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HORMONAL DETERMINANTS IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS)

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
LINDA PATRICIA CONIGLIO
1973

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HORMONAL DETERMINANTS IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS)

presented by

Linda Patricia Coniglio

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology

Lynwood R. Clemens
Major professor

Date May 3, 1973

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ABSTRACT

HORMONAL DETERMINANTS IN THE
DEVELOPMENT OF SEXUAL BEHAVIOR IN THE GOLDEN
HAMSTER (MESOCRICETUS AURATUS)

By

Linda Patricia Coniglio

The influence of estrogen or androgens during sexual differentiation upon adult sexual behaviors of male golden hamsters (Mesocricetus auratus) was investigated. In addition, age and length of postnatal treatment with testosterone were varied to determine the period of maximal susceptibility for behavioral masculinization and defeminization in male and female hamsters.

Male hamsters castrated on the day of birth (Day 1) and treated with testosterone, testosterone propionate, estradiol, estradiol benzoate or diethylstilbestrol on days 2-4 postnatally displayed mounting behavior after testosterone propionate treatment as adults. Androsterone, dihydrotestosterone or control substances given neonatally failed to induce the potential for masculine behavior. Sexual receptivity in response to adult ovarian hormones was decreased in day 1 castrated males which received testosterone propionate or estrogens neonatally. Receptivity measures of males treated with testosterone, androsterone or dihydrotestosterone were not different from controls. Treatment of the neonatally castrated male with androgens early in life resulted in

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significant development of the penile bone and cartilage, whereas the peripheral morphology of males treated neonatally with estrogen was not different from control males.

In a second experiment, male hamsters castrated at birth and intact females were treated with 100 ug testosterone on days 1-2, 3-4, 5-6, 7-8 or 9-10 postnatally. Following androgen treatment in adulthood, animals treated on days 1-2 or 3-4 were significantly higher in mounting and intromission scores than animals treated later in life. Sexual receptivity measures of females treated on days 1-2 or 3-4 were significantly lower than animals in other treatment groups.

Castrated males treated daily on days 1-10 postnatally with 50 ug testosterone were significantly higher in masculine behavior measures, as well as significantly lower in sexual receptivity measures than males treated with 100 ug testosterone daily on days 1-5 or 6-10 postnatally. Females similarly treated on days 1-5 postnatally were higher in masculine behavior measures than females in other treatment groups. Sexual receptivity was significantly lower in females treated on days 1-10 postnatally. In addition, treatment of females on days 1-5 postnatally also resulted in a reduction in sexual receptivity.

Results of the present study indicate that the presence of estrogen, or androgens which can be converted to estrogen, during sexual differentiation induces the potential for masculine sexual behavior, and suppresses the potential for feminine sexual behavior in adult male hamsters. In addition, the present findings indicate that the period of maximal susceptibility for the induction of masculine behavior and the suppression of feminine behavior in male and female hamsters by testosterone occurs during the first five days after birth.

HORMONAL DETERMINANTS IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS)

By

Linda Patricia Coniglio

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1973

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I came to take of your wisdom: And behold I have found that which is greater than wisdom. You have given me my deeper thirsting after life.

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ACKNOWLEDGEMENTS

I would like to express my sincere thanks to those who served on my academic guidance committee: Dr. Harold Hafs, Dr. John King and Dr. Robert Raisler. I would also like to thank Dr. Martin Balaban who provided critical evaluation of this research. To my Chief, Dr. Lynwood Clemens, I owe a special word of thanks for providing the sparks of excitement and enthusiasm which set ablaze the fires of academic curiosity and pursuit.

I also thank my family for the faith and encouragement which they never ceased to show. A special word of thanks is given to Trail Blazer Camps for providing the opportunity which initiated my interest and love in the mysteries of natural science. Finally, I thank my friends and fellow graduate students who have witnessed both the ebb and flood of my tide during my tenure in graduate school.

This research was supported by U. S. P. H. S. Training Grant: GM01751-01 from the National Institute of General Medical Science, and U. S. P. H. S. Research Grant: HD06760-01.

TABLE OF CONTENTS

TABLE OF CONTE

TABLE OF CONTENTS--continued

TABLE

LIST OF TABLES

FIGURE

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LIST OF FIGURES

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LIST OF FIGURES--continued

FIGURE Page

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INTRODUCTION

Patterns of adult sexual behavior in several mammalian species appear to be influenced by the presence of androgen or estrogen during early development (Phoenix, Goy, Gerall and Young, 1959; Whalen and Nadler, 1963; Levine and Mullins, 1964; Harris and Levine, 1965; Gerall, 1967; Goy, 1970; Beach, Noble and Orndoff, 1972; Carter, Clemens and Hoekema, 1972). In general, these studies have shown that the presence of androgen or estrogen during development induces the potential for masculine behavior, and reduces the potential for feminine behavior in the adult. In the absence of androgen or estrogen early in life, the feminine pattern of behavioral development occurs. Although much is known about the role of gonadal hormones in sexual differentiation of behavior in the rat and guinea pig, the hamster may be the preferred species to employ as an experimental preparation since sexual differentiation of behavior occurs postnatally in the hamster (Nucci and Beach, 1971), whereas in the rat and guinea pig this process begins prenatally (Gerall and Ward, 1966; Goy, Bridson and Young, 1964).

The male hamster, castrated on the day of birth, displays little or no adult masculine sexual behavior in response to testosterone treatment (Eaton, 1970; Swanson, 1971). Likewise, the female hamster does not mount as an adult, even after extensive testosterone treatment in adulthood (Crossley and Swanson, 1968). However, a single injection of testosterone propionate given early in life to either the castrated male or the female results in the capacity to show mounting behavior

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as an adult after testosterone treatment. Similarly, perinatal treatment of the castrated male or the female with androgen suppressed the capacity to display female sexual behavior in response to ovarian hormones (Crossley and Swanson, 1968; Eaton, 1970; Carter, et al., 1972).

Behavioral masculinization and defeminization has also been reported in female hamsters treated perinatally with the synthetic estrogen, diethylstilbestrol (Paup, Coniglio and Clemens, 1972; Coniglio, Paup and Clemens, 1973). Other investigators (Ciaccio and Lisk, 1971) have reported a disruption of gonadal function in female hamsters treated perinatally with estradiol benzoate (EB). However, no suppression of sexual receptivity was found after estrogen and progesterone replacement in adulthood in these EB treated females. The discrepancy between the results of this study and those reported by Coniglio et al., (1973) may be due to differences in hormonal action between estradiol benzoate and diethylstilbestrol.

The present study was designed to extend our knowledge of hormonal influences on the development of sexual behavior in the golden hamster (Mesocricetus auratus). The investigation focused upon three critical problems. First, studies with adult male rats have shown that masculine patterns of behavior can be induced or maintained by treatment with androgens which convert to estrogen, but not by non-convertible androgens (McDonald, et al., 1970; Luttge and Whalen, 1971). Within this framework, the in vitro conversion of androgen to estrogen reported to occur in the placenta (Ryan, 1960), fetal liver (Telegdy, 1971) and the diencephalon (Naftolin, Ryan and Petro, 1971b) emerges as a possible mechanism influencing behavior development. Thus, the hypothesis to be

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tested in the first experiment was that treatment during sexual differentiation with estrogen, or androgens which can be converted to estrogen, would induce the potential for masculine behavior, and suppress the potential for feminine behavior in the adult.

Second, perinatal androgen treatment affects the development of masculine genital structures as well as masculine behavior (Beach and Holz, 1946; Mullins and Levine, 1969; Beach, et al., 1969). There is disagreement, however, as to whether the observed changes in masculine behavior result only from alterations in genital morphology. Therefore, a second hypothesis to be tested by Experiment I was that the effects of perinatal hormone treatment upon adult masculine behavior result from modifications of the central neural systems mediating behavior, rather than modifications in peripheral morphology.

Third, the defeminization of female rats and hamsters observed with perinatal testosterone propionate treatment was not obtained when perinatal treatment of the non-propionate form of testosterone was used (Luttge and Whalen, 1971; Coniglio, et al., 1973). One possibility for this discrepancy is that the period of development during which adult sexual behavior can be influenced by testosterone treatment occurred earlier, or later than the treatment period used in previous studies. Thus, the hypothesis to be tested in Experiment II was that treatment with testosterone during the period of maximal susceptibility to modification of adult sexual behavior potential (ie. a critical period) would result in masculinization and defeminization of adult sexual behavior.

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A second alternative regarding the differences between perinatal treatment with testosterone and testosterone propionate may be that masculinization by perinatal testosterone requires a longer period of treatment than with testosterone propionate. This alternative is supported by the finding that the duration of hormone action is longer for testosterone propionate than for testosterone (Miescher, Wettstein and Tschopp, 1936). Therefore, the hypothesis tested in Experiment III was that masculinization and defeminization of adult sexual behavior would result from prolonged exposure to testosterone during early postnatal development.

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BACKGROUND

Bisexual Organization of the Vertebrate Embryo

During development, the gonad goes through a sexually undifferentiated or indifferent stage. During this period, the primordial structures for the development of either sex are present. The indifferent gonad is composed of a central core of epithelial tissue, surrounded by germinal epithelial tissue. Testicular differentiation involves greater development of the inner (medullary) portion of the gonad, while ovarian differentiation is accompanied by greater development of the cortex. The primordial germ cells become surrounded by the germinal epithelial, which grow into primary sex cords. If the gonad differentiates into a testis, the primary sex cords persist and form the seminiferous tubules of the testis. The primordial germ cells become spermatogonia. If the gonad differentiates into an ovary, the primary sex cords regress and a new growth of the cortex occurs, giving rise to secondary sex cords. The primary germ cells then multiply and become ovarian follicles containing ova. The total number of follicles is complete at birth, with no further increase in number throughout the life of the animal. Differentiation of the testis in several animals studied (rat, guinea pig and rabbit) occurs earlier and more rapidly than that of the ovary (Price and Ortiz, 1965). Evidence is unavailable as to whether differentiation of the indifferent gonad is influenced by the presence of a fetal testicular hormone (Jost, 1965).

The remaining components of the reproductive system, that is, the duct system, the urogenital sinus, and the external genitalia, also go through an

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Table 1. Homologies of male and female reproductive systems*

Indifferent	Male	Female
Gonad	testis	ovary
Mesonephric or Wolffian Duct	epididymis vas deferens ejaculatory duct	rudimentary
Mullerian Duct	rudimentary	fimbria of oviduct oviduct uterus vagina (in part)
Urogenital sinus	prostatic membranous and cavernosa urethra Bulbo-urethral gland Prostate	urethra vaginal vestibule vagina (in part) vestibular glands
Genital Tubercle	Glans Penis Corpus Penis	Glans Clitoris Corpus Clitoridis
Urethral Folds	Raphe of Scrotum penis	labia minora
Labioscrotal Swellings	scrotum	labia majora

* Modified from Nalbandov, 1962.

undifferentiated stage. The onset of sexual differentiation of these structures generally occurs after the onset of differentiation of the gonad. The differentiation of the male reproductive structures are presumed to be hormone dependent. The differentiation of homologous structures of the reproductive system are summarized in Table 1.

