Daily injections of a high dose of testosterone propionate or a low dose of estradiol benzoate beginning after the tenth day of life facilitated precocious mating behavior in male rats; however, low doses of testosterone and a high dose of estradiol were not effective. These experiments specified a minimal dose of testosterone required to accelerate the first appearance of copulation in male rats and also showed for the first time that certain doses of estradiol may have similar, facilitatory effects on the development of mating behavior.

The age at which prepuberal male rats initially received daily injections of a low dose of estradiol did not influence the first occurrence of intromission responses. However, fewer males which had first received estradiol prior to Day 11 exhibited intromission during tests administered several weeks after estradiol injections were stopped. The results of two additional experiments indicated that daily injections of a low dose of estradiol beginning on Day 11 probably affected both the sensitivity setting as well as the activation of neural mechanisms which mediate mating behavior in male rats.
Baum: Hormones and the Development of Mating Behavior in Male Rats
HORMONES AND THE DEVELOPMENT OF MATING BEHAVIOR

IN MALE RATS

by

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Genetic differences between the sexes are transformed into anatomical and functional male or female traits by the action or inaction of gonadal hormones early in life. The presence of androgen in the male vertebrate during an early "organizational" period insures (a) the differentiation of the fetal Wolffian duct system into a mature male reproductive tract (reviewed by Burns, 1961), (b) the organization of neural mechanisms which maintain tonic secretion of pituitary gonadotropins (Harris, 1964; Harris & Levine, 1965), and (c) the organization of neural mechanisms which enable the adult to display normal male sexual behavior (reviewed by Valenstein, 1968; Whalen, 1968; Young, 1961; Young, Goy, & Phoenix, 1964). The absence of gonadal hormones during this organizational period in animals of either genetic sex results in the establishment of predominantly female traits. This feminization is characterized by (a) the differentiation of the Müllerian duct system into a female genital tract, (b) the permanent establishment of a cyclic pattern of gonadotropin secretion, and (c) an enhanced capacity to exhibit female mating behavior coupled with a decreased capacity to display masculine mating behavior in adulthood.

In the several mammalian species which have been studied the organizational period occurs some time just before or just
after birth. In animals with a comparatively long gestation (guinea pigs, primates) the organizational period is prenatal; in species with a shorter gestation (rats, mice) this critical period occurs during approximately the first 10 days of life (Phoenix, Goy, & Young, 1967). Once the organizational period in development has passed, the functional role of androgen in the male is to facilitate the growth and maintenance of the reproductive tract and to insure the "activation" of the previously organized patterns of mating behavior beginning at puberty (Young et al., 1964).

"Puberty" in the male is a rather vaguely defined period during development when several events occur which are instrumental in establishing the capacity to reproduce (reviewed by Donovan & Werff ten Bosch, 1965). Central to its occurrence are changes in the function of the brain-pituitary-gonad axis which induce the testes to increase their production of steroid hormones. These augmented concentrations of gonadal hormones (primarily androgen) in the male facilitate a final spurt in body and genital organ development. Also, adult mating behavior (i.e. intromission and ejaculation patterns) usually first appears in the male during this puberal period. The role of gonadal hormones in timing the initial activation of copulation in the male is of central concern in this paper.
This problem can perhaps best be approached in the context of the influence of sex hormones on several aspects of sexual differentiation and sexual maturation in the male. Experiments employing female subjects will be discussed when their results facilitate the understanding of a particular problem under consideration in the male.

**Psychosexual Differentiation**

The administration of large quantities of testosterone propionate (TP) to pregnant guinea pigs permanently masculinized the external genital structures (see review by Burns, 1961) and altered the adult mating capacity of female offspring (Gerall, 1966; Phoenix, Goy, Gerall, & Young, 1959). These androgenized females exhibited less receptive behavior in adulthood following injections of estrogen and progesterone and subsequently displayed increased mounting behavior following injections of TP. Similar results have been obtained in the rat (Gerall & Ward, 1966; Harris & Levine, 1965) and in the monkey (Phoenix et al., 1967). The results of a recent experiment (Whalen, Edwards, Luttge, & Robertson, 1969) employing female rats which received neonatal injections of androgen confirmed the earlier findings that in adulthood androgenized females exhibited fewer lordosis responses following injections of estrogen and progesterone. However, neither prenatal nor neonatal exposure to androgen
increased the frequency of mounts with pelvic thrusting in these females following injections of TP in adulthood. For this reason these investigators suggested that in the female rat early exposure to androgen suppresses the organization of the female neurobehavioral system but does not necessarily enhance the masculine control system.

The above observations on females led several investigators to speculate that the experimental removal of androgen during the early organizational period in the male should alter its mating performance in adulthood. Experiments which confirmed this expectation were followed by attempts to identify the particular gonadal hormones which are required for the organization of masculine mating behavior. The following three sections of this paper will consider these experiments along with reports concerned with the effects of administering gonadal hormones to intact males immediately after birth.

**Perinatal androgen deprivation.** Male rats which were castrated prior to the tenth day of life and subsequently administered TP in adulthood exhibited fewer intromissions and ejaculations than than males castrated after Day 10 (Beach & Holz, 1946; Grady, Phoenix, & Young, 1965; Larsson, 1967a). Hart (1968a) performed spinal transections on adult male rats castrated on Day 4 which had failed, and on adult males castrated
on Day 12 which had successfully ejaculated following injections of TP in adulthood. Day 12 castrates subsequently exhibited normal penile reflexes after receiving daily injections of TP; however, these reflexes were severely impaired in males which had been castrated on Day 4. Hart (1968a) concluded that neonatal testicular androgen exerts an organizational influence at the spinal level on neural tissue mediating sexual reflexes. The neonatal castration procedure employed in all of the above experiments may have impaired the capacity of adult male rats to ejaculate by disrupting the early organization of essential spinal reflex mechanisms. This and other interpretations of the effects of neonatal castration on male mating performance in adulthood will be considered further on following pages.

Perinatal androgen deprivation in the male rat also affects the display of receptive behavior in adulthood. Male rats castrated neonatally (Grady et al., 1965) as well as male rats which received an antiandrogenic substance (cyproterone acetate) beginning prenatally and continuing for three weeks after birth (Neumann & Elger, 1966) exhibited lordosis in adulthood following injections of ovarian hormones. Lordosis was rarely observed in untreated males or in males which were castrated after the tenth day of life. However, it should be noted that the quality of lordosis observed in male rats
castrated neonatally was somewhat below that seen in female littermates which were ovariectomized at birth (Gerall, Hendricks, Johnson, & Bounds, 1967). In a preliminary experiment performed by Zucker and Kuehn (cited in Phoenix et al., 1967) neonatal castration of male guinea pigs failed to influence adult responsiveness to either androgen or ovarian hormones—presumably because the organizational period for mating mechanisms occurs prenatally in this species. In another preliminary experiment (cited in Phoenix, Goy, & Resko, 1968) Goldfoot found that perinatal exposure of the male guinea pig fetus to large amounts of cyproterone acetate did prevent complete masculinization of the external genital organs and disrupted the mating performance of these males following injections of TP in adulthood. However, for some reason male guinea pigs which received cyproterone acetate failed to display normal lordosis when castrated and injected with estrogen and progesterone in adulthood.

Intromission and ejaculation responses following TP injections in adulthood have been restored in male rats castrated neonatally by a single injection of 2.5 mg. TP administered on the day subjects were castrated (Whalen & Edwards, 1967). Lower doses of TP given to neonatal castrates prior to the tenth day of life were somewhat less effective in restoring intromission capacity; however, castrated males receiving as little as 10 μg.
TP on Day 4 exhibited fewer lordosis responses in adulthood following injections of estrogen and progesterone than castrates which received no steroid hormones early in life (Mullins, Jr. & Levine, 1968; Whalen & Edwards, 1967).

Androgen administered on Days 13 and 14 to male rats castrated at birth failed (a) to restore intromission capacity in response to TP replacement injections and (b) to block the performance of receptive behavior following injections of ovarian hormones in adulthood (Beach, Noble, & Orndoff, 1969).

Two of the androgens produced by the fetal rat testis are testosterone and androstenedione (Noumura, Weisz, & Lloyd, 1966; Resko, Feder, & Goy, 1968). Androstenedione administered during the first 20 days of life to male rats which had been castrated at birth facilitated the display in adulthood of both lordosis and intromission responses following injections of female hormones and androgen, respectively (Goldfoot, Feder, & Goy, 1969). Since normal male rats rarely exhibit lordosis in adulthood, even after castration and priming with ovarian hormones, it is unlikely that androstenedione is the androgen responsible for psychosexual differentiation in the male rat. Testosterone or a similar steroid yet to be identified is a more likely candidate.

The process which enables an early injection of testosterone to restore adult intromission and ejaculation
capacity in the neonatal castrate has been a topic for speculation. Androgen may act directly on the neural structures which mediate mating behavior (Hart, 1968a; Phoenix et al., 1968), or early exposure to androgen may influence mating capacity in adulthood by insuring normal development of the penis (Beach & Holz, 1946; Whalen & Edwards, 1967; Whalen et al., 1969). Regardless of the sites of action, the fact remains that the early presence of androgen in the male insures that the organization of mechanisms responsible for mating will occur long before the time when these responses normally make their first appearance.

Like androgen, estrogen administered neonatally to male rats castrated at birth may restrict the display of receptive behavior in adulthood. As little as 10 μg. estradiol benzoate (EB) administered prior to Day 4 to male rats castrated at birth disrupted the exhibition of lordosis following injections of ovarian hormones in adulthood (Feder & Whalen, 1965; Mullins, Jr. & Levine, 1968; Whalen & Edwards, 1967). Mullins, Jr. and Levine (1968) also reported that a single injection of 10 μg. or 50 μg. (but not 500 μg.) EB administered on Day 4 to male rats castrated at birth facilitated more mounting behavior following TP injections in adulthood than equivalent neonatal injections of oil or 10 μg., 100 μg., or 1000 μg. TP. These investigators considered the possibility that "...the substance
responsible during infancy for the organization of neural mechanisms which mediate male behavior [in this instance mounting] is biochemically more similar to estrogen than to testosterone [p. 342]." Although low doses of estradiol administered to male rats castrated neonatally facilitated mounting behavior following injections of TP in adulthood, neither high nor low doses of estradiol restored the capacity of these animals to intromit and to ejaculate (Mullins, Jr. & Levine, 1968; Whalen & Edwards, 1967). The finding that only neonatal injections of very large doses of TP have succeeded in this respect (Whalen & Edwards, 1967) suggests that the organization of the complete adult mating pattern in the male rat relies more on the early presence of androgen than on estrogen.

**Neonatal testosterone injections.** Although the experimental evidence available does not support the proposition, it is conceivable that the administration of exogenous TP to intact males during the early organizational period might have summated with endogenous stores of androgen to produce heightened levels of mating performance in adulthood. In fact, a single injection of a large amount of TP into intact males shortly after birth failed to affect adult sexual performance in either guinea pigs (Phoenix et al., 1959) or rats (Feder, 1967; Harris & Levine, 1965; Whalen, 1964). Daily injections of large amounts
of TP administered to male rats from birth until 30 days of age severely disrupted mating performance in adulthood (Arai & Masuda, 1968; Wilson & Wilson, 1943); however, the latter effect was probably caused by a long-lasting reduction in the output of testicular androgen in these males (details in a later section). Perhaps injections of TP in adulthood would have restored mating performance to normal levels in these subjects.

**Neonatal estrogen injections.** The administration of estrogen to intact male rats prior to the tenth day of life affects subsequent adult copulatory behavior in much the same way as neonatal castration. An injection of as little as 20 μg. EB on Day 4 or 5 significantly reduced the number of intromissions performed by male rats in adulthood; males receiving neonatal injections of higher doses of estradiol rarely intromitted in adulthood even after numerous daily injections of TP (Harris & Levine, 1962; Harris & Levine, 1965; Levine & Mullins, Jr., 1964; Whalen, 1964). Neonatal injections of estrogen did not seem to affect the frequency of mounts exhibited by intact males in adulthood; however, estrogenized males often performed mounts that were inappropriately directed toward either the side or the head of the female (Levine & Mullins, Jr., 1964; Whalen, 1964). The age at which estrogen is first administered to male rats is an important variable. Whereas 100 μg. EB given
to males at two or five days of age restricted intromission behavior in adulthood, the same dose of estradiol administered to male littermates on Day 20 had no effect on subsequent mating performance (Feder, 1967).

Neonatal exposure to estrogen may reduce levels of mating performance in adulthood by directly damaging either the penis or neural tissues which underlie copulation. Alternatively, the behavioral effects of neonatal estrogen may be indirectly mediated by a reduction in the secretion of testicular androgen during the organizational period of development.

Genital Development

Neonatal castration in the male halts further growth and development of the penis and accessory reproductive organs (including the seminal vesicles, coagulating glands, and ventral prostate). Normal genital development may be restored in the castrate only if gonadal hormones are supplied within a short time after castration. The early administration of either androgen or estrogen to intact males may hasten or delay the development of the genital organs and spermatogenesis; the effects differ depending upon the types and doses of gonadal hormones which are employed as well as upon the ages when hormones are first administered. Some of the treatments employed in the present experiments were devised on the basis of the following knowledge about how exogenous gonadal hormones affect
genital development and spermatogenesis in the intact male.

**Early testosterone injections.** Neonatal administration of TP to males may temporarily retard the subsequent growth of the genital organs. Either single or daily injections of 500 µg. TP administered to intact male rats prior to Day 10 caused a suppression of testis and accessory organ weights in subjects autopsied up to 30 days after the final injection (Johnson, Yasuda, & Sridharan, 1964; Price & Ortiz, 1944; Swanson & Werff ten Bosch, 1965). Organ weights were not suppressed in animals killed at older ages and body weights were not affected at any age. Similar results have been obtained in male mice which received 1000 µg. TP on either Day 5 or Day 10 (Barraclough & Leatham, 1959). Although these combined results constitute only circumstantial evidence, they do suggest that neonatal exposure to TP may cause a temporary reduction in the testicular production of androgens in the developing male. The finding that 250 µg. TP administered to male rats on Day 5 had no effect on the amount of testosterone in blood plasma of subjects sacrificed at 240 days of age (Frick, Chang, & Kincl, 1969) supports the conclusion that neonatal TP has no permanent influence on testicular secretion of androgen. One recent report indicates that large doses of TP administered daily to intact male rats from birth until 30 days of age suppressed the growth of all genital organs for several months (Arai &
Masuda, 1968); however, normal organ sizes might have been observed in subjects had they been autopsied at even older ages (Green, Burrill, & Ivy, 1940). Most investigators attribute the temporary suppression of genital growth to a passing inhibitory influence exerted by exogenous TP on the production or release of gonadotropins from the anterior pituitary (Davidson, 1966b).

