treated at 20 days of age with TP or ED displayed normal sexual behavior in adulthood.

Females given TP at 2 or 5 days were mounted about twice as often

as females given ED at these ages. This disparity could have been due to a) the greater number of times the TP-treated animals were at least somewhat "receptive", b) their seemingly more passive manner; the ED-treated females appeared to be hyperexcitable, often biting or attacking the male (recall that ED-treated males showed much misdirected activity), or c) a difference in the vigor of the stimulus males used for testing the treated females. Even if the last alternative were a factor, the number of times the ED-treated females were mounted (mean mounts per animal = 60/15 tests) was adequate for behavioral assessment, particularly in view of the fact that females treated at 20 days were not mounted more frequently (than females given ED at 2 or 5 days) but nevertheless had very high lordosis quotients (Table 3).

Reactions other than lordosis, such as crouching, darting and ear-quivering, were displayed rarely by females given TP or ED at 2 or 5 days, but were displayed in normal fashion by animals given these hormones at 20 days. In addition, cyclic periods of receptivity were not recorded for animals treated with either steroid within the first 5 days of life. Instead, these females were either totally refractory to mounting by the males or showed low levels of receptivity at aperiodic intervals. Cyclic patterns appeared normal in females given TP or ED at 20 days of age, but pregnancy often disrupted these patterns.

Morphology

The ovaries of females given TP or ED at 2 or 5 days were devoid of corpora lutea, indicating an anovulatory state (Figs. 5-8). Large

cysts were found on or near the ovaries of 4 of 6 females given ED at day 5. Others have noted cysts or abscesses not only in neonatally estrogen-treated females (22,56), but also in neonatal females given TP over an extended period (21,75,103).

The external genitalia were affected in different ways by the early treatments. Rupture of the vaginal membrane was precocious in all injected animals examined, confirming earlier reports (for older work see references cited in 25; for more recent work see 64,70). Females given ED at 2 or 5 days (20 day ED group not checked) or given TP at 5 or 20 days, showed vaginal canalization by the time of weaning (25 days). Those given TP at 2 days showed canalization at 33.7 days, whereas untreated animals opened at 40.6 days. The females given TP at 2 days (but not at 5 days) had vaginal orifices which were too small in adulthood for admission of a male's penis (see also 109) but the clitorides were not abnormally hypospadic. In contrast, females given ED at day 2 or 5 had adequate vaginal orifices, but the clitorides were abnormally hypospadic. Earlier workers demonstrated hypospadias in prenatally estrogen-treated female rats (52,55).

The results indicate that both hormones suppress feminine responses in adulthood, with ED being perhaps more damaging than TP. At the dosages used, ED cannot be considered a "feminizing" hormone in the neonatal female.

Experiment 3.

Consistent with previous findings (37,45,58), neonatal castration of male rats produced adults with increased female behavioral capacity.

When neonatally castrated rats were treated with estradiol in infancy, this feminine capacity was suppressed; lordoses were infrequent, the latency longer, and ear-quivering and related behaviors were not displayed. Moreover, administration of ED to neonatally intact male rats did not, as previously claimed (121), cause "feminization" (Table 4).

Qualitatively, untreated neonatally castrated males displayed a deep and prolonged arching of the back in response to mounting whereas neonatally ED injected subjects (intact or castrated) exhibited weaker and shorter lordoses.

Experiment 4.

As expected, neonatally castrated genetic males given TP in infancy had little capacity for feminine behavior in adulthood (Table 5). In this respect they resembled both normal males castrated at 20 days of age (Table 5) and genetic females given TP early in life (Table 3).

DISCUSSION

Hormones present during early mammalian development condition the neural and peripheral structures destined to mediate sexual behavior (for reviews see 30,59,133). Under physiological circumstances this conditioning or organizing process produces males capable of displaying masculine behavior and females capable of displaying feminine behavior in adulthood. The present experiments and related investigations by others have utilized techniques disruptive of the normal organization process. The results suggest that within each sex the hormonal status during a critical developmental stage largely determines whether the

gonadal hormones of the adult will activate behavioral responses characteristic of that sex.