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Hormonal Theory of Sex Differentiation

The notion that differentiation of the genital structures in the embryo is controlled by a hormone or hormones produced by the embryonic gonad was first proposed by Bouin and Ancel (1903). Their hypothesis was based upon the observation of an unusually rich interstitium in the testes of pig embryos during the period of sexual differentiation. This hypothesis suggested that the testis is active endocrinologically during development, an idea which was later confirmed empirically (see Price and Ortiz, 1965 for review).

The studies of Lillie (1916, 1917) and Keller and Tandler (1916) concerning the freemartin effect further advanced the hormonal theory of sexual differentiation. The term freemartin was applied to the female of heterosexual twins in cattle. This female, usually sterile, possessed underdeveloped ovaries which sometimes contained seminiferous tubule-like structures. The derivatives of the Mullerian ducts are more or less completely absent, and the presence of seminal vessicles is common (Jost et al., 1972). The conclusion drawn from the observation of such freemartins was that the abnormal development of the female twin calf results from a hormone produced by the gonad of the male twin. The female twin is exposed to this hormone from an early stage of development as a result of vascular anastomosis between placentas.

These hypotheses stimulated experimental embryologists to investigate the hormonal control of sex differentiation by the methods of parabiosis and orthotopic transplantation. Avian and amphibian embryos were used in these experiments since manipulation could be accomplished with relative ease. Using these methods, it was observed that gonadally secreted substances influenced the differentiation of the gonad itself (for review, see Burns, 1961). Studies of sex differentiation were further elaborated in the 1930's when purified steroid hormones became available. Morphological alterations of the gonad,

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or in some cases, complete sex reversal was possible by hormonal manipulation of various species of amphibian and avian embryos. For example, treatment of Rana temporaria tadpoles with testosterone propionate transformed all genetic females into permanent and functional males (Gallien, 1944).

In avian embryos, sex reversal was not demonstrated until purified steroids became available. Experiments of Kozelka and Gallagher (1934), Wolff and Ginglinger (1935), and Willier, Gallagher and Koch (1935, 1937) showed that estrogens affect the differentiation and transformation of the embryonic testes, whereas the embryonic ovaries were not significantly affected. Androgens, on the other hand, were less effective in transforming the ovaries of birds than those of amphibians. These results appear contradictory to results obtained with amphibians, however, it should be noted that in amphibians, the homogametic sex is the female, whereas in avians, the homogametic sex is the male. In general, the homogametic sex is the most readily reversible.

Sex differentiation in birds is further complicated by the peculiar lateral asymmetry of the genital system of the female. The left ovary develops and is functional, while the right ovary becomes rudimentary (for review, see Nalbandov, 1962). In addition, although the rudimentary right ovary can experimentally be made functional, the stage of development and hormonal treatment is particularly critical (Nalbandov, 1962).

Dramatic modification of a mammalian gonad by the administration of a steroid hormone has been observed in the opossum (Didelphis virginiana). Marsupials are so undeveloped at birth that virtually the entire course of morphological sex differentiation takes place postnatally, and pouch young are easily accessible for manipulation. Embryonic testes, treated during early development, have been transformed into ovotestes or even ovaries

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The demonstration that modification of gonadal development is possible by transplanted tissue or hormone administration merely shows that the gonadal tissues are capable of responding to external inducing agents. These results do not provide evidence that sex hormones are present and functional in the normal process of sex differentiation. A direct method of answering this question would involve removal of the fetal gonad.

Among several mammalian species, gonadectomy of the embryo results in the development of female reproductive structures, regardless of genetic sex (rabbit, Jost, 1947; mouse, Raynaud and Frilley, 1947; rat, Wells, 1950). As a test of whether hormonal influences from other sources, i.e. maternal hormones, were affecting differentiation, Mullerian ducts were isolated and cultured in vitro. These explanted Mullerian ducts from rat embryos of either sex resulted in differentiation of female structures. In addition, if embryonic testis tissue or TP were added to the culture medium, Mullerian involution occurred (Jost and Bergerard, 1949). These studies indicate that the fetal ovary has no observable effect on sexual differentiation. These results further showed that the fetal testes inhibit female reproductive structures, as well as stimulate the differentiation of male reproductive structures.

In general then, the morphological influence of sex hormones upon sex differentiation in eutherian mammals is summarized in Table 2 (for review, see Jost, 1953; Burns, 1961).

In summary, the present theory of sexual differentiation in mammals is based on the presence or absence of testicular hormones for the development of the male or female reproductive morphology. The presence of testicular hormone appears to serve a dual role: to insure development of male structures,

Table 2.

EARLY TREAT
ESTROGEN
ANDROGEN
CASTRATION

Table 2. Morphological influence of sex hormones upon sex differentiation in eutherian mammals.

EARLY TREATMENT	FEMALE SYSTEM	MALE SYSTEM
ESTROGEN	non-specific stimulation of Mullerian ducts	little influence on Wolffian ducts
	vaginal hypertropy	vaginal urogenital sinus and vagina
	female genital development	genitalia feminized
ANDROGEN	inhibits Mullerian ducts	hypertropy of Wolffian ducts
	inhibits vaginal development	male-type urogenital sinus
	enlarged clitoris	male-type genitalia
CASTRATION	development of Mullerian ducts	Wolffian ducts fail to develop
	vaginal development	vaginal development
	female genitalia	genitalia feminized

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and to inhibit the differentiation of female structures. Estrogens are not believed to play an essential role in primary sex differentiation.

Sexual Differentiation of Physiological Reproductive Function

The reproductive physiology of the normal female mammal is characterized by a cyclic secretory pattern of pituitary and ovarian hormones. These hormonal changes result in growth of ovarian follicles, the release of ova, proliferation of uterine endometrium, growth of mammary alveoli and ducts, and the occurrence of behavioral receptivity. The reproductive physiology of the male, on the other hand, is characterized by an acyclic, or tonic pattern of hormone secretion, sperm production and copulatory motivation (disregarding seasonal influences).

Since the pituitary gland was known to control the functioning of other endocrine glands, it was assumed that the male pituitary functioned differently than did the female pituitary. In 1936, Pfeiffer showed that hormonal factors operating early in development had a permanent effect upon the male and female physiological pattern. Male rats castrated at birth were implanted with ovaries in adulthood. These ovaries developed follicles and ovulated in the characteristic female pattern. Ovaries implanted into adult castrated males developed follicles, but did not ovulate. These observations led Pfeiffer to conclude that androgen early in life induced the acyclic pattern in the male pituitary, whereas the absence of androgen allowed the development of a cyclic pituitary pattern.

This hypothesis received much attention in the ensuing years. It was later found that pituitary glands transplanted from males to females functioned in a cyclic manner, and pituitaries transplanted from females to males functioned in a tonic manner. Harris in 1955, after a series of experiments on the hormonal influences of sexual differentiation, concluded that the difference

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between male and female pituitary function was due to differences in the hypothalamus. He agreed with Pfeiffer that the development of the acyclic pattern of the male is dependent upon stimulation by androgen early in life. However, he added that the androgen acted upon some hypothalamic mechanism.

Additional researches have supported this point of view. Administration of androgen to female rats during the first few days of life has resulted in anovulatory ovaries and a state of persistent estrus characteristic of tonic gonadotropin release (Barraclough and Gorski, 1962; Harris and Levine, 1962). Further, castration of the male rat shortly after birth has resulted in the potential for cyclic hypothalamic-pituitary function. Implantation of ovaries into these males results in ovulations as indicated by the presence of corpora lutea (Takewaki, 1962; Harris, 1964; Gorski and Wagner, 1965). In addition, the administration of the potent antiandrogen, cyproterone acetate, to male rats pre-and postnatally resulted in the formation of corpora lutea in ovarian grafts (Neumann and Steinbeck, 1972).

In summary, then, the present hypothesis regarding the control of gonadotrophic function proposes that the hypothalamus of the rat is undifferentiated or inherently feminine (cyclic). The presence of endogenous or exogenous androgen during sexual differentiation results in masculinization. This is manifested by the loss of cyclic activity of the anterior hypothalamic area, and the loss of the capacity for the cyclic release of ovulating amounts of luteinizing hormone (LH). Regulation of follicle stimulating hormone (FSH) is unaffected by androgen treatment, and is, therefore, maintained at a tonic level (Gorski, 1966).

Experimental Analysis of Sexual Differentiation of Behavior

Development of masculine behavior in males and females:

The hypothesis that morphological and physiological masculinization or feminization results from the presence or absence of androgen during sexual

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differentiation has recently been applied to the development of mating behavior in mammals. In a classic study of the effects of prenatal androgen on the mating responses of female guinea pigs, Phoenix and his coworkers (1959) concluded that during the period of sexual differentiation the neural tissues mediating mating behavior are organized in the direction of masculinization by androgenic stimulation. Their conclusion was based upon the observation that female offspring of androgen treated mothers had a heightened responsiveness to male hormone in adulthood, as measured by the frequency of mounting behavior, and a suppression of the capacity to estrogen and progesterone, as measured by sexual receptivity. The sexual behavior of male offspring was unaffected. A later study (Goy, et al., 1964) showed that the masculinizing effect of androgen was most effective between 30 and 35 days of gestation in the guinea pig.

The analogy which was proposed as a result of these experiments stated that behaviorally, as well as morphologically, the embryo can be considered as possessing bisexual potential. In the male, endogenous androgen, presumably by action upon neural tissues, induces development of mechanisms which, in the adult, will be responsive to androgen in the mediation of masculine sexual behavior. The absence of androgen in the female allows the development of a mechanism which will be sensitive to estrogen and progesterone in the mediation of feminine sexual behavior. This concept proposed by Phoenix and his coworkers (1959), often termed the "organizational hypothesis", has been strengthened by the finding that the administration of testosterone propionate early in life to females of several mammalian species enhanced masculine responses, and reduced feminine responses (Edwards

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and Burge, 1971; Gerall, 1967; Harris and Levine, 1965; Beach, et al., 1972; Goy, 1970; Carter, et al., 1972). The following discussion analyzes the data favoring and disfavoring the role of androgen in the organization of adult sexual behavior.

The concept of hormonal organization of reproductive behavior has been extensively examined in the rat. Several investigators have indeed found that exposure of female rats to androgen perinatally increases masculine behavior (mounting and intromission) in response to adult testosterone treatment (Koster, 1943; Harris and Levine, 1965; Nadler, 1969; Gerall and Ward, 1966). The most dramatic effect was obtained by treatment with androgen pre- and postnatally (Ward, 1969; Whalen and Robertson, 1968). Females so treated consistently showed the complete pattern of male copulatory behavior, including the ejaculatory response.