No hormonal intervention of any kind (including injections of pituitary gonadotropins) has successfully hastened the initial appearance of mature spermatozoa in the seminiferous tubules (Critchlow & Bar-Sela, 1967); however, a large dose (1000 µg.) of TP administered daily to male rats from 15 until 24 days of age did stimulate the appearance of a larger quantity of spermatogonia and spermatocytes in all stages of the spermatogenic cycle (Y. Clermont, personal communication, 1968). This effect was probably caused by direct androgenic stimulation of the seminiferous epithelium of the testes. Still larger doses of TP administered to male rats for the first 30 days of life had caused complete disruption of all stages of spermatogenesis when subjects were autopsied at 120 days of age (Arai & Masuda, 1968). However, these results do not rule out the possibility that spermatogenesis might have been restored in subjects had they been autopsied at even later ages. As with the development of the genital organs, the inhibition of spermatogenesis following
prolonged exposure to large doses of TP was probably mediated via the brain-pituitary-gonad axis which controls the secretion of gonadotropins.

**Early estrogen injections.** Neonatal administration of estrogen inhibits the growth and function of the reproductive organs in males of several different species (reviewed by Albert, 1961). As little as 30 μg. EB injected into male rats any time during the first 10 days of life severely retarded development of the testes and accessory organs as well as body growth (Kincl, Pi, & Lasso, 1963; Kincl, Pi, Maqueo, Lasso, Oriol, & Dorfman, 1965; Whalen, 1964). Whereas equal doses of androgen had no lasting effect on testicular function, estrogenized males exhibited a reduced concentration of plasma testosterone many months after a single neonatal injection (Frick et al., 1969). Daily injections of small doses of estrogen begun after the tenth day of life also inhibited genital and body development (Arai, 1964).

The same doses of estradiol which retard the development of the testes and accessory organs in the male rat are also able to inhibit the production of spermatozoa; as little as 30 μg. EB injected into male rats on Day 5 has been proven effective in this respect (Harris & Levine, 1962; Kincl et al., 1963; Kincl et al., 1965). Higher doses of estradiol
administered over longer periods during development caused severe damage to the germinal epithelium of the seminiferous tubules (Steinberger & Duckett, 1967) and have curtailed the production of spermatozoa for as long as one year (Arai, 1964).

Estrogen may retard the production of both testosterone and spermatozoa by acting directly on the cells of the testes, or its influence may be mediated via the brain-pituitary-gonad axis. The finding that injections of pregnant mare's serum (a gonadotropic hormone) reversed the inhibitory influence of neonatal exposure to estradiol on genital development and spermatogenesis (Kinc1, Maqueo, & Pi, 1964) suggests that estrogen does not affect the testes directly but instead inhibits the secretion of pituitary gonadotropins. This conclusion has been strengthened by the results of a recent experiment employing direct assays of pituitary gonadotropin concentrations. Schiavi (1968) found that the release of gonadotropins from the anterior pituitary in adult male rats can be inhibited by exposure to 100 μg. EB on the fifth day of life. The notion that prepuberal exposure to either estrogen or androgen exerts an inhibitory influence on the function of the brain-pituitary-gonad axis will be examined more carefully in the following section.
Maturation of the Brain-Pituitary-Gonad Axis

The secretion of the male gonadotropins, follicle-stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH), by the anterior pituitary are under the direct control of cells in the median eminence of the hypothalamus. These hypothalamic cells are presumably neurosecretory cells whose biochemical products (releasing factors) enter capillaries in the hypothalamus and are carried to the anterior pituitary by portal veins where they influence the synthesis and release of gonadotropins (reviewed by Turner, 1966). The hypothalamic control system in both sexes is originally organized so that after puberty pituitary gonadotropins will be secreted in a cyclic fashion (Harris, 1964). However, the presence of androgen in the male during an early critical period in development somehow alters the functioning of this hypothalamic control mechanism so that a tonic pattern of gonadotropin secretion is permanently established (Gorski & Wagner, 1965; Harris & Levine, 1965). It is probably no coincidence that in the male the critical periods for differentiation of the neural structures controlling mating and gonadotropin secretion overlap.

The production and storage of gonadotropins by the male pituitary gradually increases during the weeks following birth (Davidson, 1966b). At puberty large quantities of FSH and
ICSH are released into the blood and subsequently stimulate interstitial cell growth and the production of steroid hormones (primarily androgens) by the testes; androgens in turn facilitate a final surge in body and genital organ growth and also allow the adult copulation patterns to make their initial appearance in response to sexual stimuli. Obviously, the same neuroendocrine mechanisms which are responsible for the puberal release of gonadotropins are also ultimately in control of the amount of androgen available to activate the neuromuscular mechanisms which mediate mating. For this reason the factors which control the timing of the puberal surge in plasma gonadotropin concentration also indirectly influence the development of sexual behavior.

**Puberty: Negative feedback from gonadal hormones.**
Several experiments described earlier in this paper indicated that, depending upon the doses used and the length of treatment, injections of either androgen or estrogen to young males can retard genital development and spermatogenesis. These results suggested that exogenous steroid hormones exert an inhibitory influence on the hypothalamic cells which ultimately control the secretion of gonadotropins. It is reasonable to expect that, as in the case of injected hormones, small amounts of steroid hormones secreted endogenously by the prepuberal testes may have similar negative feedback effects on the production
and release of gonadotropins.

Donovan and Werff ten Bosch (1959) have suggested that the primary difference between the function of the brain-pituitary-gonad axis of the infant and the adult (male or female) lies in the level at which this servomechanism is set. In infants small amounts of gonadal hormones are sufficient to inhibit any appreciable release of gonadotropin; they suggest, however, that at puberty the "gonadostat" is set at a higher level. Somehow the sensitivity of hypothalamic cells to the negative feedback influence of estrogen or androgen is lowered and the puberal release of gonadotropins from the anterior pituitary occurs. The gonads subsequently respond by greatly increasing their secretion of gonadal hormones. Eventually sufficient blood concentrations of estrogen or androgen are established to regain control of the brain-pituitary secretion of FSH and ICSH. At this point the animal has passed through puberty and the brain-pituitary-gonad axis proceeds to function using the higher concentrations of gonadal and gonadotropic hormones which are characteristic of the adult (Werff ten Bosch, 1969). Although the above theory was originally based almost without exception upon results of experiments which employed female subjects (reviewed by Critchlow & Bar Sela, 1967; Donovan & Werff ten Bosch, 1965), several recent findings suggest that it may also apply to males.
The fetal and neonatal testes of rats do release detectable quantities of androgen into the blood (Resko et al., 1968); the question is whether such low titers of endogenous androgen can account for the prepuberal inhibition of pituitary gonadotropin secretion. The finding that three or four times as much exogenous TP was required to suppress plasma ICSH levels in castrated adults as in prepuberally castrated male rats (Ramirez & McCann, 1965) suggests that even small amounts of androgen may successfully inhibit the secretion of gonadotropins in the prepuberal male. In fact, the action of endogenous androgens just after birth may also increase the sensitivity of the hypothalamic cells which control gonadotropin secretion thereby augmenting the subsequent negative feedback influence of these same hormones (Morrison & Johnson, 1966). Direct implantation of TP crystals into the median eminence of the hypothalamus in male rats at 30 days of age, but not at later postpuberal ages, had caused a suppression in testis and accessory organ weights by the time subjects were autopsied 20 days later (Smith & Davidson, 1967). Pituitary FSH content was also reduced by prepuberal TP implants; however, spermatogenesis was not affected. Whereas TP implanted into the median eminence prepuberally apparently inhibited the secretion of gonadotropins, implanting cyproterone acetate (antiandrogen)
into the same structure in male rats on Day 30 resulted in a precocious increase in testis and accessory organ weights in comparison with males which received implants of cholesterol at the same age (Bloch & Davidson, 1967). Thus the puberal increase in the secretion of pituitary gonadotropins was apparently delayed when testosterone was introduced into the median eminence of the hypothalamus and was hastened when androgens were prevented from interacting with cells in this same brain region. These results suggest that endogenous gonadal hormones are probably responsible for delaying the puberal surge of gonadotropin secretion in young male rats. The next problem is to specify mechanisms which eventually neutralize the negative feedback effects of these quantities of steroid hormones so that puberty can occur.

**Puberty: Positive feedback from estrogen.** Several experiments performed with female rats may shed some light on the mechanism which initiates the puberal release of pituitary gonadotropins in the male. Apparently, brief exposure to small doses of estrogen causes precocious sexual development in female rats. Ramirez and Sawyer (1965) reported that daily subcutaneous injections of .05 µg. EB per 100 g. body weight beginning at 26 days of age stimulated precocious vaginal opening and ovulation as well as an early drop in the
pituitary content of luteinizing hormone (LH). Three independent experiments have subsequently demonstrated that exposure for several days to estradiol implanted directly into the hypothalamus of female rats during the fourth week of life also caused precociously puberty, as indicated by the same three events (above). Estrogen implants which caused precocious puberty were located in the preoptic region (Smith & Davidson, 1968), in the median eminence (Motta, Fraschini, Giuliani, & Martini, 1968), and in the ventromedial and arcuate regions of the hypothalamus (Kannwischer, Wagner, & Critchlow, 1967). Estrogen implants which were located in other parts of the brain or which were placed in the above hypothalamic locations several days prior to Day 26 failed to stimulate precocious puberty and often caused a delay in both initial ovulation and the puberal release of pituitary LH.

Corbin and Daniels (1969) have recently found that daily subcutaneous injections of .05 μg. EB per 100 g. body weight administered to female rats from Day 26 until vaginal opening hastened the puberal release of pituitary FSH into the blood by approximately one week when compared with other females receiving injections of oil, TP, or progesterone. The conclusion most readily drawn from all of the above experiments is that in addition to its widely recognized negative feedback influence on gonadotropin secretion in females of all ages,
endogenous estrogens produced by the maturing ovaries or adrenals facilitate the puberal release of large quantities of both LH and FSH.

The inhibitory influence of androgen and estrogen on the function of the brain-pituitary-gonad axis in the prepuberal male has already been reviewed (for a more complete discussion see Davidson, 1966b). Although other experimental results indicate that small doses of estrogen may facilitate gonadotropin secretion in the prepuberal female, analogous facilitatory effects have not been found in prepuberal males receiving either large or small amounts of androgen (Davidson, 1967). Experiments designed to evaluate the effects of small doses of estrogen on sexual development and maturation of the brain-pituitary-gonad axis in the prepuberal male have not yet been reported. Considering the findings in the female, it seems important to determine whether different doses of estrogen administered to the prepuberal male may exert facilitatory as well as inhibitory effects on various facets of sexual maturation including the development of mating behavior.

The Development of Mating Behavior in Males

Long before reaching puberty males of several mammalian species exhibit mounting, mounting with pelvic movements, and other signs of sexual attraction toward their peers. These preliminary responses do not rely on testicular androgens for
their occurrence; males castrated at birth display such behavior as readily as intact animals (Beach, 1965). Adult mating patterns are first exhibited toward a female at a particular time during development probably as a result of two hypothetical events: (a) an increase in the sensitivity of the neuromuscular mechanisms which underlie copulation to the activating effects of circulating androgen, and (b) a dramatic increase in the production and release of androgen by the testes. Experimental evidence relating to both of these postulated factors will be considered in the remainder of this introduction.

Behavioral reactivity to androgen in adulthood.

Experiments employing subjects of various infrahuman species as well as observations of human patients suggest that individual males require only some minimal concentration of circulating androgen to maintain a normal responsiveness to sexual stimuli. Injection of higher concentrations of androgen seldom causes any appreciable increase in sexual responsiveness or any improvement of mating performance. In this sense the reactivity to androgen of these neuromuscular mechanisms underlying mating behavior is somehow permanently established in adulthood. The first solid support for this proposition was provided from experiments performed on male guinea pigs.

Adult guinea pigs displayed many individual differences with respect to both the probability that intromissions would
occur in a test session and the number of mounts and interruptions performed during a test (Grunt & Young, 1953). Sexual performance in each subject declined after castration; however, TP replacement injections were able to restore sexual performance only to the level originally observed for each individual prior to castration. Sexual performance in individual males was not improved by injections of up to four times the dosage of TP required to restore performance to the precastration level.

The reactivity to androgen of the neural tissues which mediate mating behavior in a particular male guinea pig is probably determined by a combination of factors including inheritance, prenatal exposure to gonadal hormones, and early social experience (Valenstein, Riss, & Young, 1955). Regardless of how this sensitivity is established, androgen administered in excess of this threshold activational dose does not alter the quality or quantity of mating behavior exhibited by the adult male guinea pig (Young et al., 1964).

The neural tissues mediating mating behavior in the adult male rat responded to suprathreshold doses of TP in much the same way as those in the male guinea pig (Beach & Fowler, 1959; Larsson, 1966). However, in the male rat androgens administered to adult castrates at doses above those required to restore sexual performance to precastration levels stimulated more ejaculations during a test session as well as a more rapid
recovery of sexual responsiveness following each ejaculation (Beach & Holz-Tucker, 1949). Although some males have reportedly become more interested in females afterwards, those rats which consistently failed to exhibit intromission and ejaculation responses during successive tests could not be induced to improve their performance by injections of large amounts of androgen (Stone, 1938; Whalen, Beach, & Kuehn, 1961). Similar findings have been reported in male mice (Champlin, Blight, & McGill, 1963) and in male cats (Rosenblatt, 1965). Finally, an analogous phenomenon apparently exists in men castrated in adulthood. Beach (1965) suggested that the efficacy of administering replacement doses of androgen in order to restore sexual potency in patients depends primarily upon the "adequacy" of each man's sexual performance prior to castration. Testosterone injections are usually unable to improve sexual performance in adult men who were sexually inadequate prior to castration.

**Puberal changes in behavioral reactivity to androgen.**

The results of experiments performed on female rats and guinea pigs suggested to Beach (1948) that behavioral responsiveness to gonadal hormones is low in the neonate but increases as the animal approaches puberty. Large doses of estrogen and progesterone administered to either female rats or guinea pigs on the seventh day of life failed to facilitate lordosis
responses; by 30 days, however, females of both species responded to these hormones as readily as at later ages (Wilson & Young, 1941). In a related experiment (Goy, Phoenix, & Meidinger, 1967) female guinea pigs exhibited lordosis responses on the day of birth without injections of ovarian hormones; they were ovariectomized immediately following the test and were subsequently tested at older ages to determine when receptivity could first be elicited after priming with estrogen and progesterone. All of the females showed receptivity at 21 days of age, and the readiness with which lordosis could be elicited after an injection of progesterone continued to increase up to the ninetieth day of life. Beach (1967) suggested that the "lordosis" response observed in the female guinea pig on the day of birth is not a sexual response at all, but is instead part of an infantile excretory pattern whose activation is independent of ovarian hormones. However, the other results of this experiment confirmed the original suggestion that the reactivity to gonadal hormones of neuromuscular mechanisms which mediate mating behavior tends to increase as animals grow older.

Very few experiments have tested the validity of this hypothesis in the male (Beach, 1948; 1965). In one series of experiments which is relevant Larsson (1967b) studied the decline in the number of intromissions required to achieve ejaculation which normally occurs in male rats during the weeks
following initial copulation. These results indicated that neither physical maturation nor copulatory experience was responsible for the change in the mating pattern. It is apparently caused instead by testicular secretions which produce increased sensitivity to gonadal hormones in the tissues which mediate mating behavior. Larsson's (1967b) results gave no direct indication that androgen had altered tissue sensitivity to gonadal hormones prior to the time when mating actually first appeared. However, the finding that androgen may influence tissue sensitivity during the puberal period in the male does suggest that similar effects may occur prior to puberty.