Previous investigations have shown that behavioral capacity in rats is more susceptible to hormonal alteration during postnatal stages than during prenatal life (91,95,124,128). The present experiments support the view that maximal susceptibility to hormone treatment occurs during the first week of postnatal life in male as well as female rats. For those long gestation species studied, the period of maximal susceptibility is exclusively prenatal (43,90,133).

Testosterone administered to intact male rats during the first 5 days of life is compatible with the establishment of a substratum capable of mediating the full pattern of male behavior in adulthood (experiment 1). This finding is in agreement with work on the guinea pig (90) and with concurrent work from other laboratories (61,121). In only one study has neonatally administered testosterone suppressed masculine behavior in adulthood. Very high doses (36 mg) administered from birth until 28 days of age to intact male rats were maximally effective. The possibility that behavioral disruption was attributable to a severe suppression of testicular secretions in adulthood with resultant libidinal reduction rather than to damage of central neural or penile structures responsive to these secretions was not excluded (126). At the lower doses of testosterone used in other studies the suppression of endogenous androgen secretion in adulthood is probably less severe (64,70,109,130) or is negligible (23,46,99).

Estradiol, even when given to intact males at much lower doses than testosterone during the first 5 days of life, causes severe deficits in masculine patterns in adulthood. The damaging action of estradiol is attributable to an alteration of central (121) and/or peripheral (experiment 1) tissues mediating male behavior. In any event, it is reasonable to suppose that the estradiol interfered with endogenous testicular secretions thereby disrupting the normal process of organization. Animals incapable of responding with a display of homotypic behavior to testicular secretions or testosterone replacement therapy in adulthood were produced. The suggestion engendered by these results is that there must be adequate androgenic stimulation of males during an early critical period if the complete pattern of male behavior is to be elicited by androgens in adulthood. This receives support from investigations studying the problem with quite different techniques.

Depriving male rats of a source of testicular androgens by castration on the day of birth produces animals incapable of performing intromission and ejaculation patterns after being given androgen replacement therapy in adulthood. When the testes are left intact beyond the tenth day of life male behavioral capacity is not disrupted (18,45). Unpublished data from this laboratory indicate that testosterone adequately substitutes for testicular secretions since neonatally castrated male rats given TP from days 1 through 70 respond to testosterone treatment later in life with the display of the complete male copulatory response. Female guinea pigs (27,90) and possibly rats (60) given

testosterone during the organizational period exhibit enhanced masculine behavior in response to testosterone administered in adulthood, providing further evidence that the presence of this hormone early in development somehow establishes a male-like substratum (be it central or peripheral or a combination of both) in the adult. Testosterone elicits the display of male behavior in animals thus conditioned more readily than it does in animals of the same sex not exposed to androgen during fetal or prenatal life. Thus, androgens serve as "male hormones" in the neonatal male rat and their action is specific to the extent that estradiol cannot adequately substitute.

For the female the situation is entirely different. Unlike the male, gonadal secretions need not be present to condition the substrates destined to respond to ovarian secretions or estrogen and progesterone treatment in adulthood (16,127). On the contrary, estradiol administered during the period of organization alters these substrates in such a manner as to make them incapable of mediating female behavior either in response to endogenous ovarian secretions (experiment 2) or normally adequate replacement therapy (77,122,124). Moreover, this damaging effect of estradiol is not solely attributable to the high dosages used although it is undoubtedly influenced by this factor. Recent unpublished work in this laboratory has indicated that as low a dose as 5 μg. estradiol benzoate given to female rats in infancy causes irreversible non-receptivity.