Although these data lend credence to the concept that androgen influences the development of masculine behavior, alternative interpretations have also been proposed and results of several studies have challenged the basic assumptions of this concept. According to the organizational hypotheses proposed by Phoenix et al., (1959), females should display feminine behaviors, and not display masculine behaviors. However, Beach and Rasquin (1942) have shown that female rats display mounting behavior when tested with receptive female partners. It was further found that mounting frequency was unaffected by ovarian hormones, that is, the stage of the estrous cycle did not influence this measure. In a subsequent study, Beach (1942) showed that TP treatment in adulthood increased mounting frequency of normal female rats. Although the masculine behavior of female rats was at a lower level than that shown by

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males, the conclusion proposed was that, in the absence of neonatal androgenic stimulation, the female is capable of mediating masculine responses.

Furthermore, treatment of female rats with androgen neonatally has not, according to some investigators, enhanced mounting behavior of those females (Whalen and Edwards, 1967; Whalen, et al., 1969). These authors suggest that the neural substrate for adult masculine behavior exists in both males and females, that it is genetically determined, and that it is independent of gonadal hormones. The increase in intromission frequency observed in these females treated perinatally with TP (often referred to as androgenized females) was believed to result from clitoral enlargement as a result of neonatal androgen treatment. An alternative hypothesis, suggested by Clemens and Coniglio (1970) and Ward and Renz (1972), is that mounting behavior of female rats is a result of prenatal exposure to some masculinizing hormonal factor.

Although testosterone (or its propionate form) has been predominantly used to induce the development of masculine behavior in neonatal rodents, the induction of behavioral masculinization is not specific to androgen. Treatment of the female, as well as the neonatally castrated male rat within the first few days after birth with estradiol benzoate increased masculine behavior over that of controls when the animals were treated with androgen as adults (Levine and Mullins, 1964). Intromission and ejaculatory responses were decreased in the males. However, intromission frequency of neonatally estrogen treated females exceeded that of controls, and several estrogen treated females displayed the ejaculatory pattern (Levine and Mullins, 1964). While androgen treatment early in life influences phallic development in males and females (Beach and Holz,

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1946; Whalen and Edwards, 1967; Beach, Noble and Orndoff, 1969; Mullins and Levine, 1969), estrogen treatment has not been reported to have this effect. Thus, it appears difficult to interpret the masculinizing effects of perinatal estrogen as a result of increased phallic development.

Additional evidence challenging the hypothesis that androgens function in the organization of masculine behavior has been presented in several studies involving castration of the male early in life. According to the hypothesis of Phoenix and his coworkers, these males should show little masculine behavior as adults. However, mounting behavior of neonatally castrated male rats in response to adult TP treatment was not impaired (Beach and Holz, 1946; Grady, Phoenix and Young, 1965; Whalen and Edwards, 1967; Beach, Noble and Orndoff, 1969). Although mounting behavior was unaffected, copulatory behavior of these neonatally castrated males was not equivalent to that of adult castrates after TP treatment in adulthood. Intromission and ejaculatory responses of neonatally castrated males were significantly decreased. However, as Beach and Holz (1946) pointed out previously, neonatal castration of the male results in the development of a very small penis. These authors concluded that the decrease in intromission and ejaculation frequency was a consequence of insufficient penile stimulation. This interpretation is supported by Whalen and Edwards (1967) and Nadler (1969).

In the experiments mentioned above, removal of the testes occurred on the day of birth. However, the testis becomes functional in the rat and other mammals prior to birth (see Price and Ortiz, 1965, for review). In the rat, androgen secretion derived from the fetal testis begins at day 15 of gestation. Therefore, it is possible that the

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differentiation of the systems mediating sexual behavior are organized during the period of morphological differentiation, or approximately day 18-19 of gestation in the rat. Thus, the observation that the day 1 castrated male shows mounting behavior in adulthood may be due to in utero exposure to fetal testicular substances.

The behavioral consequences of prenatal castration of the male rat have not yet been examined. However, a "functional castration" has been attempted by prenatal administration of the synthetic anti-androgen, cyproterone acetate, to pregnant female rats. Male offspring of mothers treated with cyproterone acetate (CA) from day 13 of gestation until birth had reduced mount and intromission frequencies (Nadler, 1969). No males receiving CA prenatally achieved ejaculation. Nadler further pointed out that CA treated males had penes which appeared smaller than those of controls. Ward and Renz (1972) found similar results with prenatal CA treatment to female rats. These authors suggest that androgenic stimulation during the prenatal period seems especially critical in the development of mounting behavior.

Development of feminine behavior in males and females:

The hypothesis of Phoenix and his coworkers (1959) proposed that the presence of androgen during development inhibits the development of systems which mediate female sexual behavior. For the female guinea pig, rat and mouse, there is experimental evidence to show that the administration of TP during sexual differentiation suppressed the potential for female sexual behavior (Phoenix, et al., 1959; Harris and Levine, 1965; Gerall, 1967; Edwards and Burge, 1971). Females treated perinatally with TP do not exhibit spontaneous estrous behavior as adults, and fail to respond to exogenous

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ovarian hormones. However, defeminization can also be accomplished by neonatal treatment of the female rat with estrogen (Koster, 1943; Harris and Levine, 1962; Whalen and Nadler, 1963; Levine and Mullins, 1964; Feder and Whalen, 1965; Whalen and Edwards, 1967). Thus, the notion that the development of masculine behavior potential is dependent upon the presence of androgen during sexual differentiation may have been too restrictive in its implication of steroid specificity.

One might presume that if the presence of androgen early in life inhibits the development of feminine behavior, feminine behavior should not occur in males. However, normal male rats have been reported to assume the lordotic posture of the sexually receptive female (Beach, 1938, 1945). On the other hand, additional studies have shown that treatment with a regime of ovarian hormones sufficient to bring the spayed female into full behavioral estrus was insufficient in eliciting this behavior pattern in the male (Davidson, 1969). In addition, males rarely display the solicitation behaviors characteristic of the female in heat; that is, hopping, darting, crouching and ear-wiggling (Aren - Engelbrektsson, et al., 1970). It has also been reported that whereas both the male and female will show a minimal frequency of lordosis when treated with estrogen, treatment of the male with progesterone does not produce the lordosis facilitation which occurs in females (Davidson, 1969). In this regard, Clemens and coworkers (1969) have reported no facilitation of the lordosis response by progesterone in androgenized females, except when low doses (10 ug) of androgen were administered neonatally.

Removal of the testes prior to sexual differentiation should allow the full development of female sexual behavior in the male. Numerous experiments have demonstrated that male rats castrated on the day of birth

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and treated with ovarian hormones as adults display female sexual behavior comparable to that shown by females (Harris, 1964; Grady, et al., 1965; Feder and Whalen, 1965; Whalen and Edwards, 1967; Beach, et al., 1969). Simultaneous treatment of the neonatally castrated male rat with androgen (TP) or estrogen (EB) inhibited the potential for female behavior (Harris and Levine, 1965; Levine and Mullins, 1964; Whalen and Edwards, 1967).

Nadler (1969) inhibited the action of prenatal androgen by treatment of the pregnant female rat with the antiandrogen, cyproterone acetate (CA). Sexual receptivity scores of males treated prenatally with CA and/or castrated on the day of birth were not different from normal females. It, therefore, appears that the inhibition of female sexual behavior by gonadal hormones is accomplished during the postnatal developmental period in the male rat.

Sexual Behavior in the Golden Hamster (Mesocricetus auratus)

A general account of the copulatory behavior of the golden hamster has been reported by Reed and Reed (1946). A quantitative analysis of the copulatory performance of the male is also available (Beach and Rabedeau, 1959).

In the golden hamster, stimulation of the pudendal region is required to induce the female to assume the copulatory posture, if she is in estrus. In the receptive posture the female extends the forelegs, flexing them slightly at the elbows, spreads the hind legs and elevates the pelvis. The back is straight or slightly concave and the tail is raised. A feature of her behavior is the retention of this posture for several minutes throughout the entire copulatory sequence while the male mounts and dismounts repeatedly.

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The male mounts the female from the rear, grasps the female's flanks and executes a series of several, rapid thrusts of the pelvic region. Most males stand on one foot while copulating. This is presumed to effect maximal elevation of his pelvis for penile insertion. If vaginal penetration, or intromission is achieved, the rapid pelvic thrusting is followed by a single deep thrust while the pelvic region is held rigidly against the female. The male dismounts and grooms the genital region between each copulatory act. A succession of mounts and intromissions is culminated by a mount with intromission and ejaculation. The ejaculatory mount is similar to mount with intromission, but during the insertion the male's elevated rear leg is spasmodically flexed and extended several times (Beach and Rabedeau, 1959).

Development of masculine behavior in males and females:

Although mounting of conspecifics occurs in a wide variety of mammalian females (see Beach, 1968, for review), this behavioral response has rarely been observed in female hamsters (Crossley and Swanson, 1968; Tiefer, 1970). Prolonged treatment of TP to adult female hamsters had no facilitatory effect on this measure.

However, the potential for mounting behavior can be induced in adult female hamsters by treatment with TP early in life (Crossley and Swanson, 1968; Carter et al., 1972; Paup, et al., 1972). The intromission pattern has also been observed in androgenized females, however, the ejaculatory response did not occur (Swanson and Crossley, 1971). Prenatal treatment with TP failed to induce the potential for masculine behavior in female hamsters (Nucci and Beach, 1971). Thus, it appears that the developmental period during which the potential for masculine behavior can be induced in this species occurs postnatally.

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Castration of the male on the day of birth results in little or no masculine behavior in response to adult TP treatment (Eaton, 1970; Swanson, 1971; Carter, et al., 1972). The intromission and ejaculatory patterns did not occur in these animals, and mounting was rarely observed. Treatment of the neonatally castrated male with a single injection of TP early in life insured the development of mounting, however intromission and ejaculatory patterns occurred less frequently in experimental animals than in controls (Eaton, 1970). The presence of the testes for the first few days of life appears necessary for the development of masculine responses in the male hamster (Carter, et al., 1972).

Development of feminine behavior in males and females:

Females in natural or induced estrus show lordosis almost immediately if placed with adult male. The lordosis posture is generally maintained for several minutes during which time the male may copulate, groom, and resume copulation several times.

Treatment of the female with androgen (TP) within the first few days after birth results in a suppression of the capacity to display natural or estrogen-progesterone induced estrous behavior (Swanson, 1971; Carter, et al., 1972). Estradiol benzoate given perinatally to females also disrupted cyclic estrous behavior (Ciaccio and Lisk, 1971). However, these estrogen treated females were reported to respond normally to exogenous ovarian hormones. Ciaccio and Lisk (1971) concluded that, whereas TP affects the development of systems regulating gonadal function and behavioral responsiveness, estrogen alters only systems regulating gonadal function. The loss of natural sexual receptivity was interpreted as a secondary result of impaired gonadal function.