Precocious activation of mating behavior. Many attempts have been made to induce precocious mating in males of various species either by injecting extracts from the anterior pituitary in an effort to stimulate the endogenous production of androgen or by injecting TP directly into animals. Several of these experiments were performed during the 1930's and 1940's to demonstrate that the capacity for mating existed long before it was normally activated at puberty (reviewed by Beach, 1948). However, in addition to successfully establishing this fact, the results of these and later experiments also suggest that the amount of circulating androgen is a key limiting factor in timing the development of mating behavior in normal males.
Behavioral precocity has been experimentally induced in males of many different vertebrate species. An anterior pituitary extract administered to immature lizards caused precocious copulation (Evans, 1935), and implanting TP pellets under the skin of either intact or castrated chameleons produced complete male behavior at an early age (Noble & Greenberg, 1940). Injections of TP promoted precocious exhibition of courtship and mating patterns in guppies (Eversole, 1941). Injections of pituitary extract or TP into newborn male chicks have produced crowing as early as Day 4 and treading at 13 days of age (Domm & Van Dyke, 1932; Hamilton, 1938; Noble & Zitrin, 1942); these responses appeared at an age of 3-4 months in untreated males. Similar results have been obtained by administering TP to male turkeys (Schein & Hale, 1959). Treading was observed in two and three week old male chicks following implantation of TP into the preoptic area of the hypothalamus (Experiment by A. E. Fisher & J. E. Gardner cited in Fisher, 1966). Injections of TP induced precocious male behavior in herons of both sexes; estrogen injections produced no precocity whatsoever (Noble & Wurm, 1940). Injections of TP directly into incubating gull eggs followed by more TP injections into newly hatched males stimulated the growth of adult plumage as early as the twenty-seventh day of life and adult voice by 45 days; estrogen failed to produce these effects (Boss & Witschi, 1942).
Stone (1924) first systematically observed the simultaneous development of mating behavior, spermatogenesis, and genital organ growth in a single group of male rats. These 56 males first intromitted at a median age of 47 days (Range: 37-72) and spermatozoa were always plentiful in the external ducts after an age of 50 days. One early report claimed to have hastened the appearance of "sexual excitement" in male rats by injecting pituitary extracts (Steinach & Kun, 1940). However, in a recent unpublished experiment performed in this laboratory H. Szechtman has found that 18 daily injections of ICSH (12 Rat Units) or FSH (1 Rat Unit) administered to male rats beginning on the twenty-fourth day of life affected neither the development of intromission and ejaculation responses nor the amount of mounting behavior (an index of "sexual excitement") exhibited prior to the first intromission. Of course it is possible that larger doses of gonadotropin might have affected the development of mating.

Stone (1940) first reported that daily injections of 620 µg. TP to intact male rats beginning at 22-26 days of age produced precocious appearance of intromission patterns. With daily mating tests beginning when subjects were 29 days old, males receiving TP first intromitted at a median age of 35 days (Range: 29-78) while uninjected littermate controls intromitted at a median age of 54 days (Range: 35-77). Unfortunately
no males in this experiment received blank injections of the oil vehicle to control for the possibility that precocious mating was caused by the stress of early injections.

In a subsequent follow-up experiment four male rats received 1000 µg. TP each day beginning at 14 days of age and two littermates received daily injections of an equivalent volume of oil (Beach, 1942b). Behavioral tests were begun when males were 15 to 16 days old and continued on alternate days until all subjects receiving TP had intromitted. All six subjects exhibited mounts with pelvic thrusting prior to an age of 23 days; however, only the four males receiving TP intromitted with females (Median age of 24 days; Range: 21-29) and two of these subjects subsequently ejaculated at 27 and 29 days of age, respectively. The degree of precocity in these subjects cannot be assessed because mating tests were stopped before any oil control subjects had intromitted. Although a single experiment employing both the appropriate oil control group and a sufficient sample of prepuberal males has not been reported, the combined results of the experiments by Stone (1940) and Beach (1942b) suggest that large doses of androgen may cause a precocious activation of intromission and ejaculation patterns in male rats. Since this problem has not been pursued in recent years, it is not known whether the neuromuscular mechanisms which mediate mating in the male rat can be precociously activated by lower
doses of exogenous androgen.

Early attempts to induce precocious copulation in either castrated or intact male guinea pigs by daily administration of 500 µg. TP per 100 g. body weight from birth were unsuccessful (Gerall, 1958; Riss, Valenstein, Sinks, & Young, 1955). Mating tests in both experiments were given approximately once each week beginning when subjects were 12-15 days of age. Subsequently, in an experiment where males were tested every 2-3 days beginning at 9-12 days of age, precocious intromission and ejaculation patterns were observed in guinea pigs which received the same dose of TP used in the earlier experiments (Gerall, 1963). Subjects which received TP prenatally as well as after birth were no more precocious than subjects which received only postnatal injections of androgen.

No reports concerning the influence of androgen on the development of mating behavior in the male monkey have yet appeared. However, injection of anterior pituitary extract (Thompson & Heckel, 1938), chorionic gonadotropin, or TP (Gordon & Fields, 1942) to prepuberal boys suffering from hypogenitalism or cryptorchidism (undescended testes) caused precocious physical development along with priapism, the appearance of seminal emissions, and, reportedly, an increased interest in sex. Many clinical reports of complete physical development, including production of spermatozoa, due to
cerebral tumors or other unspecified causes are available for boys as young as 3-4 years of age (reviewed in Dorfman & Shipley, 1956; Federman, 1967). A concurrent development of strong sexual interests or activity has not been reported in these patients; however, attitude and behavior changes of this nature are rather difficult to assess. Also, social sanctions would undoubtedly tend to suppress the open expression of sexual interest in such young boys.

The previous experimental results and observations indicate that precocious mating behavior can be produced in either intact or castrated males of many species by the injection of sufficiently large doses of androgen. However, none of these experiments included prolonged observations of mating behavior to discover whether sexual responses persisted in males after the initial precocious activation of copulation and the subsequent cessation of androgen injections. Presumably the titers of androgen in the blood would decline during the days following a final injection of TP. The persistence of mating, if observed, would suggest that the sensitivity of the neural mechanisms which mediate mating to the activational influence of gonadal hormones increases after the first appearance of sexual behavior.

The Present Investigation

The present experiments investigated the role of gonadal hormones in timing the initial activation of mating behavior in
male rats. Experiments 1, 2, and 3 explored the effects of injecting various doses of testosterone and estradiol on the development of mating in intact males. Although many attempts have been made to stimulate precocious mating in males by injecting large doses of TP, no experimental treatments (other than castration) which delay the development of copulation have been devised. Since early exposure of prepuberal males to low doses of TP apparently suppresses the puberal release of gonadotropins and subsequent increases in the secretion of testicular androgen, injections of small quantities of TP might also be expected to delay the initial activation of mating. Estrogen is even more potent than androgen in inhibiting genital organ development and spermatogenesis. However, smaller doses of estradiol than those hitherto administered to prepuberal males induce precocious sexual maturation in female rats. Depending upon the doses employed, estradiol might either hasten or delay the first appearance of mating behavior.

The mechanisms which underlie the influence exerted by estrogen on the development of mating were examined in Experiments 4, 5, and 6. The objective of all of the experiments reported below was to investigate the influence of gonadal hormones on the development of sexual behavior in male rats. Therefore, with the exception of Experiment 4, hormones were not administered
to subjects during the first ten days of life when they might possibly have affected the differentiation of neural or peripheral structures necessary for the very existence of mating in adulthood.
General Method

Subjects and Rearing Conditions

The subjects (Ss) used in all six experiments were male hooded rats born in the laboratory from multiparous, pregnant females obtained from the Quebec Breeding Farm. Newborn litters were trimmed to eight members, saving male pups and using female pups to complete litters with less than eight males. Most Ss used in these experiments grew up with at most seven littermates and (in isolated instances when deaths occurred) at least five littermates. All Ss lived under reversed diurnal lighting (light: 10 p.m. -- 10 a.m., dark: 10 a.m. -- 10 p.m.), were weaned from their mothers at 21 days, and were subsequently housed two males to one cage. Male pups from a particular litter were assigned to the different treatment conditions in an experiment so that each group was comprised of Ss from several different litters. Individual Ss were identified by an ear-punch (each S received at least one ear-punch) at the age when group assignments were first made. Subjects used in Experiment 1 were born in August, 1968; Ss in Experiment 2 were born in January, 1968; and Ss for the remaining four experiments were born in January, 1969.

Apparatus

Two identical semicylindrical boxes were placed 12 inches apart on a table so that two Ss could be tested simultaneously.
These observation boxes had a diameter of 18 inches, a height of 12 inches, and a rectangular Plexiglas front. A 15-watt incandescent lamp was positioned over each box so that Ss could not see the Experimenter. The testing room was darkened except for these lights, and extraneous sounds were masked by continuous white noise. Response frequency observations were recorded on electric counters and latency data were collected using stopwatches.

**Hormones and Injections**

Solutions of .05 µg. TP and 5 µg. TP in 0.1 ml. sesame oil were prepared using a Mettler microbalance to weigh appropriate quantities of powdered TP procured from the Mann Research Laboratories, Inc. Solutions of higher TP concentrations were prepared using Perandren (supplied by the courtesy of Ciba Co. Ltd.). Estrogen solutions were prepared by weighing appropriate quantities of 17β-Estradiol 3-Benzoate (EB) (Mann Research Laboratories) which were also dissolved in sesame oil. All injections were made subcutaneously with a one inch, 25-gauge needle. Hormones were administered at noon each day approximately 3 - 5 hours prior to any behavior tests. Leakage of the injected solution onto the skin was rarely observed.
Preparation of Stimulus Females

Stimulus females were littermates of males being tested in a particular experiment, thus stimulus females were approximately the same age and size as male $s$. A large colony of females was maintained for each experiment so that individual females were used as test stimuli approximately once a week. Behavioral receptivity (even in 20-day old animals which were the youngest females used) was induced by injections of 0.2 mg. Estradiol Dipropionate (Ciba: Di-Ovocylin) three days and one day prior to a behavior test along with 1.0 mg. of progesterone (Ciba: Lutocylin) approximately 5 hours prior to testing. At least eight females were prepared for any testing session, and all females were screened with an active male for full receptivity before being used as a mating stimulus. Individual stimulus females were used to test several males; however, a fresh female was substituted if the original female became sluggish. It was inevitable that some males occasionally had less "attractive" stimulus females. However, males from a particular treatment group were never exposed just to one stimulus female; thus it is very unlikely that over a period of weeks (tests being given on alternate days) the results of any experiment could have been influenced by uncontrolled variations in female attractiveness.
Gonadectomy

Males 11 days of age were castrated by making two ventral incisions and drawing out the testes with a forceps while the animal was under ether anesthesia. Incisions were closed by two sutures. Sham-operations involved all of the castration procedures except that the testes were not removed. Particular litters were entirely comprised of some pups which received sham-operations and others which were castrated.

Behavioral Terms

Several components of male rat sexual behavior were recorded during these experiments:

1. Mount -- The male mounts the female from the rear and elicits a lordosis response. Occasionally the male performs pelvic thrusts and palpates the female's flanks. This latter behavior pattern is labelled a mount with pelvic thrusting.

2. Intromission -- The male mounts the receptive female from the rear and performs one or more pelvic thrusts with penile insertion into the vagina followed by a rapid backward lunge away from the female. In Ss where hormone treatment retarded penile development intromission refers to the above pattern of response with the omission of penile insertion.
3. Intromission latency -- Intromission latency is the time elapsed from introduction of a receptive female into the test chamber until she first intromits.

4. Ejaculation -- An ejaculation follows several intromissions and usually involves expulsion of a vaginal plug from the penis followed by a slow dismounting with forepaws raised above the female. Since expulsion of a plug is not always observed, the term ejaculation is used in this paper to refer only to an intromission followed by a slow dismounting of the female.

5. Ejaculation latency -- The ejaculation latency is the time elapsed between an initial intromission and the subsequent ejaculation.

Statistical Procedures

All behavioral observations were subjected to statistical analysis using nonparametric procedures. Overall significance of the differences among groups was first assessed with the Kruskal-Wallis one-way analysis of variance by ranks, and subsequent comparisons between individual groups were made using Mann-Whitney U Tests (two-tailed unless otherwise indicated) only if the Kruskal-Wallis H value was significant at $p < .05$. The significance of the difference among proportions for independent groups was tested by a chi square test when there
were at least two degrees of freedom and by a Fisher Exact Probability Test when only one degree of freedom existed.

Providing that sample sizes were adequately large, organ weight data were analyzed with a parametric analysis of variance. Subsequent comparisons of group means were made using Scheffé's (1953) method providing the original value of $F$ was significant at $p < .05$. Nonparametric tests were applied to organ weights when sample sizes were very small. An overall test of significance on group differences in mean body weight change over days for each experiment was made using an analysis of variance with repeated measures. Subsequent multiple comparisons of Groups and Groups X Days interactions were performed using Scheffé Tests providing the original $F$ values were significant at $p < .05$.

Unless a specific note to the contrary occurs, the data from all members of each group were included in the statistical tests performed for every experiment.
Experiment 1

The Development of Copulation in Male Rats Followed Early Exposure to Testosterone or Estradiol

Experiment 1 was designed with several objectives in mind, the first one being to verify the effect of injecting high doses of TP on the development of mating behavior in young male rats (Beach, 1942b; Stone, 1940) while employing a suitable number of subjects and an appropriate oil control group in the same experiment. A second objective was to discover whether males which initially copulated at a precocious age would continue to intromit and ejaculate even after hormone injections were stopped. A final objective was to assess the effects of a low dose of estradiol on the development of mating.

Subjects. The subjects were 65 male hooded rats born in the laboratory from 19 multiparous females. Eleven days after birth male pups were assigned to one of six groups:

1. Oil (by weight) -- 0.1 ml. sesame oil per 100 g. body weight (B. W.).

2. 5 µg. EB -- 5 µg. EB in 0.1 ml. sesame oil per 100 g. B. W.

3. .05 µg. TP -- .05 µg. TP in 0.1 ml. sesame oil per 100 g. B. W.

4. 5 µg. TP -- 5 µg. TP in 0.1 ml. sesame oil per 100 g. B. W.

5. Oil (by volume) -- 0.2 ml. sesame oil.

6. 1000 µg. TP -- 1000 µg. TP in 0.2 ml. sesame oil.
Four males from each of 11 litters were placed in Groups 1 - 4. Three of these same litters plus eight additional litters contributed pairs of littermates to Groups 5 and 6. Thus each group was comprised of 11 Ss from 11 different litters (except Group 1 where one S died).

**Procedure.** Subcutaneous injection of hormone solution or oil vehicle was begun on Day 11 for Groups 1 - 4 (day of birth is Day 0). The particular injection schedule used by Beach (1942b) with injection beginning on Day 14 was repeated in Groups 5 and 6.