The female also differs from the male in that both testosterone and estradiol administered during the organizational period produce females incapable of displaying homotypic behavior in response to endogenous gonadal secretions (experiment 2) or normally adequate replacement therapy. The irreversibility of the androgen induced non-receptivity evident in previous work by the author (36) and in independent work (44,125), indicates that behavioral disruption is not directly attributable to alterations in patterns of steroidogenesis in ovaries of treated animals (118).

Not investigated previously is the question of whether neonatally administered estradiol and testosterone might act differently on genotypic males and females in the suppression of feminine behavior. This was shown not to be the case. Injection of either estradiol or testosterone early in development suppressed feminine behavioral potential in neonatally castrated genetic males (experiments 3 and 4)(37,38) as it did in genetic females (experiment 2). Far from promoting the establishment of femininity in either sex neonatal estradiol was incompatible with the development of feminine characters in both sexes.

The present experiments and those of others indicate that the behavioral effects correspond with the morphological effects of early steroid treatment (experiments 1 and 2). Estrogen (3,69,71,79), but not androgen (10,70,126), severely disrupts spermatogenesis in neonatally treated males. Both classes of hormone, when given within the first week of life, alter the hypothalamic areas regulating ovulation in females (8,32,40,41,42,101,114).

Behavioral data also correspond with observations made by experimental embryologists (90). Genital tract structures have been shown to differentiate in a masculine direction under the influence of critically-timed testicular secretions, whereas differentiation in a feminine direction is dependent upon the absence of androgens rather than upon the presence of estrogens (for reviews see 24,66,94,119,129). Moreover, the nature of hormonal specificity is strikingly parallel for genital tract and behavioral differentiation. Testosterone simulates the action of the prenatal rat testis in causing development of the Wolffian ducts, prostate and phallus (47,48,49,50,57,120) and inhibiting the functional capacity (65,128,134) and in one instance, the morphological differentiation (53), of Müllerian duct derivatives. When testosterone is given to intact male rats during genital tract differentiation, there is no damage to the male components (52). In contrast with the consistent masculinizing action of testosterone in the fetus, estradiol has effects which have been referred to as paradoxical. It simulates some actions of the testis and testosterone when given to female rats during early development; Wolffian ducts are stimulated (51,52) and Müllerian ducts lose functional capacity (124). But estradiol administered to intact male rats prenatally causes retrogression of male genital tract components (52). Therefore, as is the case for behavior, both estradiol and testosterone alter female differentiation, whereas for the male only estradiol is disruptive of the organizational process.

It is concluded that steroids administered to the neonatal rat act by organizing the central and peripheral structures regulating mating

behavior. Gonadal hormones of adulthood activate patterns of behavior concordant with the organization established by the early hormones. For masculine sexual behavior and genital structures (18,45), adequate activation and maintenance in the adult male occurs only if functional testes were present during the organizational period. Correspondingly, normal activation of the reactions specific to the adult female occurs only in the absence of steroid-induced alterations of the differentiating genital tracts (65,124,129) and the developing substratum for behavior. Moreover, we suggest that although genetic factors may have an influence (92), the specificity manifested by adult males for androgen arises in part from the presence of androgenic steroids during a critical period of development. Administration of estrogen to genotypic males during early life interferes with specificity for androgen in adulthood. Females (or males castrated neonatally) not exposed to high titers of estrogen or androgen during the critical period develop a specificity for estrogen in adulthood. In this respect, specificity may be considered a sexually dimorphic characteristic, with its differentiation being similar to other such characteristics including behavior patterns, sexual accessory organs, and ovulatory capacity.

SUMMARY AND CONCLUSION

The expression of female behavior during adulthood was suppressed in female rats and neonatally castrated male rats treated during a critical period of early postnatal life with testosterone propionate or estradiol dipropionate. Those components of sexual behavior usually characteristic of the adult intact male, such as intromission and ejaculation, were severely inhibited in males receiving estradiol dipropionate within the first week of life. Neonatal treatment with testosterone propionate at corresponding ages did not disrupt the capacity for homotypic behavior when the males became adult.