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The female pattern of sexual behavior can readily be induced in male hamsters. Male hamsters castrated as adults and treated with ovarian hormones were reported to show "marked lordosis" when placed with vigorous males (Crossley and Swanson, 1968). Quantitative measures were not specified by Crossley and Swanson (1968), however, Tiefer and Johnson (1971) stated that the lordosis response of the male is significantly shorter than that shown by the female.

In contrast to the lack of responsiveness to progesterone observed in the male rat (Davidson, 1969) and the androgenized female rat (Clemens, et al., 1969), the adult castrated male hamster shows a facilitation in lordosis responding when given progesterone (Tiefer and Johnson, 1971). These contrasting results suggest that the hormonal events controlling behavioral differentiation are dissimilar between the male rat and hamster. The difference might be due to a smaller amount of androgen produced by the perinatal hamster testes, a lower threshold of hormone sensitivity for the structures involved in mediating lordosis in the rat, or different types of androgenic metabolites produced by these two species.

The potential for feminine behavior in the male hamster can be increased by removal of the testes early in development. Male hamsters castrated on the day of birth and treated with ovarian hormones as adults displayed lordosis which was similar in quality, latency and duration to that displayed by spayed females after ovarian hormone treatment (Eaton, 1970). Similar results were reported by Carter et al., (1972). Treatment of the neonatally castrated male with TP early in life suppressed the potential to display lordosis when treated with ovarian hormones in adulthood (Eaton, 1970; Swanson, 1971).

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Objectives of the Present Study

The experiments reported here have been conceptually divided into two parts: A and B.

Part A. Steroid specificity: Effects on the development of sexual behavior and genital morphology.

Experiment I: The objective was to determine whether behavioral masculinization during sexual differentiation of the male hamster could be achieved with estrogen, androgens which convert to estrogen, as well as androgens which do not convert to estrogen. The effects of these perinatal hormone treatments upon sexual receptivity of male hamsters was also examined. These studies also provided data relevant to the possible role of peripheral alterations resulting from perinatal hormone treatment upon adult sexual behavior.

Part B. The masculinizing and defeminizing potential of testosterone during sexual differentiation.

Experiment II: The objective of this study was to determine whether suppression of lordosis in male and female hamsters could be achieved by a short period of exposure to free testosterone. In addition, by varying the treatment period during the first ten days of life, data were provided relevant to the period of maximal susceptibility for the induction of behavioral masculinization and defeminization.

Experiment III: This study examined the possibility that suppression of lordosis in male and female hamsters requires prolonged exposure to testosterone during early postnatal development.

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METHODS

Experiment I

Subjects:

One hundred twenty three male golden hamsters (*Mesocricetus auratus*) born in the Hormones and Behavior Laboratory at Michigan State University were used. They were maintained on *ad libitum* food and water and a reversed 14-10 light-dark cycle, with lights off at 1100 hr. Pups were weaned at 21 days of age and housed in unisexual groups of 2-7 animals of the same age and treatment group. In general, hamsters in groups of 2-4 and 5-7 were housed in cages with dimensions of 7 x 10 x 6 in and 8 x 17 x 6 in, respectively.

Treatment Groups:

All males were castrated on the day of birth using cryogenic anesthesia. The abdominal incision was sutured with surgical silk (4-0) and the incision covered with flexible collodion. On days 2-4 after birth (day of birth considered day 1) all pups from a litter were injected subcutaneously with one of the following experimental substances: testosterone (25 or 100 ug/day), testosterone propionate (25 or 100 ug/day), androsterone (100 or 200 ug/day), dihydrotestosterone (200 ug/day), estradiol (2 or 25 ug/day), estradiol benzoate (2 or 25 ug/day), diethylstilbestrol (2 or 25 ug/day), sodium propionate (25 ug/day), or the vehicle, sesame oil.

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For injection, the needle punctured the skin at the lower end of the back, and the injected substance was deposited at the nape of the neck. A volume 0.03 ml was administered and the puncture site was sealed with flexible collodion to prevent leakage. All males were anesthetized with ether between 55 - 60 days of age for weighing and ear marking.

Test Procedures:

Masculine behavior: Weekly tests for masculine behavior were initiated at approximately 75 days of age. These tests were conducted in an air conditioned room under dim illumination between 1300 and 1700 hr. Ten gallon aquaria with wood shavings covering the floor were used as observation arenas. Each experimental animal was allowed 3 minutes to adapt to the test arena prior to the 10 minute presentation of a receptive stimulus female. Receptivity was induced in spayed stimulus females by daily injections of 12 ug estradiol benzoate for 3 days and 0.05 mg progesterone 4 hr prior to behavioral testing on the fourth day. Stimulus females were screened for receptive with vigorous stud males just prior to testing.

Male hamsters were tested weekly for mounting behavior for five successive weeks. After the first test (pre-test), 300 ug testosterone propionate was administered daily for 28 days and animals were tested 7, 14, 21, and 28 days after initiation of testosterone propionate treatment. The frequency of mounts was recorded and categorized as to whether the stimulus female was mounted from the rear, head or side. A mount was scored when the experimental animal clasped the stimulus female with his forelegs, accompanied by rapid pelvic

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Feminine behavior: The tests for female behavior were separated from the tests for male behavior by at least 6 weeks with no hormone treatment. Experimental males were tested for sexual receptivity following 3 days of estradiol benzoate (6 ug/day) treatment and 0.5 mg progesterone 4 hr prior to testing on the fourth day. This regime was repeated 10 days later. Each experimental male was placed with a previously adapted vigorous male for 10 minutes and behavioral responses were recorded on an Esterline-Angus event recorder.

The normal pattern of sexual behavior of the female hamster consists of a rigid posture with the back straight or slightly concave (lordosis), and the tail raised to permit vaginal penetration. Lordosis is normally maintained throughout the copulatory test, even after the male dismounts. Female behaviors recorded for each 10 minute test included total lordosis duration, total time (in seconds) the animal maintained the lordosis posture, lordosis frequency, the number of lordosis responses per test, and mean lordosis duration, calculated for each animal by dividing total lordosis duration by lordosis frequency. The behavior measures from the two lordosis tests were averaged for each animal.

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At the time of sacrifice, the males were laparotomized to ascertain the completeness of neonatal castration. Data from animals having testicular tissue were eliminated from the statistical analysis. Ano-genital distance was measured and the priapian bone and cartilage were dissected from the penis and measured.

The data were evaluated using analysis of variance and Duncans New Multiple Range Test (Kramer, 1956).

Experiment II

Subjects:

Fifty eight male and eighty female golden hamsters born in the Hormones and Behavior Laboratory at Michigan State University were used. Animals were maintained as described in Experiment I.

Treatment Groups:

All males were castrated on the day of birth according to the procedure described in Experiment I. Males and females from a litter were injected with 100 ug testosterone for 2 days: days 1 and 2, days 3 and 4, days 5 and 6, days 7 and 8, or days 9 and 10 after birth. The injection procedures were identical to those previously described.

Female hamsters were ovariectomized under ether between 60 and 65 days of age. All males were anesthetized with ether between 60 and 65 days of age for weighing and ear marking.

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Behavioral and Morphological measures:

Masculine and feminine behaviors were observed using the same procedures described in Experiment I. In addition, the occurrence of the intromission pattern was recorded. Intromission was defined as a single deep thrust of the pelvis preceeded by rapid pelvic thrusting. The intromission pattern was not always associated with vaginal penetration and thus, occurred with aberrant head and side mounts, as well as rear mounts.

The ovaries of the experimental females were fixed in Bouin's solution, fixed in paraffin, sectioned at 9 u and stained with hematoxylin and eosin. The sections were observed for the presence of corpora lutea.

At the time of sacrifice, the females and males were laparotomized to ascertain the completeness of castration. Data from animals having gonadal tissue were eliminated from the statistical analysis. Ano-genital distance was measured and the clitoral bone and cartilage of the female or penile bone and cartilage of the male were dissected out and measured.

The data were evaluated as described in Experiment I.

Experiment III

Subjects and treatment groups:

Twenty eight male and thirty seven female golden hamsters born in the Hormones and Behavior Laboratory were used. Animals were maintained as described in Experiment I. Males were castrated on the day of birth according to the procedures previously described. Entire litters were assigned to one of three treatment groups: Group 1-5

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received 100 ug testosterone subcutaneously in sesame oil daily on days 1 through 5 postnatally; Group 6-10 received 100 ug testosterone subcutaneously in sesame oil daily on days 6 through 10 postnatally; and Group 1-10 received 50 ug testosterone subcutaneously in sesame oil daily on days 1 through 10 postnatally. A volume of 0.03 ml was administered and injection procedures were identical to those previously described in Experiment I.

Female hamsters were ovariectomized under ether between 60 and 65 days of age. All males were anesthetized with ether between 60 and 65 days of age for weighing and ear marking.

Behavioral and morphological measures:

Masculine and feminine behaviors were observed using the same procedure as Experiment II. Histological preparation of the ovaries was identical to that described in Experiment II.

At the time of sacrifice, the males and females were laparotomized to ascertain the completeness of castration. Data from animals having gonadal tissue were eliminated from the statistical analysis. Anogenital distance was measured and the clitoral bone and cartilage of the female, or penile bone and cartilage of the male were dissected out and measured.

The data were evaluated as described in Experiment I.

Experiment I

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RESULTS

Experiment I

Mounting: Mounting behavior scores for male hamsters castrated on the day of birth and treated with androgen on days 2-4 are summarized in Table 3 and shown in Figure 1. After 28 days of adult TP treatment, analysis of variance indicated a significant difference among treatment groups in mean total mount frequency ($F=10.4$, $p<.001$) and mean rear mount frequency ($F=7.14$, $p<.001$). Further, animals which had neonatally received testosterone (25 or 100 µg) or testosterone propionate (25 or 100 µg) mounted at a significantly higher frequency than did control animals which had received sesame oil or sodium propionate (25 µg) ($F=9.0$. $p<.001$). Further analysis of rear mount frequencies revealed no significant differences between animals treated neonatally with testosterone and testosterone propionate at either dose level used. However, a positive dose response relation did appear between the high and low doses of testosterone and TP with the higher dose inducing a higher frequency of mounting behavior [($p<.05$) for TP].