Each S received prepuberal tests in the apparatus on alternate days during the dark part of the light-dark cycle. The first test occurred when Ss were 16 to 17 days of age. During a test S was placed in the observation box and shortly thereafter was presented with a stimulus female for 10 minutes. Several variables were recorded for each male including the occurrence of mounts with pelvic thrusting prior to the test when intromission first appeared as well as the ages when intromission and ejaculation first occurred. If during a test S intromitted with the female, it was allowed an additional 45 minutes to ejaculate. In the event that ejaculation failed to occur in that time, S was subsequently tested on alternate days until he did ejaculate. Tests and daily hormone injections were stopped when a male either ejaculated or reached an age
 Subjects which failed to intromit or ejaculate were arbitrarily assigned age at first intromission and age at first ejaculation scores of 90 and were included in the analyses of these data.

Half of the Ss from each group was randomly chosen prior to the onset of testing to receive either seven mating tests or to be sacrificed and autopsied the day following initial ejaculation. Animals that failed to ejaculate before 90 to 91 days of age were excluded from either of these procedures. Mating tests were identical to prepuberal tests except that they were given on every fourth day. Although details of each S's copulatory pattern (intromission latency, number of intromissions to ejaculate, ejaculation latency) were recorded, only the occurrence or nonoccurrence of intromission and ejaculation during each of the tests is reported in this paper. During autopsy the entire carcass was weighed, and weights for two testes, two seminal vesicles plus coagulating glands (excised of fluid), and the penis were obtained using a Mettler microbalance. Attachment of the frenulum to the glans penis was noted whenever it occurred. A smear from the tail of the epididymis was inspected for presence of spermatozoa. All Ss were weighed daily starting at 11 days of age.

Subjects in the two oil control groups (Groups 1 and 5) were compared on all behavior as well as organ and body
weight observations. Since no statistically significant differences were found (employing two-tailed Mann-Whitney U Tests for all variables except body growth which was compared using an analysis of variance), Ss were pooled into a single oil control group (n = 21).

Results. Six of the eleven animals receiving 5 µg. EB displayed mounts with pelvic thrusting toward the female during prepuberal tests prior to the test when intromission patterns were first observed. Only one or two Ss in each of the other groups exhibited this kind of behavior ($\chi^2 = 10.59$, df = 4, p < .05).

Injections of 5 µg. EB or 1000 µg. TP hastened the initial appearance of intromission and ejaculation patterns (Table 1), although in 5 µg. EB Ss the penis was too under-developed to allow actual penetration into the vagina. The overall effect of the treatments on age at first intromission was statistically significant, and subsequent comparisons showed that both 5 µg. EB ($U = 45, p < .005$) and 1000 µg. TP ($U = 62, p < .03$) Ss differed from oil controls. The overall effect on age at first ejaculation was smaller, due in part to two 5 µg. EB Ss which failed to ejaculate after intromitting, but it was still statistically significant. Again, individual comparisons showed that both 5 µg. EB Ss ($U = 65.5, p < .05$)
and 1000 µg. TP Ss (U = 54, p < .02) ejaculated earlier than control Ss. Comparisons of age at first intromission scores (U = 48, p > .05) and age at first ejaculation scores (U = 58, p > .05) revealed no significant difference between 5 µg. EB and 1000 µg. TP Ss. Subjects receiving .05 µg. TP and 5 µg. TP did not differ from the oil control Ss on either of these age variables. The proportion of males either intromitting or ejaculating before 90 to 91 days of age was approximately the same for all groups.

There were no striking qualitative differences in the copulation responses of males in the various groups. The backward lunge part of intromission responses by all 5 µg. EB Ss occasionally appeared somewhat less vigorous than those observed in Ss from the other groups. However, the disoriented head and side mounting observed in some males receiving estradiol prior to the tenth day of life (Levine & Mullins, Jr., 1964; Whalen, 1964) was never observed in the present experiment.

Animals from each group continued to intromit and to ejaculate during the seven mating tests which followed initial ejaculation (Table 2). Neither the median number of tests in which individual Ss intromitted nor the median number of tests in which Ss ejaculated was significantly affected by the hormone treatments. Even so, a tendency did exist for Ss receiving
5 \mu g. EB to ejaculate during fewer mating tests than Ss in the other groups.

Data collected from Ss autopsied one day after initial ejaculation are shown in Table 3. No overall significant difference in the age of Ss at autopsy occurred. Body weights in 5 \mu g. EB Ss were significantly retarded in comparison with weights in oil control animals ($U = 0$, $p < .01$). The development of the genital organs in 5 \mu g. EB Ss was severely retarded, even when weights were computed in terms of mg. per 100 g. B. W.: The testes, accessory organs, and penis weights in these animals were without exception lighter than corresponding organs in any oil control male. Also, the frenulum was attached to the glans penis in all 5 \mu g. EB Ss confirming the fact that penile development was retarded in these males. Subjects receiving 1000 \mu g. TP had lighter testes ($U = 3$, $p < .03$) when compared with controls. However, 1000 \mu g. TP Ss had significantly heavier accessory organs ($U = 0$, $p = .004$) and heavier penes ($U = 3$, $p = .03$) than oil control animals. Organ weights of males in the two groups which received a lower dose of TP never differed from those of oil controls. Except for the three 5 \mu g. EB Ss in which spermatids were never found, spermatozoa were observed in fresh smears from the epididymis of each autopsied animal in all of the remaining groups.
The various treatments did affect body weight as males grew older (Fig. 1). An overall analysis of variance of body weights between 14 and 50 days of age (age span during which every subject contributed body weight scores) showed a significant Groups effect ($F = 9.25, df = 4/60, p < .01$) as well as a significant Groups X Days interaction ($F = 20.19, df = 16/240, p < .01$). Subsequent comparisons using Scheffé's method showed that $5 \mu g.$ EB $S$s differed from oil control $S$s (Groups effect $p < .01$, Groups X Days interaction effect $p < .01$). The $1000 \mu g.$ TP and Oil Groups X Days interaction effect was also marginally significant ($p < .10$, Scheffé Test) indicating that the growth curves for the oil and $1000 \mu g.$ TP groups diverged. Inspection of Fig. 1 indicates that the effects of $5 \mu g.$ EB and of $1000 \mu g.$ TP on body growth became visible after males reached an age of 40 days. Growth curves for $.05 \mu g.$ TP and $5 \mu g.$ TP $S$s were not significantly different from the curve for oil controls.
Experiment 2

The Development of Copulation in Male Rats Following Early Exposure to Intermediate Doses of Testosterone

Experiment 2 actually had been performed prior to Experiment 1 in attempting to discover whether the effect of neonatal pinealectomy on the development of mating behavior in male rats (Baum, 1968) could be attributed to consequent increments in androgen titers. In addition, however, the results of Experiment 2 also helped to answer a question arising from the results of Experiment 1; namely, what minimal dosage of TP is required to allow precocious activation of intromission and ejaculation patterns. The results of Experiment 1 indicated that daily administration of 5 μg. TP per 100 g. B. W. is not sufficient whereas daily injections of 1000 μg. TP will cause precocity. Obviously, any effects on the development of mating obtained with intermediate doses of TP would be of interest, especially when compared with the capacity of various doses of estradiol to facilitate precocious copulation (see Experiment 3).

Subjects. The subjects were 34 male hooded rats born in the laboratory from seven multiparous females. Eleven days after birth males were assigned to one of three different groups:
1. Oil -- 0.1 ml. sesame oil per 100 g. B. W. (n = 11).

2. 50 μg. TP -- 50 μg. TP in 0.1 ml. sesame oil per 100 g. B. W. (n = 12).

3. 500 μg. TP -- 500 μg. TP in 0.1 ml. sesame oil per 100 g. B. W. (n = 11).

Each group was comprised of one male from each of the seven litters and one additional male from four (for Group 2, five) of these same litters.

**Procedure.** Injections of hormones and oil were begun 11 days after birth and continued until $S$ ejaculated for the first time. Prepuberal tests identical to those described for Experiment 1 were given beginning when males were 16 to 17 days old and continuing until individuals first ejaculated. Two 50 μg. TP $S$s which failed to intromit or ejaculate by 117 and 118 days of age, respectively, were assigned age at first intromission and age at first ejaculation scores of 117 days and were included in the analysis of these data. A third $S$ from this same group which did intromit but failed to ejaculate by 99 days was assigned an age at first ejaculation score of 99 and also included in the analysis. All $S$s (except for the three 50 μg. TP $S$s which failed to ejaculate) received seven mating tests identical to those in Experiment 1 following initial ejaculation.
Results. Mounts with pelvic thrusting were never exhibited by any animal prior to initial intromission. Inspection of Table 4 indicates that the TP doses used in this experiment had a slight overall effect on the ages at which intromission and ejaculation responses were initially displayed. Although neither Kruskal-Wallis H value was significant, 50 µg. TP Ss tended to intromit and ejaculate at a later median age than oil controls, and 500 µg. TP Ss tended to make both responses at an earlier median age than oil Ss. Apparently a daily TP dose somewhat higher than 500 µg. per 100 g. B. W. is required to facilitate precocious intromission and ejaculation.

Early treatment with 500 µg. TP significantly lowered the number of mating tests in which males intromitted and ejaculated (Table 5); 500 µg. TP Ss intromitted and ejaculated during less than one-half as many mating tests as oil Ss (U = 24, p < .02). Note that no animal in this experiment ever intromitted during a mating test without subsequently achieving ejaculation; thus a combined statement about the number of tests with both responses is presented in Table 5.

An analysis of variance performed on body weights of males from all three groups between 11 and 80 days of age revealed neither significant Groups nor significant Groups X Days interaction effects.
Experiment 3

The Development of Copulation in Male Rats Following Early Exposure to High and Low Doses of Estradiol

The results of Experiment 2 indicated that more than 500 μg. TP per 100 g. B. W. must be given from Day 11 onward in order to facilitate the precocious appearance of intromission and ejaculation patterns. However, in Experiment 1 precocious male behavior occurred following injections of only 5 μg. EB per 100 g. B. W. from Day 11 onward. Experiment 3 explored the effects of injecting both a higher and a lower dose of estradiol on the development of copulation.

Subjects. The subjects were 33 male hooded rats born in the laboratory from 10 multiparous females. Eleven days after birth males were assigned to one of three different groups:

1. Oil -- 0.1 ml. sesame oil per 100 g. B. W. (n = 15).
2. .05 μg. EB -- .05 μg. EB in 0.1 ml. sesame oil per 100 g. B. W. (n = 9).
3. 100 μg. EB -- 100 μg. EB in 0.1 ml. sesame oil per 100 g. B. W. (n = 9).

Each group included one male from each of the 10 litters (one .05 μg. EB and a littermate 100 μg. EB died early in the experiment) and the oil control group included one additional male from five of these litters.
Procedure. The hormone injection schedule was identical to that used in Experiments 1 and 2; however, the behavioral testing procedures were slightly different from those used previously. Examination of the median latencies to first intromission in all groups employed in both of the earlier experiments showed that the shortest median latency was 2.35 minutes (.05 μg. TP Ss, Experiment 1) while the longest median latency to initial intromission was 4.85 minutes (500 μg. TP Ss, Experiment 2). The oil control groups for Experiments 1 and 2 had median latencies of 2.85 and 3.15 minutes, respectively. Thus it appeared that little information concerning the first appearance of intromission (and subsequent ejaculation) would be lost by shortening the test sessions from 10 minutes to 5 minutes in length. On the other hand the number of animals tested during an equivalent amount of time could be doubled. Therefore the following testing procedure was used in this and subsequent experiments: Five minute tests were given on alternate days beginning when males were 16 to 17 days old. If S displayed a mount with pelvic thrusting towards the stimulus female, the test was extended for an additional 10 minutes from the time that the mount occurred. Whenever an intromission occurred S was allowed an additional 45 minutes to ejaculate. In the event that ejaculation was not achieved in that time, S was subsequently tested on alternate days until
it either ejaculated or reached an age of 90 to 91 days, at which time injections and tests were stopped. Again, Ss that failed to intromit or ejaculate were assigned age at first intromission and age at first ejaculation scores of 90 which were included in the analyses of the data.

As in Experiment 1 males from each group (in this case six per group) were randomly chosen to receive seven mating tests following initial ejaculation. Mating tests were identical to prepuberal tests except that they were given on every fourth day. The remaining animals in each group (except for Ss failing to ejaculate by 90 to 91 days) which did not receive mating tests were sacrificed and autopsied the day following initial ejaculation.

Results. During prepuberal test sessions prior to those where initial intromission occurred, six out of nine 100 μg. EB Ss displayed mounts with pelvic thrusting toward the female. Only two out of fifteen oil Ss and zero out of nine .05 μg. EB Ss showed such prepuberal thrusting behavior ($\chi^2 = 12.54, df = 2, p < .01$). Table 6 shows that estrogen treatments had no statistically significant overall effect on ages at first intromission or first ejaculation. Even so, the median ages at initial intromission and ejaculation for .05 μg. EB Ss were lower than those for oil control Ss. The same was true of the median age at first intromission for 100 μg. EB Ss. This latter
result was due primarily to two $S$s (93-4 and 78-4) which first intromitted at 21 and 22 days of age, respectively. These are by far the most sexually precocious males ever observed in this laboratory, although Beach (1942b) reported one intromission at 21 days in a male $S$ which received 1000 µg. TP from Day 14. Curiously, 52 days was the next lowest intromission age for any male receiving 100 µg. EB. Several 100 µg. EB $S$s exhibited mounts with pelvic thrusting during this 22 - 52 days age span; however, none achieved intromission. Subject 93-4 also ejaculated on Day 22 (Beach first observed ejaculation at 27 days), but $S$ 78-4 did not ejaculate until it was 57 days of age. Littermates of each of these animals neither intromitted nor ejaculated any earlier than other members of their respective groups. Neither $S$ ever displayed intromission or ejaculation patterns during the seven mating tests following initial ejaculation, suggesting that for these two $S$s this dose of estradiol exerted a powerful activating influence in the short term, but a debilitating long-term influence on the ability to copulate.

Oil control males in this experiment intromitted and ejaculated at a later age than oil $S$s in Experiments 1 and 2. A possible explanation for this discrepancy was the use of shortened, 5-minute prepuberal tests in this experiment. However, H. Szechtman using 5-minute sessions performed an experiment in this laboratory two months prior to Experiment 3 in which $S$s
injected with saline (n = 15) first intromitted and ejaculated at a median age of 60 days. This result is very similar to median age values for oil Ss in Experiments 1 and 2. One conclusion from these facts is that appropriate control groups must be included each time a new longitudinal experiment is performed, and the effects of any treatment on the development of mating should be weighed only in comparison with the control group tested concurrently.

Response data for males receiving mating tests are shown in Table 7. Subjects in both estradiol groups tended to intromit during fewer tests than oil controls; however, this result was not statistically significant. Estradiol did significantly reduce the number of mating tests during which Ss ejaculated; individual group comparisons showed that only 100 µg. EB Ss ejaculated during significantly fewer tests than the oil control Ss (U = 5, p = .042). This latter difference can be attributed to four out of six 100 µg. EB Ss; the other two Ss from this group ejaculated during all but one mating test.

