# SENESCENT ALTERATIONS OF LH AND TESTOSTERONE REGULATION AND HYPOTHALAMIC CATECHOLAMINES IN THE MALE RAT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
ANNA ELIZABETH MILLER
1976

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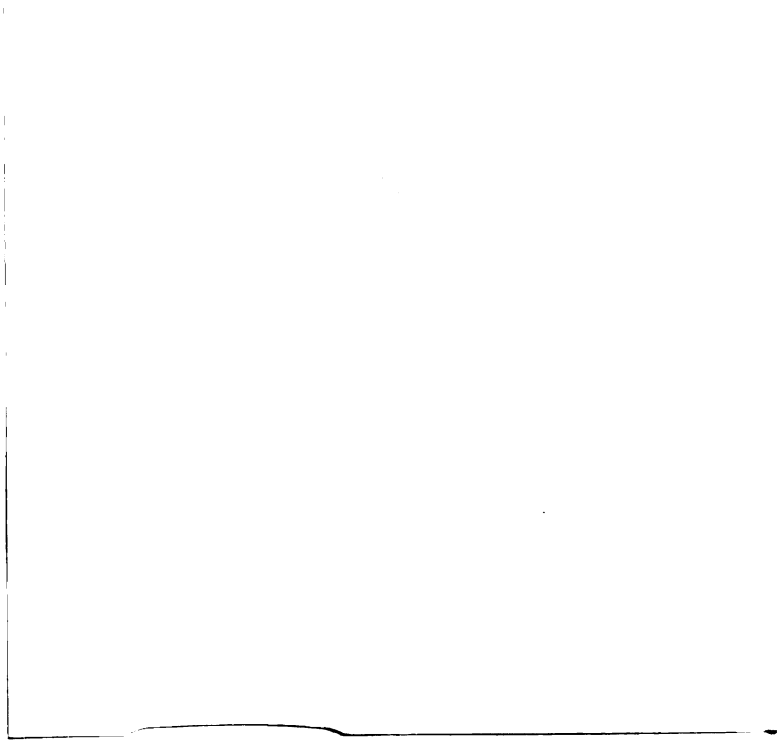

ABSTRACT

# SENESCENT ALTERATIONS OF LH AND TESTOSTERONE REGULATION AND HYPOTHALAMIC CATECHOLAMINES IN THE MALE RAT

By

Anna Elizabeth Miller

The effects of advanced age on the reproductive control system was studied in male rats by examining changes in serum testosterone and LH concentrations and alterations which occur in the response to administration of Luteinizing Hormone Releasing Hormone (LHRH), Human Chorionic Gonadotropin (HCG), and L-dopa, and in hypothalamic catecholamine concentrations. Young adult (3-6 mo) and aged (20-30 mo) male Long-Evans rats were used in these studies.

The effect of HCG on serum testosterone was studied in two trials. In the first experiment, 23 young and 27 aged male rats were assigned to one of three groups which received jugular vein injections of 0.5 ml of physiological saline or 0.5 ml of saline containing 1 or 5 IU of HCG. Testosterone was measured by radioimmunoassay in serial blood samples taken by orbital sinus puncture before injection and at 15, 30, and 60 minutes after treatment.

Resting serum testosterone concentrations were found to be significantly lower in aged male rats than in young male rats. In the saline treated groups, testosterone remained stable throughout the sampling period. After treatment with HCG, serum testosterone was steadily increased in both age groups over the sampling period. This increase in testosterone was approximately twice as great in young as in aged males, and the 5 IU dose of HCG stimulated a greater increase in testosterone than did 1 IU in both young and aged groups.

In the second trial, groups of 32 young and 31 aged male rats were assigned to one of 4 groups and received intravenous injections of 0.5 ml of physiological saline or 0.5 ml of saline containing 1, 5, or 20 IU of HCG. Serial blood samples were taken from each rat prior to and at 45, 90, and 150 minutes after the HCG treatment. Serum testosterone was increased in both young and aged rats following all 3 doses of HCG. The increases after each HCG treatment were smaller in aged rats compared to young rats. The increase in serum testosterone concentrations following HCG injection was sustained throughout the sampling period in both age groups.