In contrast to the above treatment groups, androsterone (100 or 200 µg doses) and dihydrotestosterone, both of which do not aromatize to estrogen, were not significantly different from control groups ($F=2.1$, $p>.05$). The level of mounting displayed by the animals treated with 100 µg androsterone was due entirely to one litter of animals in

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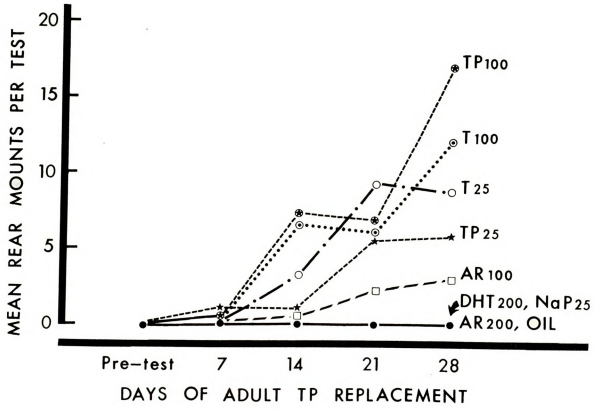

Figure 1. Mean rear mounts as a function of daily treatment with testosterone propionate in adult male hamsters castrated on the day of birth and injected perinatally with androgen. The following abbreviations are used: T, testosterone; TP, testosterone propionate; AR, androsterone; DHT, dihydrotestosterone; NaP, sodium propionate. Subscript numbers indicate microgram dosage given on days 2-4 postnatally.

Table 3. Mounting behavior in male hamsters castrated on the day of birth and treated on days 2-4 after birth with androgens or estrogens.

Neonatal Treatment	N	Dose/day (ug/.03 ml)	Mean Mount Freq. (total)*	% Animals Responding

Table 3. Mounting behavior in male hamsters castrated on the day of birth and treated on days 2-4 after birth with androgens or estrogens.

Neonatal Treatment	N	Dose/day (ug/.03 ml)	Mean Mount Freq. (total)*	% Animals Responding
Sesame Oil	11	-	0.63	36.3
Sodium Propionate	5	25	0.0	0.0
Testosterone	5	25	16.20	80.0
Testosterone	5	100	18.40	80.0
Testosterone Propionate	6	25	9.22	100.0
Testosterone Propionate	9	100	20.90	89.9
Androsterone	12	100	3.75	33.3
Androsterone	10	200	0.10	10.0
Dihydrotestosterone	8	200	2.25	62.5
Estradiol	8	2	1.77	55.5
Estradiol	10	25	14.30	70.0
Estradiol Benzoate	9	2	15.11	100.0
Estradiol Benzoate	5	25	22.80	100.0
Diethylstilbestrol	11	2	11.10	81.8
Diethylstilbestrol	8	25	17.62	100.0

* Sum of rear, head and side mounts after 28 days of adult TP treatment.

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that group. The remaining animals within that group, as well as the animals treated with 200 ug androsterone, showed virtually no mounting behavior, nor did control animals receiving either sodium propionate or sesame oil early in life (Table 3, Figure 1).

Mounting behavior scores for animals which received estrogen neonatally are summarized in Table 3 and shown in Figure 2. Analysis of mean frequency of rear mounts after 28 days of adult TP treatment indicated that all estrogen treatments, except estradiol 2 μg, resulted in significantly higher levels of mounting than control treatments ($F=4.86$, $p<.01$ for rear mounts; $F=6.12$, $p<.001$ for total mounts). Further analysis of rear mounts revealed no significant differences among the 25 μg dose of estradiol, estradiol benzoate and diethylstilbestrol ($p>.05$). Similarly, no significant difference was found between 2 μg of diethylstilbestrol and estradiol benzoate ($p>.05$).

Lordosis: Female receptivity scores for male hamsters castrated on the day of birth and treated with androgen neonatally are shown in Figure 3. Testosterone propionate (25 or 100 μg doses) given early in life suppressed total lordosis duration ($p<.05$) and mean lordosis duration ($p<.05$) in response to adult treatment with estrogen and progesterone. Lordosis suppression was greater for the 100 μg TP treated males than for the 25 μg TP treated males ($p<.05$). Males treated neonatally with testosterone (25 or 100 μg), androsterone (100 or 200 μg) or dihydrotestosterone were not different from control animals in duration of lordosis response. Lordosis frequency among androgen treated males was not statistcally different ($F=2.4$, $p>.05$).

Figure 4 presents sexual receptivity scores for male hamsters

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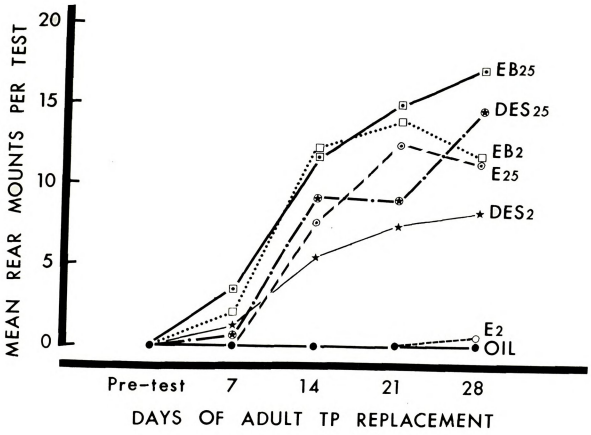
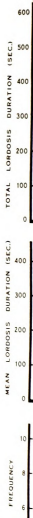


Figure 2. Mean rear mounts as a function of daily treatment with testosterone propionate in adult male hamsters castrated on the day of birth and injected perinatally with estrogen. The following abbreviations are used: E, estradiol; EB, estradiol benzoate; DES, diethylstilbestrol. Subscript numbers indicate microgram dosage given on days 2-4 postnatally.



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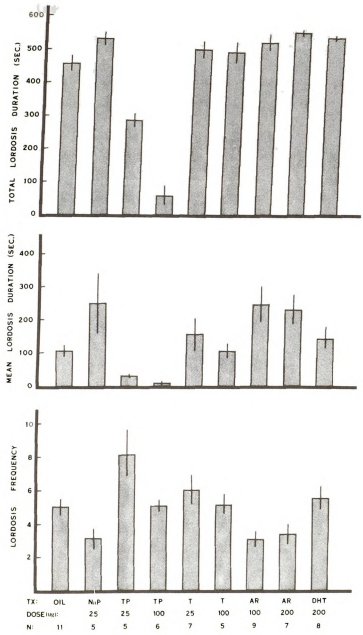

Figure 3. Effect of perinatal androgen treatment upon parameters of adult sexual receptivity in male hamsters castrated on the day of birth

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castrated on the day of birth and treated with estrogen on days 2-4 of postnatal life. Analysis of variance indicated a significant treatment effect across all estrogen treatments on total lordosis duration ($F=5.42$, $p<.001$), mean lordosis duration ($F=5.93$, $p<.001$) and lordosis frequency ($F=2.84$, $p<.05$). Duncan's New Multiple Range Test revealed estradiol benzoate (2 or 25 µg) and diethylstilbestrol (2 or 25 µg) treated animals were significantly lower than oil controls in total lordosis duration ($p<.05$) whereas estradiol benzoate (2 or 25 µg) diethylstilbestrol 25 µg, and estradiol 2 µg treated animals were significantly lower than controls in mean lordosis duration ($p<.05$). Although free estradiol 25 µg and diethylstilbestrol 2 µg decreased mean lordosis duration, this difference was not statistically significant from controls. Nor was total lordosis duration in estradiol treated males significantly different from controls. Lordosis frequency in diethylstilbestrol 25 µg treated animals was significantly higher than controls ($p<.05$), but was not different from estradiol benzoate 25 µg treated animals.

Morphological Measures: Ano-genital distance of neonatally treated male hamsters, measured at the time of sacrifice, are presented in Table 4. Analysis of variance among androgen treated animals revealed no significant differences ($F=2.49$, $p>.05$). A significant variation was found among estrogen treated males ($F=3.63$, $p<.02$). Further analysis indicated that animals treated early in life with 25 µg estradiol benzoate had significantly greater ano-genital distance than did other estrogen treated animals or control animals ($p<.05$). However, the 25 µg estradiol benzoate treated males had received daily TP treatment for 21 days prior to sacrifice. No other group received this additional androgenic stimulation.

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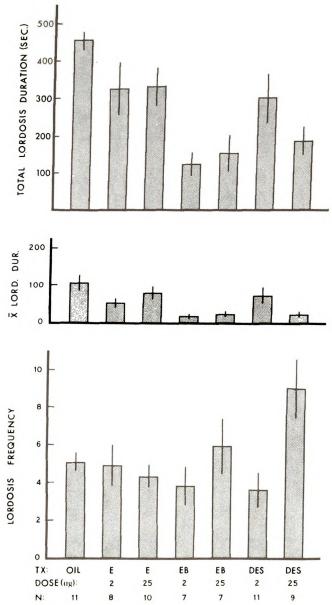

Figure 4. Effect of perinatal estrogen treatment upon parameters of adult sexual receptivity in male hamsters castrated on the day of birth.

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Table 4. Mean ano-genital distance of adult male hamsters castrated at birth and treated neonatally with androgens or estrogens.

Neonatal Treatment	Dose/day (ug/.03 ml)	A-G distance mean length (mm) ± SE
Sesame Oil	-	12.73 ± 0.24
Sodium Propionate	25	13.82 ± 0.01
Testosterone	25	12.91 ± 0.22
Testosterone	100	13.06 ± 0.22
Testosterone Propionate	25	14.31 ± 0.59
Testosterone Propionate	100	14.12 ± 0.17
Androsterone	100	12.24 ± 0.60
Androsterone	200	13.24 ± 0.51
Dihydrotestosterone	200	13.48 ± 0.46
Estradiol	2	13.36 ± 0.24
Estradiol	25	12.86 ± 0.42
Estradiol Benzoate	2	12.68 ± 0.30
Estradiol Benzoate	25	14.42 ± 0.38
Diethylstilbestrol	2	12.88 ± 0.33
Diethylstilbestrol	25	12.40 ± 0.42

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The effect of neonatal hormone treatment on priapian bone and cartilage length is shown in Fig. 5. All androgen treatments significantly increased priapian growth ($F=57.4$, $p<.001$). Further analysis indicated that TP resulted in significantly greater priapian development than other androgens ($p<.05$). Estrogen treatment early in life did not increase priapian growth over oil controls.

Experiment II

Masculine behavior of males: Masculine behavior scores for male hamsters castrated on the day of birth and treated with testosterone postnatally are summarized in Table 5 and shown in Figure 6. After 28 days of adult TP treatment, analysis of variance indicated a significant difference among treatment groups in mean total mount frequency ($F=8.07$, $p<.001$) and mean rear mount frequency ($F=4.57$, $p<.01$). Further analysis revealed that mounting frequency of males treated with testosterone on days 1-2 was not statistically different from mounting frequency of males treated on days 3-4. These two groups were, however, significantly higher in mean total mount frequency than all other treatment groups ($p<.05$). There were no significant differences in mount frequency in males treated on days 5-6, 7-8 or 9-10. It should be noted that some animals in all treatment groups displayed mounting behavior. However, the percentage of animals responding was below 50% in groups treated after day 7 postnatally (Table 5).