Ovulation was suppressed in females receiving the estrogen or androgen in early postnatal life, whereas spermatogenesis was suppressed in males receiving the estrogen, but not the androgen, at corresponding periods.

The present experiments were interpreted in the context of related investigations into sexual differentiation. It was concluded that testosterone is compatible with the normal development of male sexual characteristics but that estradiol is not compatible with the development of female sexual characteristics when administered during a critical period of differentiation. The specificity for androgen in adult males is postulated to be partly due to the presence of androgens during the critical period. The specificity for estrogen shown by

females and neonatally castrated males in adulthood is thought to be dependent upon the absence of estrogen and androgen during a corresponding developmental stage. Not completely ruled out by the present study is the possibility that either or both of these gonadal steroids could be present during the critical period in concentrations too low to produce a measurable deficiency in adult sexual conduct. Nevertheless, the results suggest nothing that would contradict the interpretation that the hormonal specificity seen in adult males and females is a secondary sexual characteristic that is organized by the actions of early hormones.

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Table 1. Neonatal Androgen and Estrogen Administration and the Sexual Behavior of Intact Male Rats in Adulthood.

	1.25 mg TP			100 µg ED			Untreated
	Day 2 (Group I)	Day 5 (Group II)	Day 20 (Group III)	Day 2 (Group IV)	Day 5 (Group V)	Day 20 (Group VI)	- (Group VII)
N (Animals)	5	7	7	7	8	7	9
N (Tests)	35	49	49	49	56	49	63
Mounting With Thrusts (Mean Rate Per Test)	10.1	17.4	16.8	1.1*	12.1	16.4	13.6
Intromissions (Mean Rate Per Test)	8.2	7.7	10.0	0*a	0.1*a	7.0	7.6
% Tests With Ejaculation	51.4	34.7	57.1	0*	0*	51.0	47.6
% Subjects Ejaculating	80.0	85.7	100	0†	0†	100	77.8

[a] When rate of intromission was < 1 per test animal was assigned a score of 0. * Significantly different ($p < .05$) from untreated animals. † Significantly different from untreated animals by Fisher exact probability test ($p < .01$). There were no other statistically significant differences among groups.

Table 2. Glans Lengths and Incidence of Hypospadias in Adult Males Given Estrogen at Various Neonatal Ages.

Time of Estrogen Treatment (100 µg ED)	N	Mean Length of Glans (mm)	Range	% Hypospadic
2 days	7	4.1	(1-6)	85.7
5 days	8	6.4	(6-8)	0
20 days	5	8.4	(8-9)	0
Untreated	6	8.3	(8-9)	0

Table 3. Neonatal Androgen and Estrogen Administration and the Sexual Behavior of Intact Female Rats in Adulthood.

	1.25 mg TP Day 2 (Group I)	Day 5 (Group II)	Day 20 (Group III)	100 µg ED Day 2 (Group IV)	Day 5 (Group V)	Day 20 (Group VI)	Untreated (Group VII)
N (Animals)	8	8	9	6	6	8	8
N (Tests)	120	120	135	90	90	120	120
% Animals Showing Lordosis	62.5	87.5	100	33.3†	0†	100	100
% Tests With Lordosis	11.1	19.2	[a]	2.2△	0△	[a]	[a]
Mean Lordosis Quotients	8.6[b]*	18.0[c]*	87.3	7.3[d]*	0[e]*	86.1	80.2

† Significantly different from untreated females and females treated at 20 days by Fisher exact probability test ($p \leq .05$). △ Significantly different from females treated with TP at 2 or 5 days by χ^2 test ($p < .02$). * Significantly different from untreated females and females treated at 20 days ($p < .05$). [a] Not calculated since pregnancy and pseudopregnancy interfered with normal cyclicity. [b,c,d] Based on 20, 105, and 2 lordosis responses respectively. [b] and [c] did not differ significantly from each other. Comparisons with [d] and [e] were not made because of insufficient number of lordoses.