In a second experiment, 17 aged and 24 young male rats received 3 intravenous injections of 500 ng LHRH at 75-minute intervals. Serum LH was measured by radioimmunoassay in serial blood samples taken before each LHRH injection and 15 minutes following each drug treatment.

Young males were found to have higher LH concentrations and higher serum LH concetrations 15 minutes after the first LHRH injection than aged male rats. However, serum LH levels were similar in both age groups before and after the second and third LHRH injections.

In a third experiment, groups of 8 aged and 8 young male rats received intravenous injections of 500 ng of LHRH. Serum testosterone was measured in serial blood samples taken before and 15, 30, and 60 minutes after LHRH injection and was found to be progressively increased following LHRH injections in the young group while not being significantly increased over saline injected controls in the aged group.

A fourth experiment examined hypothalamic catecholamine content of young and aged male rats. Groups of 16 aged and 16 young male rats were decapitated and their hypothalami collected and weighed. Hypothalamic norepinephrine and dopamine were determined from hypothalamic extracts by microflourescence after alumina absorption. Hypothalamic dopamine and norepinephrine content were both found to be about twice as great in young as in the aged rats.

In a fifth experiment, groups of 19 aged and 30 young male rats were given an intravenous injection of 500 ng LHRH prior to and shortly after 10 days of treatment with L-dopa. Serum LH was measured in blood samples taken before and at 15 and 45 minutes after LHRH injection.

Although the LHRH response prior to L-dopa treatment showed that serum LH was higher 15 minutes after LHRH injection in the young than in the aged rats, serum LH 15 minutes after LHRH treatment at the end of the L-dopa injection regime was similar for both ages.

These data suggest that with age, functional deterioration of gonadotropin control occurs at the level of the gonad, pituitary, and at the hypothalamus. Although aged male rats have lower serum concentrations of LH and testosterone than young males, the responsiveness of the testes and pituitary to LH and LHRH stimulation indicates that these tissues are capable of maintaining higher levels of secretion. These data are interpreted to indicate that the primary dysfunction in the gonadal control system in the aged male rat occurs in the hypothalamus or other neural regulatory tissues.

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Anna Elizabeth Miller

A THESIS

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

MASTER OF SCIENCE

Department of Physiology

1976

## ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Gail D. Riegle, my major advisor, for his patient assistance and inspiring example as both a scientist and a person.

I am also grateful to the other members of my guidance committee, Dr. Harold Hafs and Dr. Edward Convey for their interest and advice in the final stages of my masters program.

I would also like to express my appreciation to Dr. E. M. Bogdanove at the Medical College of Virginia who kindled my interest in endocrinology and showed me how exciting scientific research can be.

The friendship and helpfulness of Sandra M. Wood and Donald W. McKay have been of immeasurable importance to me, along with the rest of the Endocrine Research Unit staff.

I must also thank my parents, Loentine V. Goff and the late John B. Beltz for giving me the financial opportunities and the emotional support to be all I can be.

And I must certainly thank my husband, Michael E. Miller, for his concern and understanding, and for always providing the confidence I lack.

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# INTRODUCTION

While man has historically been fascinated with the changes that accompany advanced age and has time and again been haunted with hopes of reversing or retarding this apparently inevitable process, the state of experimental gerontology is still at the descriptive stage, a relative infant in the bio-medical research field.