Intromission frequency in mount-positive animals showed a significant variation across treatment groups ($F=5.73$, $p<.005$). Further analysis revealed males treated on days 1-2 or 3-4 were significantly higher in frequency of intromission pattern than males treated on days 5-6 or 7-8

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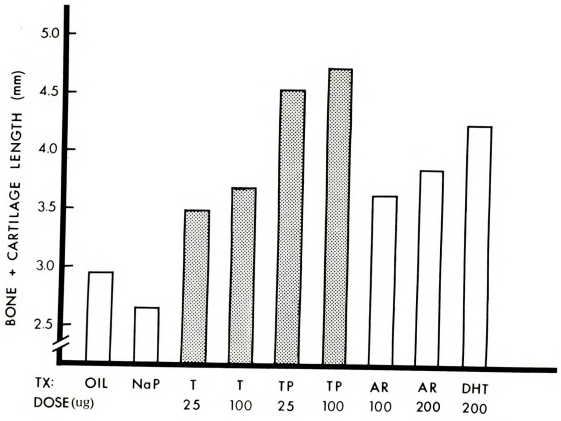


Figure 5. Mean length of priapian bone and cartilage of adult male hamsters castrated on the day of birth and treated perinatally with androgen. The shaded columns represent treatment groups which displayed masculine sexual behavior.

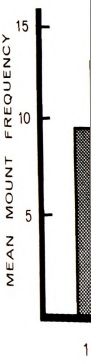
Table 5. Masculine behavior in male hamsters castrated on the day of birth and treated with testosterone postnatally.

Table 5. Masculine behavior in male hamsters castrated on the day of birth and treated with testosterone postnatally.

Day of Treatment*	N	Rear mount Freq. ± SE	% Animals Mount - Pos.	Intromission Freq.** ± SE	% Animals Intro.- Pos.**
1 - 2	9	5.4 ± 3.4	78	3.4 ± 1.7	71.1
3 - 4	11	5.8 ± 0.9	100	5.1 ± 0.9	91
5 - 6	13	.85 ± 0.4	61.5	.25 ± .16	25
7 - 8	18	.27 ± .19	44.5	.37 ± .26	25
9 - 10	7	.86 ± .46	43	2.3 ± 0.6	100

* Day of birth considered Day 1.

** Mount positive subjects only.



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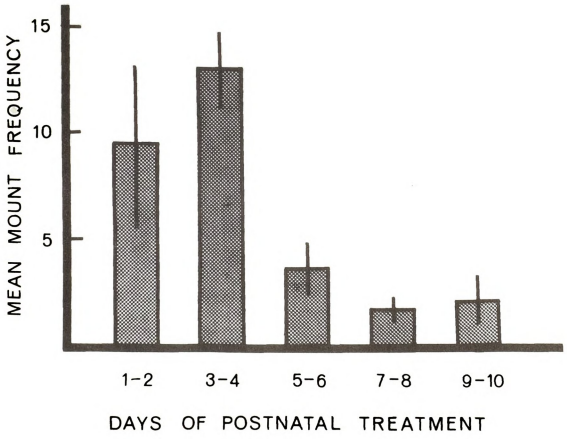

Figure 6. Mean mount frequency in adult male hamsters castrated at birth and treated with testosterone on days 1-2, 3-4, 5-6, 7-8 or 9-10 postnatally.

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($p<.05$). Males treated on days 9-10 were not significantly different in intromission scores from any other treatment group.

Masculine behavior of females: Masculine behavior scores for female hamsters treated with testosterone postnatally are summarized in Table 6 and shown in Figure 7. In general, the results obtained for females are similar to those obtained for males. After 28 days of adult TP treatment, analysis of variance indicated a significant difference among treatment groups in mean total mount frequency ($F=5.4$, $p<.01$) and mean rear mount frequency ($F=6.05$, $p<.01$). Further analysis revealed that mount frequency of females treated on days 1-2 was not statistically different from that of females treated on days 3-4. However, females treated on days 1-2 or 3-4 were significantly higher in mean total mount frequency than those treated on days 5-6, 7-8 or 9-10 ($p<.05$). No significant differences in mount frequency were found among females treated on days 5-6, 7-8 or 9-10.

Intromission pattern scores for females were considerably lower than the scores for males. No significant differences in intromission frequency of mount-positive animals were found among the treated females.

Lordosis behavior of males: Lordosis behavior scores of males castrated on the day of birth and treated with testosterone neonatally are presented in Figure 8. No significant differences were found among the treatment groups in any measure of female sexual behavior. The lordosis scores achieved by these males were comparable to those obtained with testosterone treatment in Experiment I.

Lordosis behavior of females: Lordosis behavior scores of females treated neonatally with testosterone are presented in Figure 9. Analysis of variance indicated a significant difference among the

Table 6. Masculine behavior in female hamsters treated with testosterone postnatally

Table 6. Masculine behavior in female hamsters treated with testosterone postnatally

Day of Treatment*	N	Rear mount Freq. ± SE	% Animals Mount - Pos.	Intromission Freq.** ± SE	% Animals Intro.- Pos.**
1 - 2	14	3.28 ± 0.94	78.6	1.36 ± 0.76	36.3
3 - 4	17	5.29 ± 1.50	100	1.77 ± 0.66	66
5 - 6	19	1.05 ± 0.45	68.4	0.53 ± 0.33	23
7 - 8	17	0.41 ± 0.21	53	0.11 ± 0.10	11
9 - 10	13	0.62 ± 0.33	46.2	1.50 ± 0.72	83.3

* Day of birth considered Day 1.

** Mount positive subjects only.

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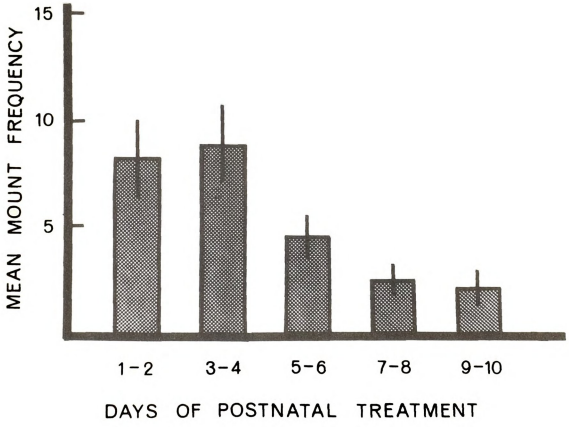

Figure 7. Mean mount frequency in female hamsters treated with testosterone on days 1-2, 3-4, 5-6, 7-8 or 9-10 postnatally.

Figure 8. Effects of adult type of

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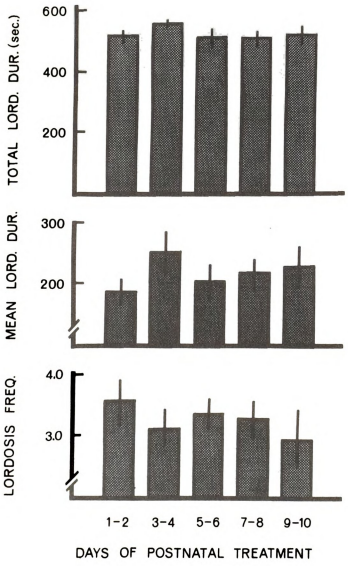

Figure 8. Effect of testosterone treatment neonatally upon parameters of adult sexual receptivity in male hamsters castrated at birth.

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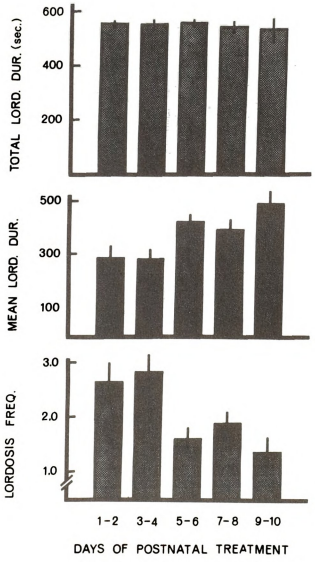

Figure 9. Effect of testosterone treatment neonatally upon parameters of adult sexual receptivity in female hamsters.

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treatment groups in mean lordosis duration ($F=5.10$, $p<0.005$), and in lordosis frequency ($F=5.59$, $p<0.001$). No significant difference was found in total lordosis duration among the treated females. Duncan's New Multiple Range Test further indicated that females treated on day 1-2 and 3-4 showed significantly shorter mean lordosis responses than females treated on days 5-6 or 9-10 ($p<0.05$). Females treated on days 1-2 and 3-4 were also significantly higher in lordosis frequency than females treated on days 5-6 or 9-10 ($p<0.05$).

Morphological measures: Ano-genital distance of neonatally treated male hamsters, measured at the time of sacrifice, are presented in Table 7. Analysis of variance revealed significant differences among the treatment groups ($F=4.6$, $p<0.005$). Further analysis indicated that males treated on days 9-10 had significantly shorter ano-genital distances than males treated at earlier ages ($p<0.05$). No significant differences were found among males treated on days 1-2, 3-4, 5-6 or 7-8.

Length of penile bone and cartilage (Table 7) varied significantly among treatment groups ($F=18.2$, $p<0.001$). Penile bone and cartilage of males treated on days 1-2 were significantly longer than any other treatment group ($p<0.05$). In addition, males treated on days 3-4 or 5-6 were significantly longer in bone and cartilage length than males treated at later ages ($p<0.05$).

Morphological measures of female hamsters were consistent with those seen in males (Table 8). Ano-genital distance varied significantly across treatment groups ($F=15.26$, $p<0.001$). Further analysis indicated females treated on days 1-2 had significantly longer ano-genital distances than other treatment groups ($p<0.05$). In addition, females treated on days 3-4 had greater peripheral development than other treatment groups ($p<0.05$).

Table 7.

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* Day of birth

Table 7. Morphological measures of male hamsters castrated on the day of birth and treated neonatally with testosterone.

Day of Treatment*	A - G distance mean length (mm) ± SE	Penile bone and cartilage mean length (mm) ± SE
1 - 2	15.84 ± 0.47	3.94 ± 0.07
3 - 4	16.06 ± 0.33	3.63 ± 0.03
5 - 6	16.50 ± 0.34	3.72 ± 0.04
7 - 8	15.53 ± 0.37	3.36 ± 0.07
9 - 10	14.47 ± 0.14	3.27 ± 0.04

* Day of birth considered Day 1.

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Table 8. Morphological measures of female hamsters treated neonatally with testosterone

Day of Treatment*	A - G distance mean length (mm) ± SE	Clitoral bone and cartilage mean length (mm) ± SE
1 - 2	9.40 ± 0.22	3.48 ± 0.054
3 - 4	8.34 ± 0.30	3.49 ± 0.054
5 - 6	7.33 ± 0.18	3.13 ± 0.031
7 - 8	7.47 ± 0.15	3.09 ± 0.044
9 - 10	6.82 ± 0.18	2.94 ± 0.005

* Day of birth considered Day 1.