Table 4. Suppressive Effect of Neonatal Estrogen on Female Sexual Behavior of Male Rats Tested in Adulthood.

Group	Neonatal Treatment	N Animals	N Tests	Mean Latency to Lordosis Elicited by Males (hr)	Mean Latency to Lordosis Elicited by Fingering (hr)	Tests Positive for Crouching, Ear-Wiggling (%)	Mean Lordosis Quotient
I	Castrated + no hormone	8	24	6.1*	7.5*	70.8*	40.0*
II	Castrated + 100 μg ED	9	27	11.2	13	0	6.4
III	Non-castrated + 100 μg ED	9	27	9.9	13	0	14.0
IV	Non-castrated + no hormone	8	24	9.6	13	8.3	6.6

* Significantly different from all other groups ($p < .05$). Groups II, III and IV did not differ from one another in any measure taken.

Table 5. The Suppression of Feminine Behavioral Capacity in Neonatally Castrated Rats by TP Administration in Infancy.

Group	Neonatal Treatment	N (Animals)	N (Tests)	% Tests with Lordosis	Lordosis Quotient	% Animals Responding to Fingering
I	Castration at day 1 + no hormone	10	40	77.5†	45.9*	100*
II	Castration at day 1 + TP	12	48	16.7	16.5	0
III	Castration at day 20 + no hormone	7	28	14.3	18.3	0

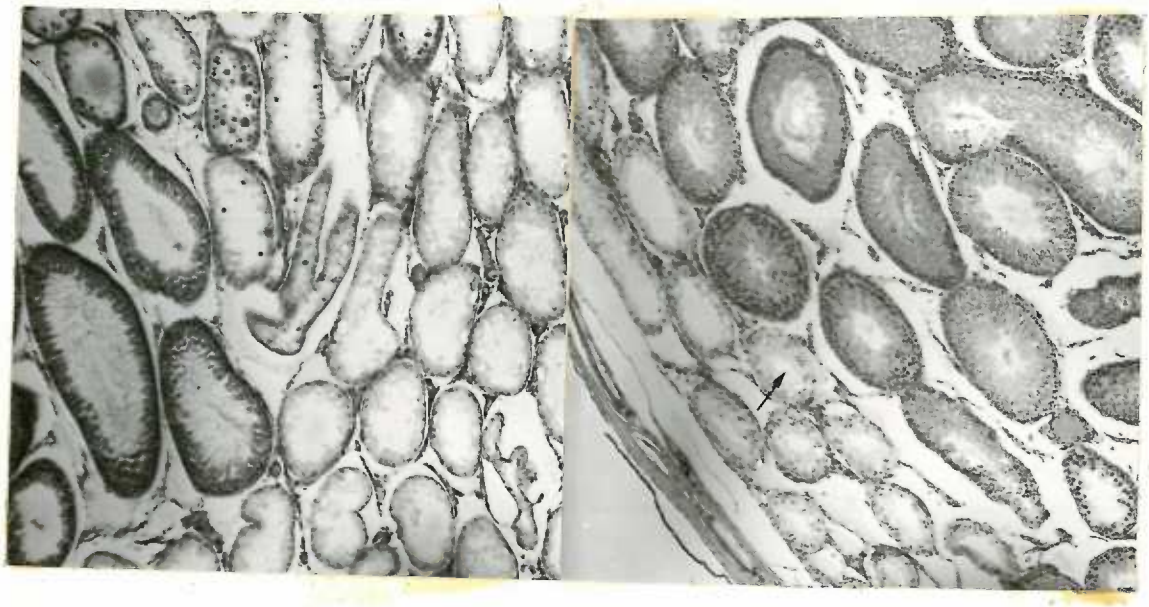
† Significantly different from groups II and III by χ^2 ($p < .001$). * Significantly different from groups II and III ($p \leq .05$).