The progression of life from conception through growth, development, physiological aging and the onset of diseases leading to death, while primarily under genetic control, clearly involves physiological control systems. Gerontologists have long been fascinated by the possibility of a direct relationship between aging and changes in hormonal function. Many investigators have related changes in endocrine control system function to aging. Reduction of reproductive function with increasing age has been a universal observation in mammalian species. A large amount of recent experimental work has shown that reproduction is regulated by complex control systems involving the central nervous system, the anterior pituitary gland and the gonads. Most hormones secreted by these tissues have been shown to be influenced by the aging phenomenon. Alterations in

certain response thresholds and changes in the function of the components of these control systems also appear to be involved with the loss of reproductive function. It is hoped that a better understanding of the effects of aging on the endocrine neurophysiological control mechanisms will be applicable to other neurophysiological control systems and increase our knowledge of the biology of aging.

The research described here was done in an effort to characterize the major sources of the alterations occurring with age in the reproductive control system of the male rat. The rat was selected as a model primarily because of its short life span and the relative ease in acquiring modest numbers of senescent animals in a short time. The reproductive control system was regarded as an appropriate model for an aging study since it has measurable impairments occurring with age and the normal neuroendocrine control system has been quite thoroughly established in this species.

# LITERATURE REVIEW

## Gonadotropin Control

The literature published in recent decades concerning reproductive control mechanisms is voluminous and frequently conflicting. No attempt will be made here to comprehensively review this topic. The purpose of this review is to summarize recent findings and the relationships relevant to our study of the control of male reproductive function with age. Many recently published reviews have given more exhaustive and comprehensive consideration to the control of reproduction (Greep and Astwood, 1973, 1974, 1975).

Testicular activity has been clearly shown to be controlled by the pituitary gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). It was initially thought that FSH was primarily concerned with the regulation of spermatogenesis, while LH was the primary regulator of Leydig cell growth and androgen secretion (Greep et al., 1936; Greep and Fevold, 1937). The finding that the administration of testosterone alone is sufficient to maintain spermatogenesis in hypophysectomized animals (Nelson and Merckel, 1938) somewhat complicated this theory.

Also, a linear relationship between the capacity of the Leydig cells to produce testosterone and the serum levels of gonadotropins has not been demonstrated. It is now thought that LH directly controls the secretion of androgens by the testes and, along with FSH, then indirectly influences spermatogenesis through its control over steroidogenesis.

Pituitary gonadotropins have also been shown to control growth of the testes. Steinberger and Steinberger (1972) have shown that at puberty in rats, increases in plasma levels of LH and FSH are associated with dramatic increases in testicular weight which largely reflects growth of the seminiferous tubules. These workers also found that in neonatal rats estrogen administration sufficient to block gonadotropins also blocks growth of the seminiferous tubules. However, it has also been shown that several androgens, especially testosterone, can stimulate growth of the seminiferous tubules without the presence of gonadotropins, although the degree of stimulation is significantly less than that seen with gonadotropins (Steinberger and Steinberger, 1972). The growth of the testes appears to be a complex process, involving several different tissues, and it appears that the hormonal control may be similarly complicated, involving both the gonadotropins and testosterone.

It is now accepted that the anterior pituitary secretion of gonadotropins is largely controlled by the hypothalamus. In male rats, median eminence lesions

result in atrophy of the testes and accessory organs, with a dramatic decrease in plasma FSH and LH, and an increase in prolactin secretion (DeVoe et al., 1965). The existence of specific releasing factors that control pituitary secretions is also well established. Highly purified LH-releasing hormone (LHRH) is active in nanogram doses to release LH in vivo and in vitro (Fawcett and McCann, 1971). Luteinizing Hormone Releasing Hormone (LHRH) has been found to act directly on the pituitary gland to release LH, since it is active (a) when injected into the peripheral circulation in animals with lesions in the median eminence that eliminate neural control of the gland, (b) when microinjected into the interstitial tissue of the pituitary, (c) when perfused directly into a hypophysial portal vessel, or (d) when added to pituitary incubates in vitro (McCann, 1970).

Moore and Price (1932) were perhaps the first to recognize that secretion of gonadotropins by the pituitary is normally held in check by the feedback action of gonadal steroids. When rats are orchidectomized, both plasma and pituitary LH show a rapid and prolonged rise. Kalra et al. (1971) demonstrated that administration of gonadal steroids in castrates inhibits gonadotropin secretion. Single injections of low doses of testosterone are capable of rapidly lowering plasma LH.