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Further more, females treated on days 5-6 or 7-8 were found to have greater clitoral bone and cartilage development than females treated on days 9-10 ($p<0.05$).

Ovarian histology: Histological analysis of the ovaries revealed no modification of normal function in any treatment group. One hundred percent of females in each group were found to have normal follicular and corpora lutea development.

Experiment III

Masculine behavior of males: Masculine behavior scores for male hamsters castrated on the day of birth (Day 1) and treated with testosterone days 1-5, 6-10 or 1-10 postnatally are summarized in Table 9 and shown in Figure 10. After 28 days of adult TP treatment, analysis of variance indicated a significant difference among treatment groups in mean total mounting frequency ($F=7.7$, $p<0.005$), mean rear mounting frequency ($F=11.4$, $p<0.001$) and intromission frequency in mount-positive animals ($F=14.15$, $p<0.001$). Further analysis indicated that mounting and intromission frequency of males treated on days 1-10 was significantly higher than that of males treated on days 1-5 or 6-10 ($p<0.05$). No significant differences were found between males treated on days 1-5 and 6-10 in any measure of masculine behavior.

Masculine behavior of females: Masculine behavior scores for female hamsters treated with testosterone on days 1-5, 6-10 or 1-10 postnatally are summarized in Table 10 and shown in Figure 10. After 28 days of adult TP treatment, analysis of variance indicated a significant difference among treatment groups in mean total mounting frequency ($F=6.35$, $p<.01$) and mean

Table 9. Masculine behavior of male hamsters castrated on the day of birth and treated with testosterone on days 1 - 5, 6 - 10 or 6 - 10.

Table 9. Masculine behavior of male hamsters castrated on the day of birth and treated with testosterone on days 1 - 5, 6 - 10 or 6 - 10.

Day of Treatment*	Dose (ug)/day	N	Rear mount Freq. ± SE	% animals mount - pos.	Intromission Freq** ± SE	% animals intro - pos.**
1 - 5	100	10	3.4 ± 1.4	80	3.37 ± 1.13	75
6 - 10	100	10	1.8 ± 0.82	60	1.16 ± 0.47	66
1 - 10	50	8	15.87 ± 3.95	75	10.0 ± 1.59	100

* Day of birth considered Day 1.

** Mount - positive subjects only.

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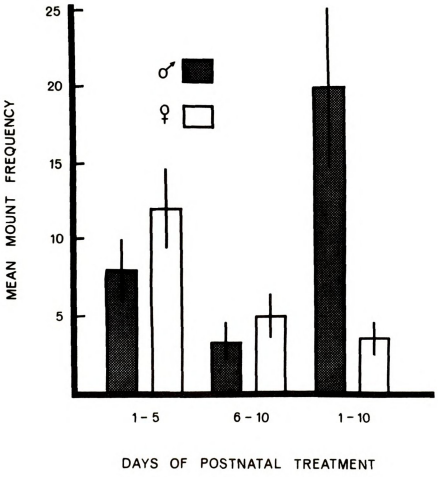

Figure 10. Effect of neonatal testosterone treatment upon mean mount frequency in male hamsters castrated at birth and female hamsters.

Table 10. Masculine behavior of female hamsters treated with testosterone on days 1 - 5, 6 - 10 or 1 - 10.

Table 10. Masculine behavior of female hamsters treated with testosterone on days 1 - 5, 6 - 10 or 1 - 10.

Day of Treatment*	Dose (ug)/day	N	Rear mount Freq. ± SE	% animals mount - pos.	Intromission Freq** ± SE	% animals intro - pos.**
1 - 5	100	11	4.63 ± 1.16	100	1.27 ± 0.44	54.5
6 - 10	100	13	1.38 ± 0.43	92.4	0.91 ± 0.45	41.7
1 - 10	50	13	1.23 ± 0.51	77	0.50 ± 0.40	20.0

* Day of birth considered Day 1.

** Mount - positive subjects only.

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rear mounting frequency ($F= 6.69$, $p<.005$). Further analysis revealed that females treated on days 1-5 were significantly higher in mounting frequency than females treated on days 6-10 or 1-10 ($p<.05$). No significant differences in mounting frequency were found between females treated on days 6-10 and 1-10 postnatally. Analysis of mount-positive animals revealed no significant differences in intromission frequency among the treated females ($F=0.74$, $p>.05$).

Lordosis behavior of males: Lordosis behavior scores of male hamsters castrated at birth and treated with testosterone on days 1-5, 6-10 or 1-10 postnatally are shown in Figure 11. Analysis of variance indicated a significant difference among treatment groups in total lordosis duration ($F=5.24$, $p<.025$). Further analysis revealed that males treated on days 1-10 postnatally were significantly lower in total lordosis duration than other treatment groups ($p<.05$). No significant difference was found between males treated on days 1-5 and 6-10 postnatally. In addition, scores for mean lordosis duration and lordosis frequency did not differ significantly among the treated males.

Lordosis behavior of females: Lordosis behavior scores of females treated with testosterone on days 1-5, 6-10 or 1-10 after birth are presented in Figure 12. Analysis of variance indicated a significant difference among treatment groups in total lordosis duration ($F=226.8$, $p<.001$), mean lordosis duration ($F=14.93$, $p<.001$) and lordosis frequency ($F=14.21$, $p<.001$). Further analysis revealed that females treated on days 1-10 were significantly lower in total and mean lordosis duration than other treatment groups ($p<.05$). In addition, females treated on days 1-5 were significantly lower in total and mean lordosis duration than females treated on days 6-10 postnatally ($p<.05$). Lordosis

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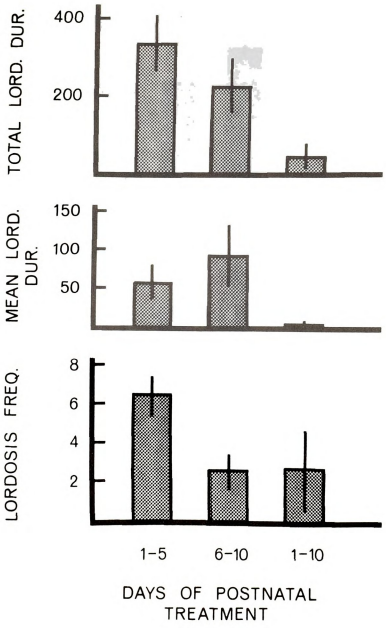

Figure 11. Adult sexual receptivity measures in male hamsters castrated at birth and treated with testosterone on days 1-5, 6-10 or 1-10 postnatally.

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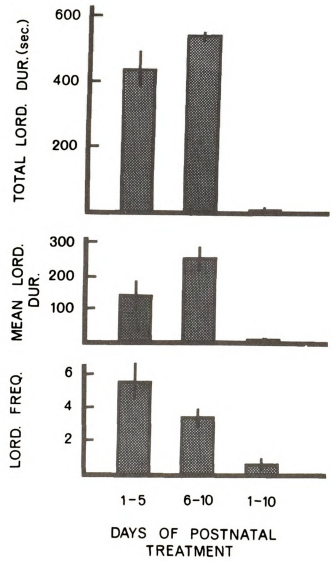

Figure 12. Adult sexual receptivity measures in female hamsters treated with testosterone on days 1-5, 6-10 or 1-10 postnatally.

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frequency was significantly higher in females treated on days 1-5 than other treatment groups ($p<.05$). Females treated on days 6-10 were higher in lordosis frequency than those treated on days 1-10 ($p<.05$). However, only 23% of the females treated on days 1-10 displayed lordosis responses, whereas 100% of the females treated on days 1-5 or 6-10 displayed lordosis.

Morphological measures: Ano-genital distance measures of males castrated at birth and treated with testosterone postnatally are presented in Table 11. No significant differences were found among the treated males in ano-genital distance, measured at the time of sacrifice.

Length of the penile bone and cartilage (Table 11) varied significantly among the treated males ($F=129.1$, $p<.001$). Further analysis revealed that castrated males treated on days 1-10 postnatally had significantly greater penile development than did other treatment groups ($p<.05$). In addition, castrated males treated on days 1-5 had significantly greater penile development than did males treated on days 6-10 postnatally ($p<.05$).

Morphological measures of female hamsters treated with testosterone postnatally are presented in Table 12. Ano-genital distance, measured at the time of sacrifice, varied significantly across treatment groups ($F= 8.85$, $p<.005$). Subsequent analysis indicated that females treated on days 6-10 postnatally had significantly shorter ano-genital distances than females in other treatment groups ($p<.05$). No significant difference was found in ano-genital distance between females treated on days 1-5 and 1-10 postnatally.

Table 11. Morphological measures of male hamsters castrated on the day of birth and treated with testosterone on days 1-5, 6-10 or 1-10 postnatally.

Table 11. Morphological measures of male hamsters castrated on the day of birth and treated with testosterone on days 1-5, 6-10 or 1-10 postnatally.

Day of Treatment*	Dose (ug/day)	A-G distance mean length (mm) ± SE	Penile bone and cartilage mean length (mm) ± SE
1 - 5	100	15.19 ± 0.55	4.40 ± 0.053
6 - 10	100	15.45 ± 0.35	3.68 ± 0.046
1 - 10	50	16.64 ± 0.32	4.75 ± 0.050

* Day of birth considered Day 1.

Table 12. Morphological measures of female hamsters treated with testosterone on days 1-5, 6-10 or 1-10

Table 12. Morphological measures of female hamsters treated with testosterone on days 1-5, 6-10 or 1-10 postnatally.

Day of Treatment*	Dose (ug/day)	A-G distance mean length (mm) ± SE	Clitoral bone and cartilage mean length (mm) ± SE
1 - 5	100	9.79 ± 0.33	3.86 ± 0.065
6 - 10	100	8.62 ± 0.39	2.86 ± 0.315
1 - 10	50	10.27 ± 0.17	3.93 ± 0.042

* Day of birth considered Day 1.

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A significant variation was found in clitoral bone and cartilage length among the treated females ($F=18.91$, $p<.001$). Further analysis indicated that females treated on days 6-10 postnatally were significantly shorter in clitoral length than other treatment groups ($p<.05$). No significant difference was found in clitoral length between females treated on days 1-5 and 1-10 postnatally.

Ovarian histology: Histological analysis of the ovaries revealed that 75% of the females treated with testosterone on days 1-5 had no corpora lutea. Furthermore, 42% of these females were found to have cystic ovaries.

Ovaries of females treated with testosterone on days 1-10 were all found to have no corpora lutea. In addition, 86% of these females had ovaries containing cystic follicles.

In contrast, all females treated with testosterone on days 6-10 were found to have normal ovaries, containing follicles in various stages of development and corpora lutea.