Data collected from Ss autopsied one day after initial ejaculation are shown in Table 8. Only one ejaculating 100 µg. EB S was available for autopsy, thus statistical comparisons (two-tailed Mann-Whitney U Tests) were made between scores obtained from Ss in the .05 µg. EB and oil groups. None of
these comparisons reached statistical significance. The findings that body and organ development for § 80-3 were severely retarded would have been predicted from the effects of daily injections of 5 μg. EB per 100 g. B. W. (Experiment 1) as well as from gross genital inspection of other 100 μg. EB Ss which received mating tests. Spermatozoa were observed in fresh epididymal smears from every male except 80-3 which had received 100 μg. EB.

Overall analysis of variance of body weights between 11 and 50 days of age (Fig. 2) showed a significant Groups effect ($F = 26.20$, $df = 2/30$, $p < .01$) and also a significant Groups X Days interaction ($F = 52.87$, $df = 8/120$, $p < .01$). Subsequent individual comparisons of treatment and oil control groups showed that body growth was in fact significantly retarded only in 100 μg. EB Ss ($p < .01$, Scheffé Tests for Groups effect and for Groups X Days interaction).

Experiment 4

The Development of Copulation and Adult Mating Performance in Male Rats Receiving Estradiol from Birth Onward

In previous experiments hormone treatments were never initiated before 11 days of age. As stated earlier, this procedure was adopted to avoid any interference, particularly by estradiol injections, with the organization of the mechanisms
required for the eventual display of mating behavior in adulthood. However, the surprising behavioral precocity observed in males receiving 5 µg. EB from Day 11 onward (Experiment 1) suggested the following question: Will initiation of estradiol treatment prior to the eleventh day facilitate an even more precocious appearance of copulation patterns, or will it undermine the animal's capacity to perform intromission and ejaculation responses? Experiment 4 was designed to answer this question by examining both the development of mating behavior as well as adult sexual performance in males which first received estradiol prior to the eleventh day of life.

Subjects. The subjects were 31 male hooded rats born in the laboratory from 11 multiparous females. At birth males were assigned to groups on the basis of what treatment they would receive on each of the first ten days of life:

1. Oil -- 0.1 ml. sesame oil per 100 g. B. W. (n = 11).
2. 5 µg. EB -- 5 µg. EB in 0.1 ml. sesame oil per 100 g. B. W. (n = 10).
3. 100 µg. EB -- 100 µg. EB in 0.1 ml. sesame oil on Day 4; 0.1 ml. sesame oil per 100 g. B. W. on the other 10 neonatal days (n = 10).

Each group included one male from each of 11 litters, except for Groups 2 and 3 where one male died early in the experiment. Pups in each litter were individually identified at birth by
clipping a nail on the right forepaw; ears of animals were subsequently punched at 11 days of age for easier identification.

**Procedure.** The various treatment injections were given from birth (Day 0) to Day 10, inclusive. All Ss received one injection of some kind on each of the 11 (including day of birth) neonatal days. The estradiol dose given to Group 2 animals was merely an extension to the day of birth of the original estradiol dose which had induced precocious copulation in Experiment 1. Levine and Mullins, Jr. (1964) had found that the particular EB dose used in Group 3 (100 μg. EB on Day 4) restricted the ability of adult males to intromit. Beginning on Day 11 all males in the present experiment received 5 μg. EB per 100 g. B. W. daily until individual Ss first intromitted, at which time injections were stopped. Levine and Mullins, Jr. (1964) did not include this latter procedure in their experiment; however, the continuation of estradiol injections beyond Day 11 might be expected to intensify any deleterious effects on mating behavior in adulthood.

Each subject received prepuberal tests identical to those described in Experiment 3 beginning at 16 to 17 days of age and continuing on alternate days until S first intromitted. Tests were stopped after one intromission, and Ss were returned to their home cages prior to the administration of sexual performance tests starting at 110 days of age. These adult tests
were comprised of four 15-minute sessions on alternate days. During each session $S$ was presented with a receptive female and then the number of mounts (with or without pelvic thrusting), intromissions, and ejaculations made by $S$ was recorded. Using a method described by Whalen and Edwards (1967), the data in each behavior category were summed over the four sessions for each $S$, and the following performance scores were calculated:

(a) A mount frequency score consisting of the ratio of the total mount frequency to the number of tests in which mounting was displayed at least one time, and (b) an intromission frequency score consisting of the ratio of the total intromission plus ejaculation frequencies divided by the number of tests in which $S$ intromitted. All animals were sacrificed and autopsied at 117 days of age to assess any differential effect of neonatal estradiol on adult organ size.

The reasons for modifying the prepuberal and mating test procedures from those used previously should be clarified. Prepuberal tests and injections were stopped after initial intromission (not after initial ejaculation) to minimize the number of estradiol injections given each male while still using a meaningful endpoint (age at first intromission) to study the effect of neonatal estradiol injections on the development of male behavior. Mating tests given in earlier experiments had been designed to determine whether intromission and ejaculation
patterns disappeared or remained intact immediately after hormone treatments were stopped. In the present experiment animals were tested several weeks after treatments had been stopped because the objective was to assess the effect of neonatal estradiol on adult performance, not on changes in sexual responsiveness immediately following the termination of injections.

Results. One 5 µg. EB Ss and two 100 µg. EB Ss died about two weeks after initial intromission and therefore were excluded from the latter parts of the experiment. Several Ss in all three groups displayed mounts with pelvic thrusting during prepuberal tests prior to the one when intromission first occurred ($\chi^2 = 1.48, df = 2, n.s.$). An overall analysis showed that extending estradiol injections to the first ten days of life was neither more nor less effective than first injecting estradiol on Day 11 in accelerating the first appearance of intromission patterns (Table 9). Although a control group receiving only oil from Day 11 until initial intromission was not included in this experiment, casual comparison of the median ages at first intromission (Table 9) with the median age of 67 days for the oil control group in Experiment 3 (Experiments 3 and 4 were performed concurrently) suggests that all of the treatments used in the present experiment probably facilitated precocious intromission to some degree.
The results of the adult sexual performance tests (Table 10) indicated that long term exposure to estradiol begun neonatally did not affect the capacity of males to mount stimulus females. Early estradiol did significantly lower the proportion of Ss in these groups which intromitted during the tests; however, median intromission frequency scores, whether calculated to include all Ss or only those Ss which showed the response, were not significantly influenced.

Comparison of the weights of organs from adult animals (Table 11) indicates that exposure to estradiol during the first ten days retarded genital growth, at least up to 117 days, even though Ss in all three groups received 5 µg. EB from Day 11 until initial intromission. The testes ($p < .05$, Scheffé Test) and accessory organs ($p < .01$, Scheffé Test) were always lighter in 5 µg. EB and in 100 µg. EB Ss than in oil Ss. Also, the penes of 5 µg. EB Ss ($p < .05$, Scheffé Test) and of 100 µg. EB Ss ($p < .01$, Scheffé Test) were lighter when compared with penes of oil Ss, and the frenulum was attached to the glans penis in a significantly larger proportion of the males from the two estradiol groups. Spermatozoa were observed in the epididymal smears taken from animals in all three groups.

Significant differences in body weight among the groups did not occur at any point during the experiment.
Experiment 5

The Development of Copulation in Prepuberally Castrated Male Rats Following Early Exposure to Estradiol

Experiments 5 and 6 were designed in an attempt to understand better how a small dose of estradiol facilitated the precocious appearance of mating behavior in intact male rats (Experiment 1).

Ball (1939) and Beach (1942a) both reported intromission behavior following the administration of estrogen to a small number of male rats castrated prepuberally. In Beach's (1942a) experiment the single male which did intromit after receiving estrogen had failed to copulate during repeated pretests without estrogen. More recently Lisk and Suydam (1967) have reported intromission and ejaculation responses in three out of seven adult males (castrated at 3-5 days) with estradiol and progesterone implanted in the preoptic anterior hypothalamus; surprisingly, implants of testosterone were not effective in stimulating male behavior. These results all suggest that estradiol may be expected to support some intromission and perhaps ejaculation patterns in the prepuberal castrate.

As stated earlier in this paper, the neuromuscular mechanisms which mediate mating behavior are probably organized very early in life. Ordinarily these mechanisms can be successfully activated by sexual stimuli in the environment.
only if they are exposed to sufficient quantities of androgen, which is either produced by the testes or injected experimentally. Since estradiol can apparently support some mating behavior in adult males which were castrated prepuberally, it is possible that exogenous estradiol alone facilitated precocious copulation (Experiment 1) by exerting an activating influence on these same mating mechanisms. If this was the case, and the contribution made by endogenous testicular secretions to the development of male behavior in 5 μg. EB Ss was insignificant, then males castrated prepuberally and given 5 μg. EB per 100 g. B. W. from Day 11 onward would be expected to copulate as readily as sham-operated animals receiving the same dose of estradiol.

**Subjects.** The subjects were 20 male hooded rats born in the laboratory from 10 multiparous females. Eleven days after birth two males from each litter either were castrated or received a sham-operation. Thus the castrated (n = 10) and sham-operated (n = 10) groups included a total of ten littermate pairs.

**Procedure.** Injections of 5 μg. EB per 100 g. B. W. were administered starting on Day 11 (same day as surgical operation) and continued daily until each male ejaculated for the first time or reached an age of 90 days, at which time injections were stopped.
Each $S$ received prepuberal tests identical to those described in Experiment 3 beginning at 16 to 17 days of age and continuing on alternate days until $S$ either ejaculated or reached an age of 90 to 91 days. Subjects which failed to intromit or ejaculate by 90 to 91 days were arbitrarily assigned age at first intromission and age at first ejaculation scores of 90. All $S$s subsequently received seven mating tests which were identical to prepuberal tests except that they occurred every fourth day. If intromission patterns were observed in castrated $S$s during either of the last two mating tests, mating tests were continued (after the regular seven tests) on every fourth day to see if these $S$s would continue to copulate.

**Results.** Four out of ten sham-operated $S$s and one out of ten castrated $S$s performed mounts with pelvic thrusting in prepuberal tests prior to initial intromission (n.s., Fisher Exact Probability Test). As anticipated, some castrated males did intromit and ejaculate following injections of 5 $\mu$g. EB per 100 g. B. W. Both of these behavior patterns were of a similar quality in castrated and sham-operated $S$s. Despite the presence of severely underdeveloped penes in all $S$s in the experiment, pelvic movements and backward lunges following intromission were usually vigorous and well-executed. Although the difference did not reach statistical significance, a higher proportion of sham-operated than castrated $S$s showed intromission
and ejaculation patterns during prepuberal tests (Table 12). Subsequent comparison of the median ages at first intromission revealed no difference between the groups; however, the four castrated Ss which ejaculated did so at a slightly earlier median age than sham-operated Ss.

No significant differences between groups were observed either in the number of mating tests with intromission or in the number of mating tests with ejaculation (Table 13). Two castrated Ss last showed intromission and ejaculation patterns 12 and 20 days after 5 μg. EB injections were stopped. Two other castrates intromitted as long as 52 and 60 days after injections were stopped. Neither S intromitted during either of two subsequent mating tests. Six out of seven sham-operated Ss intromitted during the seventh mating test given 28 days after 5 μg. EB injections were stopped.

Body growth was significantly accelerated in sham-operated Ss in comparison with castrated Ss (Fig. 3). Both the Groups (F = 4.98, df = 1/18, p < .05) and Groups X Days interaction (F = 3.03, df = 6/108, p < .01) effects were significant. This divergence in the body weight curves strongly suggests that testicular production of androgen in sham-operated males was not totally suppressed during the period that estradiol was being injected.
Experiment 6

Behavioral Reactivity to Testosterone Following Early Castration
and Subsequent Priming with Testosterone or Estradiol

Whereas Experiment 5 tested the possibility that a low dose of estradiol facilitated precocious mating behavior by duplicating the activational function ordinarily performed by androgen, Experiment 6 was designed to find out whether exposing intact prepuberal males to low doses of estradiol (Experiment 1) could possibly have increased the sensitivity of neuromuscular mechanisms mediating copulation to the activation-al effects of androgens already being secreted by the testes. If this hypothesis is at all valid, then administering 5 μg. EB, 5 μg. TP, or oil vehicle to prepuberal males for a discrete time period immediately following castration should differentially affect the readiness with which intromission and ejaculation patterns are later facilitated by replacement injections of a low dose of TP. Subjects receiving 5 μg. EB would be expected to copulate after fewer TP replacement injections. As in previous experiments, castration and hormone treatments were performed after the tenth day of life to avoid any possible disruption of Ss' potential to copulate.

Subjects. The subjects were 30 male hooded rats born in the laboratory from the same 10 mothers used in Experiment 5. Eleven days after birth all animals were castrated and
subsequently three littermates were each assigned to one of three groups:

1. Oil -- 0.1 ml. sesame oil per 100 g. B. W. (n = 10).
2. 5 µg. TP -- 5 µg. TP in 0.1 ml. sesame oil per 100 g. B. W. (n = 10).
3. 5 µg. EB -- 5 µg. EB in 0.1 ml. sesame oil per 100 g. B. W. (n = 10).

Procedure. Subjects in the three groups received their respective injections daily for 20 days beginning at 11 days of age (the day Σs were castrated). No hormones were administered between 31 and 55 days of age. Each male received 50 µg. TP in 0.1 ml. sesame oil daily from 56 days onward until Σ first ejaculated or until 60 such injections had been given. This particular dose of TP is apparently close to the minimal amount of TP which will maintain ejaculation patterns in most castrated adult males which copulated successfully prior to castration (Beach & Holz-Tucker, 1949).

Beginning at 16 to 17 days of age and continuing on alternate days each Σ was placed in the test apparatus with a receptive female for one minute. During these habituation sessions male mounts without pelvic thrusting were occasionally observed. Actual behavioral tests began when each Σ reached an age of 55 days (one day prior to the first TP replacement injection) and continued on alternate days until Σ first
ejaculated or until S had received 60 injections of 50 µg. TP. These tests were identical in detail to the five minute prepuberal tests described in Experiment 3, except that Ss were allowed 60 minutes to ejaculate following an initial intromission. If any male failed to ejaculate within 60 minutes, it was subsequently retested two days later. Subjects which failed to intromit or ejaculate even after 60 TP replacement injections were arbitrarily assigned scores of 60 for the number of TP injections prior to first intromission and first ejaculation. All Ss were sacrificed and autopsied one day following initial ejaculation, or at 116 days of age for four Ss not ejaculating. Since an a priori hypothesis concerning the behavioral outcome of this experiment had been formulated, one-tailed tests were used to compare group scores, providing the original Kruskal-Wallis H value was significant at $p < .05$.

Results. No mounting behavior was exhibited by animals from any group during the first behavioral test at 55 days of age. Whereas members of estradiol groups in all previous experiments had performed mounts with pelvic thrusting during prepuberal tests prior to initial intromission, no S in the present experiment ever made this type of response, even after 50 µg. TP injections were begun. As shown in Table 14, Ss which had received 5 µg. EB early in life did in fact intromit after
fewer injections of 50 µg. TP than did oil Ss ($U = 26, \ p < .05$) or 5 µg. TP Ss ($U = 17, \ p < .01$). Although comparison of the median number of injections required to produce ejaculation in each group revealed a definite trend analogous to that for intromission behavior, the overall Kruskal-Wallis $H$ value was not statistically significant.