Fig. 1. Testis from adult male given ED at 2 days of age (x51). Spermatogenesis is severely suppressed except in a few tubules which were located in the central portion of the testis. Of 400 tubules counted 34% (range 0-59) were of the completely degenerated type shown in the right side of the plate (100 tubules counted/animal).

Fig. 2. Testis from adult male given ED at 5 days of age (x51). Peripheral portion of testis is pictured, showing some tubules containing sperm and others (arrow) in which spermatogenesis is severely disrupted. Of 900 tubules counted 19% (range 0-100) were pathological in this treatment group (100 tubules counted/animal). Most of the damaged tubules were located peripherally.

Fig. 3. Testis from adult male given ED at 20 days of age (x51). Peripheral portion of testis is pictured, showing normal spermatogenesis. Six other males in this treatment group also had no impairment of spermatogenesis.

Fig. 4. Testis from adult male given TP at 2 days of age (x51). All seven animals given TP at 2 or 5 days showed no disruption of spermatogenesis. The severely abnormal tubules shown in Figs. 1 and 2 were not present.



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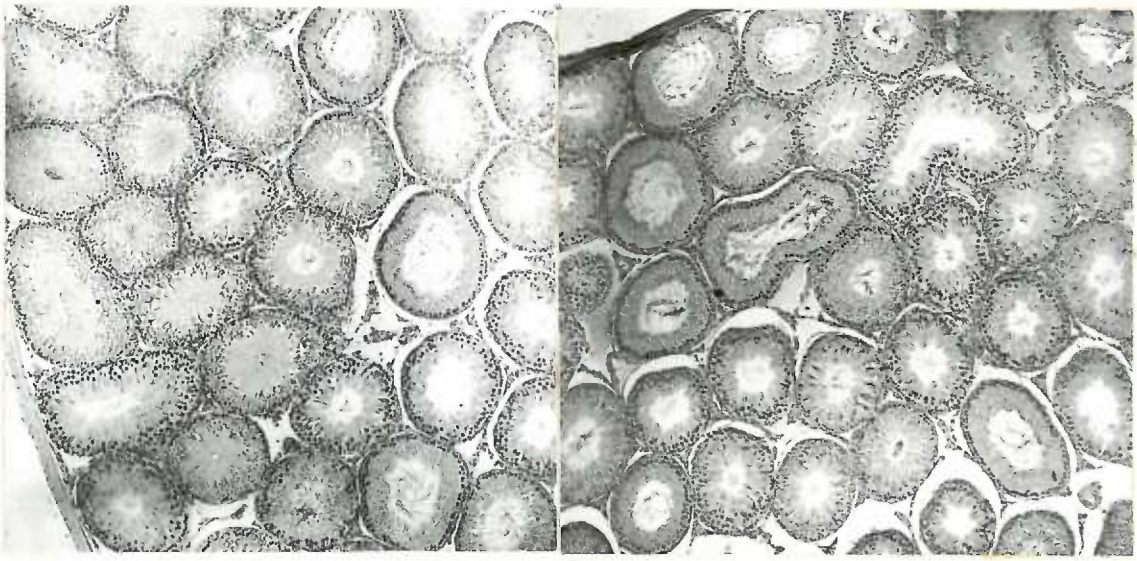