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DISCUSSION

The probability of masculine sexual behavior in adult male hamsters castrated on the day of birth was increased by neonatal treatment with estradiol, estradiol benzoate, diethylstilbestrol, testosterone, or testosterone propionate. The androgens androsterone and dihydrotestosterone failed to induce the potential for masculine sexual behavior. Adult masculine behavior potential was greater in animals treated with testosterone prior to day 5 postnatally, than in animals treated with testosterone later than day 5 postnatally.

Castration of the male hamster on the first day of postnatal life resulted in high levels of female sexual behavior in the estrogen-progesterone treated adult. Female sexual behavior was reduced in day 1 castrates when they were treated with estradiol, estradiol benzoate, diethylstilbestrol, testosterone or testosterone propionate, but not with androsterone or dihydrotestosterone. However, suppression of adult sexual receptivity in males or females by testosterone required prolonged hormone exposure during postnatal development.

Treatment of the day 1 male castrate with androgen early in life enhanced the development of the penile bone and cartilage, whereas perinatal treatment with estrogens or control substances resulted in little effect on the growth capacity of the phallus after adult hormone treatment. Androgenic stimulation prior to day 5 postnatally induced a greater growth capacity of the penile or clitoral structure than treatment with androgen after the fifth day of postnatal development.

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These findings extend our information concerning several variables in the ontogeny of sexual behavior: 1. specificity of hormone -behavior relations, 2. morphological virilization and its relation to behavioral masculinization, 3. male - female differences in hormone induced sexual behavior, and 4. period of maximal susceptibility to masculinization and defeminization.

Specificity of hormone - behavior relations:

The present findings indicate that behavioral masculinization during perinatal development in the hamster is hormone specific. Adult mounting was observed only in animals treated early in life with estradiol, estradiol benzoate, diethylstilbestrol, testosterone or testosterone propionate, but not with androsterone or dihydrotestosterone. Since the androgens which induced mounting can be aromatized to estrogen, this might suggest that conversion to estrogen is a step in behavioral masculinization by androgens. This is supported by the finding that treatment with 2 ug estradiol benzoate or diethylstilbestrol on days 2-4 of life resulted in a frequency of mounting comparable to that observed in animals receiving 25 ug testosterone on days 2-4 of life. Estradiol benzoate has also been shown to be more potent than testosterone propionate in causing the "androgen sterilization" syndrome in rats (Gorski, 1963). In vitro aromatization of androgens to estrogens has been reported for anterior hypothalamic tissue of male and female rats (Naftolin, et al., 1972), as well as limbic and hypothalamic tissue of human male fetuses (Naftolin, et al., 1971a, 1971b). These observations strengthen the possibility of androgen conversion as a mechanism involved in behavioral masculinization.

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The results of the present study extend this hypothesis in that conversion of androgen to estrogen may be important in the suppression of adult sexual receptivity by early androgen treatment. Lordosis suppression was achieved in animals treated neonatally with estrogens or testosterone propionate (Experiment I). In addition, treatment with testosterone on days 1-5 or 1-10 postnatally also resulted in lordosis suppression. The present study demonstrated that administration of testosterone for a longer period of neonatal development was as effective in lordosis suppression as treatment with testosterone propionate for a brief period during neonatal development. This is consistent with the finding that the duration of hormone action is longer for testosterone propionate than for testosterone (Miescher, et al., 1936). This may explain why earlier studies in which testosterone was administered neonatally for a shorter time failed to reduce estrogen-progesterone induced lordosis in adult female hamsters (Coniglio, et al., 1973) or female rats (Luttge and Whalen,1971). However, Edwards (1970) has reported a decrease in sexual receptivity in female mice as a result of neonatal treatment with free testosterone.

Morphological virilization and its relation to sexual behavior:

Although behavioral masculinization occurs postnatally in the hamster (Nucci and Beach, 1971), morphological virilization begins prenatally (Bruner and Witschi, 1946; Ortiz, 1945). Ano-genital distance, one measure of morphological virilization, has been established in the male by the time of birth, and was minimally influenced by postnatal treatment, as indicated by the present finding that this measure

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was significantly affected in only one group of animals (Experiment II). However, the phallus was responsive to postnatal treatment. Depriving the male of postnatal androgen by castration, or delaying treatment with androgen until day 7 postnatally, reduced the normal growth capacity of the phallus. Similar findings have been reported for the male rat (Beach and Holz, 1946). Growth and ossification of the priapian cartilage were enhanced by neonatal treatment with all androgens used in this study. The difference in penile development between castrated males receiving no androgen neonatally and those which had received androgen neonatally may be a result of differences in potential for phallus growth in response to adult testosterone propionate treatment. Since measurements were taken after adult hormone treatment, it is not clear whether these differences would have been present prior to adult hormone treatment. However, the postnatal capacity of the male phallus for growth was clearly androgen dependent, since estrogen treatment neonatally was ineffective in promoting phallus growth.

The notion has been advanced that intromission behavior is facilitated in the rat by early androgen stimulation as a result of enhanced phallic development (Beach and Holz, 1946; Whalen and Edwards, 1967; Nadler, 1969). While this concept may be applicable to the rat, it does not seem to be the case for the hamster. Although the results of Experiment II seemed to indicate that animals treated at earlier ages postnatally showed a higher level of masculine behavior and were more extensively virilized, this relationship was not a consistent one. In Experiment II, males treated on days 3-4 were statistically higher in intromission frequency than males treated on days 5-6. However,

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there were no significant differences in the morphological measures of these two groups. In addition, females treated with testosterone on days 1-10 postnatally showed the greatest peripheral virilization, however they were significantly lower than other treatment groups in masculine behavior measures. Although the genital tissues and behavioral mechanisms sensitive to hormone treatment are competent to respond during the same period of development, they may not be causally related. Thus, the degree of penile or clitoral development is not related to the probability of masculine behavior in male and female hamsters.

Male - female differences in hormone-induced sexual behavior:

Male hamsters castrated as adults and treated with estrogen and progesterone do exhibit lordosis (Crossley and Swanson, 1968; Tiefer, 1970). However, these responses, as Tiefer pointed out (1970), were significantly shorter than those of females under similar conditions. These findings are consistent with the hypothesis that endogenous testicular secretions during postnatal development have a suppressive effect on adult sexual receptivity in the male hamster. Although several studies (Carter, et al., 1972; Eaton, 1970) have shown that castration of the male hamster on the day of birth increases his potential to display lordosis in response to adult ovarian hormone treatment, results of the present study indicate that this response is quantitatively different from that shown by the normal female hamster in response to exogenous ovarian hormones.

A comparison of lordosis scores between the results of Experiment II with males and similarly treated female hamsters (Coniglio, et al., 1973) shows that the mean lordosis duration per 10 min test for female

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hamsters treated with sesame oil days 2-4 after birth was 418 sec., whereas for the day 1 castrated male hamster treated with sesame oil it was 108 sec. Lordosis frequency for the female was 2.0, while for the male it was 5.2. Thus, while the male readily displays the lordosis response, he does not maintain the response as long as the female does. Several alternatives can be suggested to account for this behavioral difference between male and female hamsters. Since the fetal testis is active in the male (Price and Ortiz, 1965), prenatal exposure to endogenous androgen may affect the maintenance of the lordosis response. On the other hand, the stud male may respond differently to a castrated male than to a female, particularly since intromission by the stud male is not achieved with the castrated male. Further, the stimulation of vaginal penetration received by the female may influence the maintenance of the lordosis response. In this regard, Diakow (1970) has shown that cervical stimulation prolonged lordosis in the female rat. This apparent difference between the lordosis response of male and female hamsters requires further investigation to determine the factors which may be contributing to this difference.

Additional male - female differences are apparent in the masculine behavior results of Experiment II. While treatment of castrated males with testosterone (50 µg/day) on days 1-10 postnatally resulted in high levels of masculine behavior in adulthood, similar treatment of females resulted in very low levels of masculine behavior in adulthood. Several alternatives can be suggested to account for this difference. The male may be more sensitive to testosterone than the female. Raising the dose level of hormone for the 10 days of treatment for the female may

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result in a level of masculine behavior comparable to that shown by the male. Alternatively, in vitro studies have shown that, in both rats and rabbits, males had a higher level of aromatization in hypothalamic tissue than did females (Ryan, et al., 1972). Since aromatization may be involved in behavioral masculinization, this could account for the higher level of masculine behavior in males treated on days 1-10 than that observed in females. Finally, it has been shown that liver homogenates of male rats maintain testosterone in unmetabolized form longer than female rat liver (Ota, et al., 1971). Thus, the maintenance of testosterone in active form may be responsible for the higher levels of masculine behavior observed in the male hamster.

Period of maximal susceptibility to masculinization and defeminization:

Studies of the rat and guinea pig have shown that the presence of androgen at a particular stage of fetal development or very soon after birth affects the potential to display adult sexual behaviors (Phoenix, et al., 1959; Goy, et al., 1964; Grady, et al., 1965; Beach, et al., 1969). This study shows the same to be true for the golden hamster. In both male and female hamsters, the presence of androgen during the first 4 days of life insured the development of masculine behavior potential in the adult. Treatment with androgen at later ages resulted in a very low probability of masculine behavior performance in the adult.

Treatment with testosterone during the first days of postnatal life was significantly more effective in reducing the potential for sexual receptivity in the adult male or female than treatment on days

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6-10 postnatally. Furthermore, extending the period of testosterone treatment to include the first 10 days of postnatal life virtually eliminated the potential for sexual receptivity in the adult. Thus, while it appears that days 1-5 may be more sensitive to defeminization by testosterone than days 6-10, mechanisms responsible for adult lordosis behavior appear to be influenced throughout the first 10 days of postnatal life, if continuous hormone treatment is administered.

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CONCLUSION

The development of behavioral masculinization and the suppression of adult sexual receptivity have been shown to result from neonatal treatment with estrogen or androgens which can be converted to estrogen. It is therefore suggested that the aromatization process may be involved in the mechanism responsible for the development of adult sexual behavior potential in hamsters. Since variations in the level of masculine behavior did not relate to variations in peripheral morphology, the hypothesis that the behavioral modifications associated with perinatal hormone treatment result from changes in morphological parameters is not supported by this study.

The study further demonstrated that the period of maximal susceptibility to the hormonal induction of masculine behavior and the suppression of feminine behavior occurs within the first 5 days of postnatal life in both male and female hamsters. However, masculine behavior potential can be further facilitated in male hamsters by extending the duration of hormone treatment, whereas such facilitation was not demonstrated by extended hormone exposure to females. In addition, mechanisms responsible for adult sexual receptivity are sensitive to prolonged hormone treatment (days 1-10 postnatally), resulting in the absence of lordosis in the adult of both sexes.

Previous findings that testosterone failed to suppress adult sexual receptivity were shown to be inconsistent with the present results. Prolonged administration of testosterone was as effective

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It was but yesterday we met in a dream.
You have sung to me in my aloneness,
and I of your longings
have built a tower in the sky.
But now we must part,
And if, in the twilight of memory, we
should meet in another dream
we shall build another tower in the sky.

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