The only lasting effect of early exposure to 5 µg. EB still evident when Ss were sacrificed (Table 15) was reduced body weight and attachment of the frenulum to the glans penis in eight out of ten animals. Mean accessory organ and penis weights did not differ significantly among the groups.

Injections of 5 µg. EB per 100 g. B. W. for 20 days did affect body growth (Fig. 4). An overall analysis of variance revealed significant Groups ($F = 5.16, \ df = 2/27, \ p < .05$) and Groups X Days interaction ($F = 6.39, \ df = 6/216, \ p < .01$) effects. Subsequent comparisons of growth curves for 5 µg. EB and oil Ss showed a significant Groups ($p < .05$, Scheffé Test) effect as well as a significant Groups X Days interaction ($p < .01$, Scheffé Test). The growth curve for 5 µg. TP Ss was not significantly different from the curve for oil Ss.

It is possible that males receiving 5 µg. EB required fewer TP replacement injections to facilitate initial intromission because they weighed less than Ss in other groups at
the age when injections were begun (see Fig. 4). Lighter 5 μg. EB Ss were receiving approximately 3 - 4 μg. TP per 100 g. B. W. more per day than oil or 5 μg. TP Ss which were heavier. If this slight discrepancy in TP doses can account for the precocious appearance of intromission in 5 μg. EB Ss, then a significant correlation between body weight at 55 days of age and the number of 50 μg. TP injections prior to initial intromission should exist without any reference to early hormone treatment (i.e., light Ss in all groups should have intromitted after fewer injections than heavy Ss). Without making any reference to group assignments, all 30 Ss were ranked according to their body weight at 55 days of age (lightest S received rank of 1, etc.) and these ranks were then correlated with the ranks of the number of injections prior to first intromission (first S to intromit received rank 1, etc.). The Spearman rank correlation coefficient ($\rho_s$) for body weights and injections prior to first intromission was: $\rho_s = 0.224$. When $n = 30$, $\rho_s$ must equal or exceed 0.306 for $p < .05$, one-tailed probability. Therefore the fact that they had significantly lighter body weights at the time TP replacement injections were begun cannot readily account for the earlier appearance of intromission patterns in males which had previously received 5 μg. EB per 100 g. B. W.
Discussion

It may be helpful to summarize briefly the main findings of the present experiments before discussing the results in detail:

1. Daily injections of 1000 μg. TP or 5 μg. EB per 100 g. B. W. begun after Day 10 significantly hastened the first appearance of intromission and ejaculation responses in intact male rats.

2. Neither lower doses of TP nor higher or lower doses of EB accelerated mating to a significant degree. Although the difference did not reach statistical significance, subjects receiving .05 μg. EB per 100 g. B. W. did tend to intromit and ejaculate at an earlier median age than oil control subjects.

3. Males receiving daily injections of estradiol beginning either during the first ten days of life or on Day 11 first intromitted at approximately the same median age. However, significantly fewer subjects which had first received estradiol during the first ten days displayed intromission during any of four sexual performance tests given several weeks after estradiol injections had been stopped.

4. Males castrated at 11 days and subsequently administered 5 μg. EB per 100 g. B. W. each day first
copulated almost as readily as intact littermates receiving the same dose of estradiol.

5. Castrated males receiving 5 μg. EB per 100 g. B. W. for 20 days beginning on Day 11 first intromitted after significantly fewer replacement injections of TP than castrated littermates which had been primed with either 5 μg. TP per 100 g. B. W. or with oil.

A major portion of the interpretation which follows relates closely to these experimental observations; however, it should be stated at the outset that the theoretical exposition which is included in the later sections of this paper is based on a small amount of empirical evidence and as such is quite speculative.

Precocious Copulation Facilitated by Testosterone or Estradiol

Testosterone injections begun on Day 11 or Day 14.

Although the effect failed to reach statistical significance, males receiving 500 μg. TP per 100 g. B. W. beginning on Day 11 tended to intromit and ejaculate for the first time at an earlier median age than subjects which received oil (Table 4); daily injections of 1000 μg. TP beginning on Day 14 significantly facilitated the precocious activation of both mating patterns (Table 1). Daily injections of 50 μg. TP per 100 g. B. W. or even smaller doses of TP beginning on Day 11 all failed to accelerate the appearance of mating behavior even to a small
degree. These results along with the evidence collected earlier by Beach (1942b) and Stone (1940) strongly suggest that daily administration of more than 500 μg. TP per 100 g. B. W. beginning after the tenth day of life will facilitate precocious mating in male rats.

Beach (1942b) observed intromission responses as early as Day 21 in male rats receiving 1000 μg. TP; however, in Experiment 1 males receiving the same dose of TP first intromitted and ejaculated at 34 days of age. This discrepancy in the readiness with which 1000 μg. TP injections facilitated precocious mating may be due to the genetic differences between the strains of rats which were employed in the two experiments. However, the important finding is that in comparison with respective control subjects, males receiving 1000 μg. TP in both experiments first copulated at a precocious age.

These results suggest that in the normal male the amount of circulating androgen constitutes one factor which regulates the time during development when mating first occurs. In subjects where precocious copulation was observed, androgen may have accumulated in the blood until it reached a concentration capable of facilitating previously unreactive neural tissues mediating mating behavior to respond to a receptive female.

The sensitivity of tissues which mediate mating to the activating influence of androgen appears to be lower in
prepuberal than in adult males. In Experiment 1 mating first occurred following at least three weeks of daily injections of 1000 µg. TP (and after much longer exposure to lower doses of TP); however, daily administration of only 50 - 75 µg. TP to male rats castrated in adulthood was sufficient to maintain mating performance in subjects which had exhibited copulation prior to castration (Beach & Holz-Tucker, 1949). Also, changes in the responsiveness to androgen of tissues mediating mating behavior seemed to occur during the mating tests which followed initial ejaculation: In Experiment 1 males continued to intromit and ejaculate during as many mating tests as oil control subjects even after injections of 1000 µg. TP had been stopped (Table 2). It is fairly safe to assume that the concentration of circulating androgen in intact males declined following the final injection of TP. Much of the accumulated androgen was probably metabolized in the liver, and it is unlikely that each day the testes could have secreted as much as 1000 µg. testosterone into the bloodstream (Bardin & Peterson, 1967). Therefore it appears that less androgen was required to activate mating patterns during the seven mating tests than at the time when subjects first ejaculated.

Although an increase in the sensitivity to androgen of the tissues mediating copulation may have been caused by nonhormonal factors such as physical maturation or even
previous mating experience, Larsson's (1967b) results (see p. 26) suggested that androgen in itself may affect the sensitivity of these same tissues during the puberal period of development. It is also conceivable that androgen (especially when it is injected in large doses) acts to lower response thresholds in mating mechanisms long before the time when intromission or ejaculation patterns normally first appear.

The reader has probably noticed that unlike 1000 µg. TP subjects in Experiment 1, males receiving 500 µg. TP per 100 g. B. W. in Experiment 2 failed to intromit and ejaculate during as many mating tests as oil control subjects (Table 5). Since autopsies were not performed on any of these subjects immediately after the first ejaculation, there is no way of knowing whether injections of 500 µg. TP per 100 g. B. W. retarded growth of the testes. However, testicular growth was retarded in males receiving 1000 µg. TP (Experiment 1) without a corresponding reduction in the exhibition of intromission and ejaculation during mating tests. Thus it is not likely that the deficient mating performance in 500 µg. TP subjects was caused by a reduction in the secretion of androgen by the testes.

Estradiol injections begun on Day 11. Five µg. EB per 100 g. B. W. administered daily beginning on Day 11 had paradoxical effects on genital organ development and on the development of mating behavior in intact males. Although
injections of estradiol severely retarded both genital development and the production of spermatozoa (Table 3), intromission and ejaculation first occurred at a precocious age (Table 1). In the female rat small doses of exogenous estrogen may stimulate a precocious puberal surge in the secretion of pituitary gonadotropins. However, precocious mating behavior observed in males receiving 5 μg. EB per 100 g. B. W. was probably not caused by a precocious increase in the secretion of gonadotropins which in turn might have stimulated the production of testicular androgen. If the latter sequence of physiological events had in fact occurred, the development of the genital organs in 5 μg. EB subjects would have been accelerated instead of retarded.

Although none of the males receiving any of the different doses of estradiol employed in Experiments 1 and 3 ever exhibited grossly aberrant mounting or intromission patterns, subjects which received 5 μg. EB or 100 μg. EB per 100 g. B. W. experienced more difficulty than males in other groups in achieving ejaculation once intromissions were occurring. For example, two 5 μg. EB subjects (Experiment 1) failed to ejaculate once prior to an age of 90 days even though they had consistently intromitted during tests given on alternate days for a period of weeks. Among all of the groups included in Experiment 1, 5 μg. EB subjects ejaculated during the lowest
median number of mating tests (Table 2). Although neither
result reached statistical significance, 100 µg. EB subjects
intromitted at an earlier median age than oil control males
but subsequently ejaculated at a later median age (Table 6);
after injections of 100 µg. EB per 100 g. B. W. were stopped,
subjects ejaculated during significantly fewer mating tests
than oil control males (Table 7). Also, the two 100 µg. EB
subjects which intromitted at a very precocious age each managed
to ejaculate once but subsequently exhibited neither intromission
nor ejaculation during any mating test. The daily administration
of 5 µg. EB or 100 µg. EB per 100 g. B. W. severely retarded the
growth of the penis (Tables 3 & 8). Although direct verification
is not available, it is quite possible that this phallic under-
development caused a reduction in the amount of sensory input
received by the penis during mating and consequently made
ejaculation more difficult to achieve (Hart, 1968b; Whalen &

It is somewhat surprising that although genital organ
development and body growth were retarded in several instances,
none of the hormone regimens employed in Experiments 1, 2, or 3
effectively delayed the development of mating behavior. Initial
intromission and ejaculation were delayed slightly in males
receiving 50 µg. TP per 100 g. B. W. (Table 4), and the median
age at first ejaculation was slightly retarded in subjects which
received 100 μg. EB per 100 g. B. W. (Table 6); however, neither difference approached statistical significance. Perhaps none of these treatments was able to reduce the concentrations of circulating gonadal hormones in experimental males to levels below the threshold quantities needed to activate the neural mechanisms which mediate mating. Hormone regimens which may have effectively lowered the net quantity of circulating gonadal hormones (in comparison with injections of oil vehicle) apparently also caused an increase in the sensitivity of mating mechanisms which compensated for any hormone deficiency.

Estradiol injections begun prior to Day 11. The age at which intact male rats first received injections of 5 μg. EB per 100 g. B. W. did not influence the occurrence of the first intromission response. The initiation of estradiol injections prior to the eleventh day of life affected neither the probability that subjects would be able to intromit nor the age at which intromission first occurred (Table 9). However, a significantly lower proportion of males which had first received estradiol during the first ten days displayed intromission responses during four sexual performance tests administered several weeks after estradiol injections were stopped (Table 10). In earlier experiments (reviewed on p. 10) a single injection of estradiol to intact males on Day 4 or 5 lowered the probability that intromissions would occur in adulthood and
reduced the frequency of intromission and ejaculation in the few males which did respond. Intromission frequencies in oil control subjects in Experiment 4 might have been higher than those observed in males which first received estradiol prior to Day 11 had subjects receiving oil during the first 10 days of life not also received daily injections of 5 μg. EB per 100 g. B. W. beginning on Day 11.

The findings that males which initially received estradiol during the first 10 days exhibited significantly lighter penes and a higher incidence of attachment of the frenulum to the glans penis than subjects receiving oil during the neonatal period (Table 11) corroborate Feder's (1967) report that an injection of estradiol on either Day 2 or 5, but not on Day 20, retarded penile development. It is possible that underdevelopment of the penis may have contributed to the inability of several males in Experiment 4 to intromit during sexual performance tests. However, all males in Experiment 4 were able to intromit while receiving injections of estradiol. The presence of underdeveloped penes may have disrupted intromission behavior in adulthood, but injections of estradiol apparently compensated for any structural deficiencies at the time when subjects first intromitted.
Possible Explanations for the Action of Estradiol

The results of Experiments 5 and 6 shed some light on the processes underlying the strong facilitatory influence which daily injections of 5 μg. EB per 100 g. B. W. exerted on the development of mating in intact males (Experiment 1).

Temporary activation of neural mechanisms mediating mating. Injections of estradiol were almost equally capable of facilitating the initial activation of intromission and ejaculation in either castrated or sham-operated males (Table 12). Although the results of Experiment 1 suggested that prepuberal injections of estradiol suppress testicular function, sham-operated subjects receiving estradiol in Experiment 5 were producing enough endogenous androgen to have stimulated more rapid body growth than that observed in castrated males (Fig. 3). Still, the behavioral results suggest that testicular androgens in sham-operated males (and by implication, in intact males in Experiment 1) receiving 5 μg. EB per 100 g. B. W. contributed very little to the initial activation of mating.

The possibility does exist that androgens produced by the adrenal cortex in castrated males (Experiment 5) interacted with exogenous estradiol to facilitate the development of mating. If this did occur, then it becomes difficult to claim that testicular androgen was inessential to the activation of mating.
behavior in sham-operated males. Male rats with intact adrenals which were castrated prepuberally and subsequently given several mating tests over a period of months failed to copulate (Beach, 1942a); however, injections of gonadal hormones subsequently facilitated mating behavior in these castrates. Also, Bloch and Davidson (1968) have recently confirmed an earlier conclusion by Young (1961) that in the adult male rat adrenalectomy does not accentuate the decline in sexual performance which follows castration. Although an unequivocal answer can come only from a new experiment employing males which are both castrated and adrenalectomized prepuberally, the available evidence suggests that the small amounts of endogenous androgen which may have been present in castrated males probably contributed very little to the initial activation of male behavior.

The finding that intromission and ejaculation responses exhibited by castrated males eventually disappeared after estradiol injections were stopped also suggests that this steroid was responsible for the activation of mating in these subjects. Since the androgens present in sham-operated males apparently contributed very little to the activation of mating, it appears that estradiol played an activational role in these subjects (and in intact 5 µg. EB Subjects in Experiment 1) as well as in castrated males. However, once estradiol
injections were stopped, endogenous androgen in males possessing testes probably facilitated the exhibition of intromission and ejaculation patterns during subsequent mating tests.