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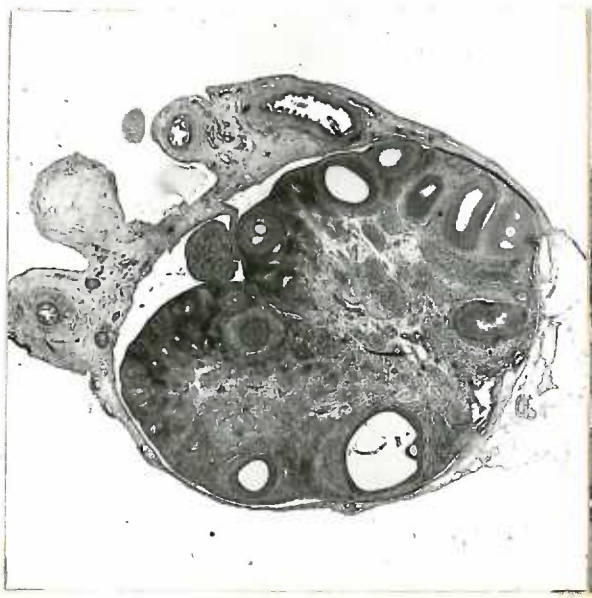
Fig. 5. Ovary from adult female given ED at 2 days (x16). Note follicular development and absence of corpora lutea. Ovarian sections from three other females given ED at 2 days and from six females given ED at 5 days were made. A corresponding suppression of ovulation was evident in all such specimens.

Fig. 6. Ovary from adult female given TP at 2 days (x16). Note large vesicular follicles and absence of corpora lutea. Sections from one other female given TP at 2 days and from eight females given TP at 5 days were made. Ovulation was inhibited by both treatments.

Fig. 7. Ovary from adult female given ED at 20 days (x15). Note extensive luteinization. Ovariectomy was performed on this animal during lactation following successful fertilization and normal parturition. Seven other females given ED at 20 days and three females given TP at 20 days also had large numbers of corpora.

Fig. 8. Ovary from untreated adult female (x14). Ovariectomy was performed three weeks after unsuccessful insemination by a stimulus male. Corpora were present in all eight control females observed.



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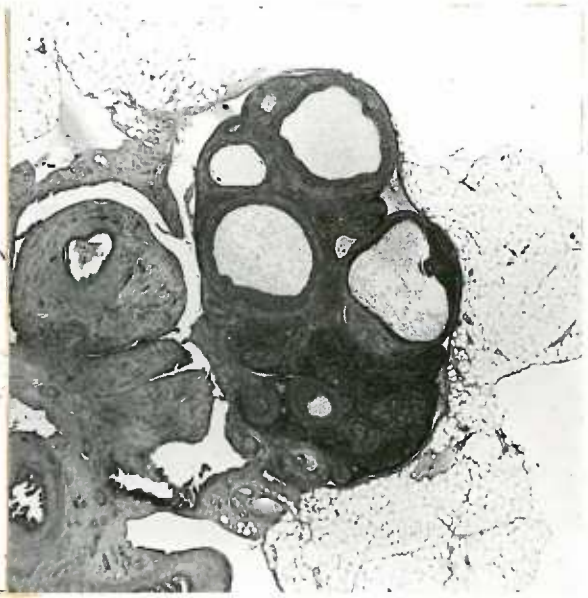

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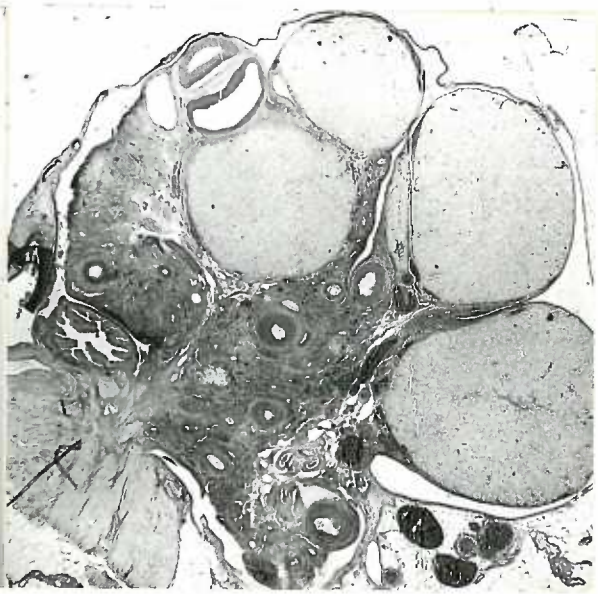

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