The discovery that injections of 5 µg. EB per 100 g. B. W. facilitated initial mating as readily in castrated as in sham-operated males is puzzling. The results of experiments employing either male rats (Davidson, 1969; Young, 1961) or male guinea pigs (Antliff & Young, 1956) castrated in adulthood suggested that androgen is always more effective than estrogen in restoring sexual performance to precastration levels. If daily injections of 50-75 µg. TP are generally required to maintain mating behavior in male rats castrated in adulthood (Beach & Holz-Tucker, 1949), it is surprising that daily injections of 5 µg. EB per 100 g. B. W. (a less effective hormone than androgen for maintaining mating in the adult male) were able to facilitate mating behavior in six out of ten castrated males. Somehow the neural mechanisms underlying mating in these males were unusually sensitive to the activating influence of small amounts of estradiol. The results of Experiment 6 indicate that it is very likely that these same injections of estradiol drastically increased the sensitivity of neural tissues mediating intromission so that, in the presence of sexual stimuli, even small amounts of estradiol (in castrated males) or small amounts of estradiol aided perhaps by endogenous androgens
(in sham-operated or unoperated males) would successfully facilitate mating.

**Long-lasting sensitivity bias of mating mechanisms.** The results of Experiment 6 suggest that the daily administration of 5 μg. EB per 100 g. B. W. to castrated males between the ages of 11 and 30 days increased the sensitivity of the neural mechanisms which underlie intromission to the activational influence of exogenous TP when it was provided several weeks later. Males receiving daily injections of 5 μg. EB per 100 g. B. W. first intromitted after significantly fewer TP replacement injections than subjects which had previously received either 5 μg. TP per 100 g. B. W. or oil (Table 14). The fact that early injections of 5 μg. TP had no particular effect on subsequent behavioral reactivity to TP replacement injections indicates that at the dosages employed, the facilitatory effects of estradiol were specific to that hormone and could not be duplicated by an androgenic steroid.

As suggested earlier, the sensitivity to androgen of neural mechanisms mediating mating behavior may also have been heightened in the prepuberal male by daily injections of 1000 μg. TP (Experiment 1). The results of Experiment 6 merely suggest that less estrogen than androgen is required to alter these neural thresholds. If males in Experiment 6 had received doses of TP greater than 5 μg. per 100 g. B. W., they might have
subsequently exhibited intromission in response to TP replacement injections as readily as subjects which originally received estradiol. Nonhormonal factors such as early social experience may also influence the reactivity of neural mechanisms which mediate mating in male rats (Gerall, Ward, & Gerall, 1967; Gruendel & Arnold, 1969). In the present experiments social rearing conditions were the same for all subjects so that, hopefully, this factor was unable to differentially influence any result.

The ejaculation pattern in males primed previously with estradiol failed to appear after significantly fewer injections of 50 µg. TP. The absence of a significant effect on ejaculation is consistent with trends observed in earlier experiments where intact males receiving estradiol often experienced difficulty in ejaculating even though intromissions were occurring regularly. At autopsy a large proportion of 5 µg. EB subjects exhibited attachment of the frenulum to the glans penis (Table 15) suggesting that penile development was incomplete in these males. Mullins and Levine (1969) recently reported that a single injection of estradiol given neonatally to castrated male rats decreased the sensitivity of cornified papillae on the surface of the glans penis to the growth inducing influence of exogenous TP in adulthood. They also observed attachment of the frenulum to the glans penis in many of these same males. It is true
that males in Experiment 6 were neither castrated nor first injected with estradiol until Day 11 whereas Mullins and Levine (1969) had performed both procedures by Day 4. However, when their results are combined with autopsy results obtained in Experiments 4 and 6 it becomes at least plausible to claim that the difficulty experienced by several 5 μg. EB males (experiment 6) in ejaculating may have been caused by a deficiency in the sensitivity of the penis to sensory input. This deficiency may in turn have restricted the accumulation of input from successive intromissions needed to trigger ejaculation.

It is not likely that the kind of activational influence which estradiol exerted on castrated males in Experiment 5 was also present in castrated males receiving 5 μg. EB per 100 g. B. W. in Experiment 6. Five weeks intervened between the last injection of 5 μg. EB per 100 g. B. W. and the earliest age at which any male in that treatment group first intromitted. Also, no mounting was observed in males of any group during tests administered one day before injections of 50 μg. TP were begun.

**Precocious Mating: Effects of Estradiol and Testosterone Compared**

Daily injections of 5 μg. EB per 100 g. B. W., but not .05 μg. TP, 5 μg. TP (Experiment 1) or 50 μg. TP (Experiment 2) per 100 g. B. W., accelerated the first appearance of intromission and ejaculation in intact males. The notion that small doses of estradiol affected both the sensitivity setting as well as the
activation of neural mechanisms underlying mating behavior helps to explain this finding. Doses of TP which failed to facilitate precocious mating in these experiments probably all exerted a small activating influence on neural tissues which mediate copulation; however, the response thresholds in these mechanisms were apparently well above the androgen concentrations attained in males receiving the ineffective doses of TP. In contrast, injections of 5 µg. EB per 100 g. B. W. facilitated precocious mating by somehow sensitizing these same tissues to the activational effects of whatever gonadal hormones were present in the bloodstream. Although a trend was observed for 500 µg. TP subjects (Experiment 2), only 1000 µg. TP subjects (Experiment 1) copulated precociously. Presumably in the prepuberal male much larger concentrations of androgen than estrogen are required to lower tissue sensitivity to the point where circulating hormones may facilitate mating.

Aside from two males which first copulated at a very precocious age, prepuberal administration of 100 µg. EB per 100 g. B. W. failed to produce precocious mating (Table 6) and, even after injections were stopped, had a severe debilitating effect on the ability of subjects to ejaculate during mating tests (Table 7). The two males which copulated after a comparatively small number of daily injections of estradiol may have possessed mating mechanisms which, because of
inheritance or some other nonhormonal factor, were very sensitive to the activating influence of large amounts of estradiol. Prolonged exposure to this large dose of estradiol in other subjects which were less responsive to its activational effects may have caused damage to either peripheral or neural structures mediating ejaculation or intromission so that the first appearance of mating was delayed in several of these males.

Finally, a significantly higher number of intact males receiving daily injections of 5 μg. EB (Experiment 1) or 100 μg. EB (Experiment 3) per 100 g. B. W. beginning on Day 11 exhibited mounts with pelvic thrusting prior to the test when a subject first intromitted. Animals receiving injections of oil or TP seldom displayed these responses alone. In these subjects mounts with pelvic thrusting almost always culminated in intromission patterns. The effects of estradiol and testosterone on the initial occurrence of mounts with pelvic thrusting do not correspond with their effects on initial intromission: Sufficiently high doses of TP did accelerate the first appearance of mating almost as readily as injections of 5 μg. EB per 100 g. B. W.; however, no dose of TP ever duplicated the effects of estradiol on the occurrence of mounts with pelvic thrusting. No explanation for these differential effects of estradiol and testosterone is immediately obvious.
The "Organization" of Mating Mechanisms: Conceptual Relevance
for the Present Findings

The results of several experiments (reviewed on pp. 3-11) suggest that prenatal or neonatal exposure to androgen organizes the neural mechanisms which mediate mating behavior in adulthood. What does the phrase "organization of neural mechanisms" actually mean? In dealing with this question a recent review paper (Phoenix et al., 1968) employed several of the same hypothetical concepts used in the present discussion to account for the precocious development of mating in males receiving injections of gonadal hormones. Specifically, these investigators proposed that in the male rat exposure to androgen up to 10 days after birth sets a bias on the mechanisms which mediate mating so that a differential sensitivity and responsiveness to later stimulation by androgen is built into the animal. The exact anatomical locations for the neural mechanisms involved have not been specified; they may lie in tissue situated anywhere from the lowest levels of the spinal cord on up to higher centers in the cerebrum. Beach, Noble, and Orndoff (1969) have demonstrated rather conclusively that early exposure to androgen sensitizes the penis of the male rat so that later in life it will react to androgenic stimulation by growing to a normal size. However, it is worth noting that these investigators label as "speculative" the application of this kind of reasoning to explain the effects
of early androgen on the neural mechanisms responsible for mating.

Phoenix et al. (1968) were attempting to describe in physiological terms the way in which early hormonal experience may influence sexual performance in the adult; they were not particularly interested in the events surrounding the initiation of adult mating behavior. Neither the results of the present experiments nor the present interpretations of those results conflict with previous findings or earlier theoretical statements. The present conclusions do suggest, however, that although their influence may not be "organizational" in the strictest sense, both estrogen and to a lesser degree androgen retain well beyond the tenth day of life the ability to alter the sensitivity to gonadal hormones of the neural mechanisms which mediate intromission behavior in the male rat.

This addition must not be allowed to detract from the fact that androgen in the male performs very important functions early in development. Androgen deprivation imposed by neonatal castration or by the injection of large doses of estrogen prior to Day 10 may permanently damage the capacity of the male to copulate in adulthood. In the latter instance further alterations in tissue sensitivity caused by subsequent prepuberal exposure to estrogen, androgen, or even social stimuli become irrelevant because irreparable damage to mating mechanisms has already occurred.
Possible Regulatory Roles for Endogenous Estrogens on Sexual Maturation in the Male Rat

Three different estrogenic hormones including estrone, estradiol-17\alpha and estradiol-17\beta have been detected in the urine of both prepuberal and adult male rats (Ketz, 1961). Although unusually large amounts were found in the stallion, males of most species tested (including the rat) excreted only small quantities of urinary estrogen (see reviews by Albert, 1961; Velle, 1966). The results of several experiments employing males of species other than the rat suggest that endogenous estrogens are produced primarily by cells in the testis and to a lesser degree by cells in the adrenal cortex (Velle, 1966). The functional significance in the male of such comparatively small amounts of circulating estrogen has not yet been clarified; however, two tenable hypotheses are available.

Maturation of the brain-pituitary-gonad axis. Noting the potent ability of small doses of estradiol to inhibit the secretion of pituitary gonadotropin, Davidson (1966b) suggested that in the male, endogenous estrogens may constitute a primary source of negative feedback control on the hypothalamic mechanisms regulating the secretion of pituitary gonadotropins. Since endogenous estrogens are present in the prepuberal male rat, they may in conjunction with circulating androgen exert the negative feedback required to delay the functional resetting of
the brain-pituitary-gonad axis which occurs at puberty. Recent evidence reviewed earlier in this paper indicated that in the female rat very small quantities of estrogen may have a temporary, facilitatory influence on the hypothalamic nuclei responsible for the puberal surge in the synthesis and release of LH and FSH. It is conceivable that endogenous estrogens in the puberal male may also exert this type of temporary, positive feedback effect in addition to a long term, inhibitory influence on pituitary function.

The development of mating behavior. The results of the current experiments suggest a second regulatory role for estrogenic steroids in the prepuberal male. Endogenous estrogens along with larger amounts of circulating androgen which become available as prepuberal males grow older may be factors responsible for a gradual lowering of response thresholds in neural mechanisms mediating mating behavior. Once these thresholds are low enough and the testes have been stimulated by gonadotropins to produce adequate amounts of androgen, gonadal hormones subsequently facilitate the response of these mating mechanisms to sexual stimuli in the environment. The results of Experiment 5 suggested that estradiol, like androgen, is able to exert a direct activating influence on these same mechanisms. However, the detection of only small quantities of urinary estrogen in male rats suggests that the concentrations
of circulating estrogens are also very low. Therefore it is quite unlikely that enough endogenous estrogens are ever present in the bloodstream to exert significant activational effects.

Gonadal hormones may facilitate the actual exhibition of adult male mating behavior in at least two ways. One possibility is that androgens somehow attenuate the inhibitory influence exerted by higher cerebral structures upon sexual reflex mechanisms in the spinal cord (Beach, 1967). As a result these reflexes (including pelvic movements and intromission) can be successfully activated in the presence of a receptive female. Sensory input to the penis resulting (in the rat) from a series of intromissions has an additive effect at some cerebral location, and it eventually reaches a threshold level where the spinal ejaculation reflex is briefly disinhibited from cortical control (Hart, 1967; 1968b).

Despite their great importance, the possession of reflex mechanisms involved in mating does not in itself insure that copulation will occur. Sexual reflexes can be successfully activated only after the male has solicited, chased, and mounted a receptive female. Implanting androgen directly into the hypothalamus of castrated male rats in which mating behavior had disappeared restored sexual performance to levels exhibited prior to castration (Fisher, 1956; Davidson, 1966a; Lisk, 1967). In each instance the restoration of sexual performance included
a revival of sexual interest and aggressiveness in males toward receptive females as well as the revival of intromission and ejaculation responses. These results in conjunction with Beach's (1967) proposals suggest that in the adult male rat androgen maintains a readiness to respond in neural structures which mediate preliminary sexual behavior. When a sexual object becomes available these preliminary responses are activated and in conjunction with androgen facilitate the release of appropriate spinal reflexes from cerebral inhibition.

The present hypothesis asserts that small amounts of estrogen circulating in the prepuberal male may gradually increase the readiness with which adult-sized concentrations of endogenous androgen are able to activate mating for the first time. More specifically, early exposure to estrogen for several weeks may make either the cerebral inhibitory structures or the hypothalamic "sexual motivation" mechanisms more sensitive to the activating influence of androgen. Exposure to social stimuli throughout the prepuberal period or the appearance of comparatively large concentrations of circulating androgen just prior to initial copulation may also influence the final threshold setting of the neural mechanisms which mediate mating. Estrogen, however, appears to be more effective than androgen in altering neural sensitivity in the prepuberal male. An empirical comparison of the abilities of estrogen and social experience in this respect
is not available. In any event, the ages at which intromission
and ejaculation patterns first appear in a normal male rat
reflect the amount of time required for the combined effects
of these hormonal and social factors to occur. The puberal
male is first able to copulate when the thresholds of neural
mechanisms mediating mating have fallen to a level matched by
the concentration of circulating gonadal hormones.
Summary

Interest in the functional significance of gonadal hormones for masculine mating behavior in the male has centered around (a) the role of androgen in directing psychosexual differentiation during prenatal or early postnatal life, and (b) the ability of androgen to facilitate male sexual behavior in adulthood. The influence of exogenous estrogen administered neonatally or in adulthood on mating in the adult male has also been examined; however, few experiments have been reported in which the intent was to elucidate the behavioral significance of endogenous estrogen in males of various ages.

Several investigators have injected large doses of androgen into males of several species to discover whether masculine mating patterns were available for activation prior to puberty. However, this paper describes the first systematic studies of the control exerted by different gonadal hormones in timing the development of mating behavior in the male rat. Daily injections of a high dose of testosterone propionate or a low dose of estradiol benzoate beginning after the tenth day of life facilitated precocious mating behavior in male rats; however, low doses of testosterone and a high dose of estradiol were not effective. These experiments specified a minimal dose of testosterone required to accelerate the first
appearance of copulation in male rats and also showed for the first time that certain doses of estradiol may have similar, facilitatory effects on the development of mating behavior.

The age at which prepuberal male rats initially received daily injections of a low dose of estradiol did not influence the first occurrence of intromission responses. However, fewer males which had first received estradiol prior to Day 11 exhibited intromission during tests administered several weeks after estradiol injections were stopped. The results of two additional experiments indicated that daily injections of a low dose of estradiol beginning on Day 11 probably affected both the sensitivity setting as well as the activation of neural mechanisms which mediate mating behavior in male rats.
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### TABLE 1

Ages at First Intromission and First Ejaculation for Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>21</td>
<td>57.25</td>
<td>44-90</td>
<td>17/21</td>
<td>58.00</td>
<td>45-90</td>
<td>17/21</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>11</td>
<td>38.25</td>
<td>34-77</td>
<td>11/11</td>
<td>48.75</td>
<td>34-90</td>
<td>9/11</td>
</tr>
<tr>
<td>.05 µg. TP</td>
<td>11</td>
<td>52.00</td>
<td>43-90</td>
<td>9/11</td>
<td>52.00</td>
<td>47-90</td>
<td>9/11</td>
</tr>
<tr>
<td>5 µg. TP</td>
<td>11</td>
<td>55.33</td>
<td>40-90</td>
<td>10/11</td>
<td>55.33</td>
<td>46-90</td>
<td>10/11</td>
</tr>
<tr>
<td>1000 µg. TP</td>
<td>11</td>
<td>48.00</td>
<td>34-88</td>
<td>11/11</td>
<td>48.25</td>
<td>34-88</td>
<td>11/11</td>
</tr>
</tbody>
</table>

\[ H^a = 11.77** \quad \chi^2 = 4.74 \quad H^a = 9.96* \quad \chi^2 = 2.80 \]

\(^a\) Kruskal-Wallis

\(* p < .05\)

\(** p < .02\)
## Table 2

Performance During 7 Mating Tests in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median number of tests with intromission</th>
<th>Range</th>
<th>Median number of tests with ejaculation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>5.88</td>
<td>0-7</td>
<td>5.88</td>
<td>0-7</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>6</td>
<td>6.00</td>
<td>1-7</td>
<td>4.00</td>
<td>0-7</td>
</tr>
<tr>
<td>.05 µg. TP</td>
<td>5</td>
<td>6.00</td>
<td>5-7</td>
<td>6.00</td>
<td>5-7</td>
</tr>
<tr>
<td>5 µg. TP</td>
<td>5</td>
<td>6.68</td>
<td>3-7</td>
<td>5.25</td>
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</tr>
<tr>
<td>1000 µg. TP</td>
<td>6</td>
<td>5.50</td>
<td>0-7</td>
<td>5.50</td>
<td>0-6</td>
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<tr>
<td>Kruskal-Wallis H</td>
<td>1.51</td>
<td></td>
<td></td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>N</td>
<td>Median age (days)</td>
<td>Body (g.)</td>
<td>Two testes (mg./100g.B.W.)</td>
<td>Two accessory organs (mg./100g.B.W.)</td>
</tr>
<tr>
<td>---------------</td>
<td>----</td>
<td>-------------------</td>
<td>-----------</td>
<td>---------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Oil</td>
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<td>268.6</td>
<td>1106.8</td>
<td>162.5</td>
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<td>3</td>
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<td>151.0</td>
<td>182.7</td>
<td>74.1</td>
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<td>.05 µg. TP</td>
<td>4</td>
<td>55.5</td>
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<td>5 µg. TP</td>
<td>5</td>
<td>57.0</td>
<td>253.0</td>
<td>1197.2</td>
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<tr>
<td>1000 µg. TP</td>
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<td>53.0</td>
<td>209.4</td>
<td>903.6</td>
<td>485.6</td>
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<td>2.39</td>
<td>11.21*</td>
<td>14.77***</td>
<td>15.99***</td>
<td>13.00**</td>
</tr>
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</table>

* p < .05
** p < .02
*** p < .01
TABLE 4

Ages at First Intromission and First Ejaculation for Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>59</td>
<td>42-83</td>
<td>11/11</td>
<td>62</td>
<td>50-83</td>
<td>11/11</td>
</tr>
<tr>
<td>50 μg. TP</td>
<td>12</td>
<td>67</td>
<td>45-117</td>
<td>10/12</td>
<td>67</td>
<td>45-117</td>
<td>9/12</td>
</tr>
<tr>
<td>500 μg. TP</td>
<td>11</td>
<td>51</td>
<td>39-72</td>
<td>11/11</td>
<td>51</td>
<td>39-72</td>
<td>11/11</td>
</tr>
</tbody>
</table>

\[ H^a = 4.70 \quad \chi^2 = 4.03 \quad H^a = 5.18 \quad \chi^2 = 5.81 \]

\(^a\) Kruskal-Wallis H
TABLE 5

Performance During 7 Mating Tests in Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median number of tests with intromission and ejaculation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>5.00</td>
<td>2-7</td>
</tr>
<tr>
<td>50 µg. TP</td>
<td>9</td>
<td>5.10</td>
<td>3-7</td>
</tr>
<tr>
<td>500 µg. TP</td>
<td>11</td>
<td>2.33</td>
<td>0-5</td>
</tr>
</tbody>
</table>

Kruskal-Wallis $H = 8.95^*$

* $p < .02$
TABLE 6

Ages at First Intromission and First Ejaculation for Experiment 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
<th>Median age</th>
<th>Range</th>
<th>Proportion responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
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<td>67.00</td>
<td>44-90</td>
<td>14/15</td>
<td>67.00</td>
<td>44-90</td>
<td>14/15</td>
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<tr>
<td>.05 µg. EB</td>
<td>9</td>
<td>57.00</td>
<td>45-75</td>
<td>9/9</td>
<td>57.00</td>
<td>46-75</td>
<td>9/9</td>
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<tr>
<td>100 µg. EB</td>
<td>9</td>
<td>57.25</td>
<td>21-90</td>
<td>8/9</td>
<td>70.75</td>
<td>22-90</td>
<td>7/9</td>
</tr>
</tbody>
</table>

\[ H^a = 3.29 \quad \chi^2 = 0.94 \quad H^a = 3.92 \quad \chi^2 = 2.58 \]

\(^a\) Kruskal-Wallis \( H \)
TABLE 7

Performance During 7 Mating Tests in Experiment 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median number of tests with intromission</th>
<th>Range</th>
<th>Median number of tests with ejaculation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>6</td>
<td>6.9</td>
<td>3-7</td>
<td>6.9</td>
<td>3-7</td>
</tr>
<tr>
<td>.05 µg. EB</td>
<td>6</td>
<td>4.5</td>
<td>3-7</td>
<td>4.5</td>
<td>2-7</td>
</tr>
<tr>
<td>100 µg. EB</td>
<td>6</td>
<td>2.0</td>
<td>0-7</td>
<td>1.5</td>
<td>0-7</td>
</tr>
<tr>
<td>Kruskal-Wallis H</td>
<td></td>
<td>4.64</td>
<td></td>
<td>6.00*</td>
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</tr>
</tbody>
</table>

* p < .05
TABLE 8

Median Organ Weights for Subjects in Experiment 3 Sacrificed Following Initial Ejaculation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median age at autopsy (days)</th>
<th>Body (g.)</th>
<th>Two testes (mg./100g. B. W.)</th>
<th>Two accessory organs (mg./100g. B. W.)</th>
<th>Penis (mg./100g. B. W.)</th>
<th>Proportion of subjects with attachment of frenulum to penis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil (n = 8)</td>
<td>85.5</td>
<td>301.7</td>
<td>1035.8</td>
<td>152.6</td>
<td>66.5</td>
<td>0/8</td>
</tr>
<tr>
<td>.05 µg. EB (n = 3)</td>
<td>64.0</td>
<td>233.3</td>
<td>1130.2</td>
<td>134.3</td>
<td>80.5</td>
<td>0/3</td>
</tr>
<tr>
<td>Subject 80-3 (100 µg. EB)</td>
<td>82.0</td>
<td>161.2</td>
<td>115.2</td>
<td>79.7</td>
<td>24.2</td>
<td>1/1</td>
</tr>
</tbody>
</table>

Note: Comparisons of values for Oil and .05 µg. EB groups were made using Mann-Whitney U tests.

a Fisher Exact Probability
### TABLE 9

Age at First Intromission for Experiment 4.

<table>
<thead>
<tr>
<th>Neonatal treatment</th>
<th>N</th>
<th>Median age at first intromission</th>
<th>Range</th>
<th>Proportion responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>58.75</td>
<td>34-69</td>
<td>11/11</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>10</td>
<td>60.50</td>
<td>35-87</td>
<td>10/10</td>
</tr>
<tr>
<td>100 µg. EB (Day 4)</td>
<td>10</td>
<td>52.00</td>
<td>25-77</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Kruskal-Wallis $H = 0.99$
TABLE 10
Mount and Intromission Frequency Scores for Adult Males in Experiment 4.

<table>
<thead>
<tr>
<th>Neonatal treatment</th>
<th>N</th>
<th>Median mount frequency score</th>
<th>Proportion mounting once during any test</th>
<th>Median intromission frequency score</th>
<th>Proportion intromitting once during any test</th>
<th>Median intromission score for those subjects intromitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>31.00</td>
<td>11/11</td>
<td>10.00</td>
<td>11/11</td>
<td>10.00</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>9</td>
<td>24.00</td>
<td>9/9</td>
<td>5.75</td>
<td>5/9</td>
<td>8.00</td>
</tr>
<tr>
<td>100 µg. EE (Day 4)</td>
<td>8</td>
<td>34.00</td>
<td>8/8</td>
<td>11.00</td>
<td>6/8</td>
<td>11.50</td>
</tr>
</tbody>
</table>

\[ H^a = 4.88 \quad \chi^2 = 0 \quad H^a = 4.63 \quad \chi^2 = 6.08^* \quad H^a = 1.64 \]

\(^a\) Kruskal-Wallis H

\(^*\) \( p < .05 \)
TABLE II

Organ Weights for Subjects in Experiment 4 Sacrificed at 117 Days of Age.

<table>
<thead>
<tr>
<th>Neonatal treatment</th>
<th>N</th>
<th>Body (g.)</th>
<th>Two testes (mg./100g.B.W.)</th>
<th>Two accessory organs (mg./100g.B.W.)</th>
<th>Penis (mg./100g.B.W.)</th>
<th>Proportion of subjects with attachment of frenulum to penis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>11</td>
<td>302.1 ± 9.0</td>
<td>990.5 ± 22.7</td>
<td>193.7 ± 22.7</td>
<td>59.6 ± 3.2</td>
<td>3/11</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>9</td>
<td>280.0 ± 16.6</td>
<td>739.7 ± 57.9</td>
<td>89.0 ± 9.0</td>
<td>48.0 ± 2.5</td>
<td>6/9</td>
</tr>
<tr>
<td>100 µg. EB (Day 4)</td>
<td>8</td>
<td>287.7 ± 22.8</td>
<td>769.8 ± 95.2</td>
<td>76.5 ± 8.4</td>
<td>44.4 ± 3.2</td>
<td>8/8</td>
</tr>
</tbody>
</table>

\[ F = 0.55 \quad F = 5.87^{**} \quad F = 15.47^{**} \quad F = 7.31^{**} \quad \chi^2 = 10.41^{*} \]

Note: Data are expressed as mean ± standard error of the mean.

* p < .02

** p < .01
<table>
<thead>
<tr>
<th>Group</th>
<th>Sexual First intromission</th>
<th></th>
<th>Sexual First ejaculation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median age</td>
<td>Proportion responding</td>
<td>Median age for those responding</td>
<td>Median age</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>48.50</td>
<td>9/10</td>
<td>48.00</td>
<td>57.16</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(35-90)</td>
<td>(35-71)</td>
<td>(43-90)</td>
<td>(43-89)</td>
</tr>
<tr>
<td>Castrated</td>
<td>74.00</td>
<td>6/10</td>
<td>46.00</td>
<td>89.67</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(39-90)</td>
<td>(39-89)</td>
<td>(39-90)</td>
<td>(39-59)</td>
</tr>
<tr>
<td></td>
<td>$\bar{y}^a = 37$</td>
<td>n.s.$^b$</td>
<td>$\bar{y}^a = 25$</td>
<td>$\bar{y}^a = 39$</td>
</tr>
</tbody>
</table>

Note: Ranges are indicated in parentheses beneath each median value.

$^a$ Mann-Whitney U

$^b$ Fisher Exact Probability


<table>
<thead>
<tr>
<th>Group</th>
<th>Median number of tests with intromission</th>
<th>Range</th>
<th>Median number of tests with ejaculation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>5.25</td>
<td>2-7</td>
<td>3.00</td>
<td>0-7</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrated</td>
<td>3.16</td>
<td>3-6</td>
<td>2.83</td>
<td>2-3</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mann-Whitney U</td>
<td>7.5</td>
<td></td>
<td>13.0</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 14

The Number of Daily Injections of 50 ug. TP Prior to First Intromission and Ejaculation in Experiment 6.

<table>
<thead>
<tr>
<th>Treatment (Days 11--30)</th>
<th>N</th>
<th>Median number of injections</th>
<th>Range</th>
<th>Proportion responding</th>
<th>Median number of injections</th>
<th>Range</th>
<th>Proportion responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>10</td>
<td>42</td>
<td>14-60</td>
<td>9/10</td>
<td>42</td>
<td>18-60</td>
<td>9/10</td>
</tr>
<tr>
<td>5 μg. TP</td>
<td>10</td>
<td>43</td>
<td>24-60</td>
<td>8/10</td>
<td>44</td>
<td>24-60</td>
<td>8/10</td>
</tr>
<tr>
<td>5 μg. EB</td>
<td>10</td>
<td>28</td>
<td>14-40</td>
<td>10/10</td>
<td>29</td>
<td>14-60</td>
<td>9/10</td>
</tr>
</tbody>
</table>

\[ H^a = 6.80^* \]
\[ \chi^2 = 2.22 \]

\[ H^a = 2.84 \]
\[ \chi^2 = 1.19 \]

\(^a\) Kruskal-Wallis \(H\)

\(^*\) \(p < .05\)
TABLE 15

Organ Weights from Subjects in Experiment 6 Sacrificed One Day After Initial Ejaculation

<table>
<thead>
<tr>
<th>Treatment (Days 11--30)</th>
<th>N</th>
<th>Age (days)</th>
<th>Body (g.)</th>
<th>Two accessory organs (mg./100g.B.W.)</th>
<th>Penis (mg./100g.B.W.)</th>
<th>Proportion of subjects with attachment of frenulum to penis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>10</td>
<td>94 ± 4</td>
<td>311.1 ± 13.5</td>
<td>113.4 ± 9.2</td>
<td>57.3 ± 1.9</td>
<td>0/10</td>
</tr>
<tr>
<td>5 µg. TP</td>
<td>10</td>
<td>100 ± 2</td>
<td>338.7 ± 15.7</td>
<td>108.7 ± 12.3</td>
<td>50.1 ± 2.9</td>
<td>0/10</td>
</tr>
<tr>
<td>5 µg. EB</td>
<td>10</td>
<td>89 ± 3</td>
<td>272.3 ± 10.4</td>
<td>115.7 ± 14.2</td>
<td>56.6 ± 2.1</td>
<td>8/10</td>
</tr>
</tbody>
</table>

\[ F = 1.18 \quad F = 6.22^* \quad F = 0.09 \quad F = 2.06 \quad \chi^2 = 19.85^* \]

Note: Data are expressed as mean ± standard error of the mean.

* \( p < .01 \)
Fig. 1. Body Growth in Experiment 1.
Fig. 2. Body Growth in Experiment 3.
Fig. 3. Body Growth in Experiment 5.
Fig. 4. Body Growth in Experiment 6.