Several workers have implanted minute amounts of steroids into either the hypothalamus or the anterior

pituitary in an effort to localize their site of action in modifying gonadotropin release (Rose and Nelson, 1957; Davidson and Sawyer, 1961; Lisk, 1962; Bogdanove, 1963; Chowers and McCann, 1965). Evidence indicates a direct effect of steroids on the anterior pituitary, along with a hypothalamic site of feedback such that the hypothalamic content of LHRH is lowered. Furthermore, alterations in the level of hypothalamic releasing factors have been shown to follow castration (Meites, 1970).

Several studies have shown that hypothalamic control of gonadotropin secretion by the anterior pituitary involves the monoamine containing neurons of the hypothalamus as well as other neural mechanisms whose actions may be independent of or directly associated with the effects of the catecholamines. Ungerstedt (1971) has shown that catecholaminergic tracts enter the hypothalamus and innervate many anatomical regions. Lesion and stimulation studies have shown these catecholamine tracts to be involved in hypothalamic control of anterior pituitary secretions. Fuxe (1969) has proposed that dopaminergic tracts terminating in the region of the primary plexus of the hypothalamic-pituitary portal blood system control the secretion of releasing factors into the portal blood.

Direct evidence of catecholaminergic regulation of hormone release has been provided by studies correlating catecholamine administration with hormone secretion. Dopamine injection into the third ventricle of estrogen

primed rats resulted in increased LHRH in portal blood (Kamberi et al., 1969), and in systemic blood (Schneider and McCann, 1970a), and increased serum LH in rats (Schneider and McCann, 1970b; Kamberi et al., 1970). Systemic injection of L-dopa, the precursor for dopamine, has been shown to increase serum LH concentrations in female rats (Watkins et al., 1975). However, these data do not indicate the mechanism by which L-dopa stimulates an increase in LH. The recent studies of Sawyer et al. (1974) and Cocchi et al. (1974) suggest that the release of hypothalamic LHRH is stimulated by norepinephrine. These reports would suggest that the systemically-injected L-dopa was converted to norepinephrine for biological activity. Following castration, Wurtman et al. (1969) found that norepinephrine synthesis is increased and Donoso et al. (1967) reported increased hypothalamic norepinephrine content.

On the other hand, not all investigators agree with the forementioned data. Fuxe and Hokfelt (1969) concluded that catecholamines inhibit gonadotropin secretion and did not detect norepinephrine changes following castration. The molecular mechanism of catecholamine regulation of the release of hypothalamic factors regulating gonadotropins and comprehension of how multiple stimulatory and inhibitory inputs may effect this system remain to be identified.

# Aging

## Introduction

The physiological control system mechanisms governing reproduction have received considerable attention in recent years, particularly regarding the location, specificity, sensitivity, and mechanisms of action of the reproductive control system components. While interest in the effects of aging on reproductive control systems is increasing, insight into the causes and origins of fundamental alterations and deterioration of reproductive function associated with advanced age is still minimal. Much of the work done in this field continues to be oriented toward descriptive and quantitative data concerning the effects of age on reproduction, with the causative factors and mechanisms yet to be elucidated.

While this report is concerned only with the effects occurring in the male with advanced age, a majority of the aging studies to date concern females, and thus the particulars of reproductive control system alterations with age are more thoroughly known in females. Since many of the components of the male reproductive control system have equivalent counterparts in the female which are often ultimately found to operate in a similar manner, the effects of aging on both the male and female reproductive control systems will be reviewed here.

Female--General Considerations

Decreased reproductive output has long been recognized as a consequence of advanced age in mammalian species. Several studies have shwon a decrease in litter size of aging laboratory rodents (Blaha, 1964; Adams, 1970; Thorneycroft and Soderwall, 1969a). Rugh and Wohlfromm (1967) reported a decrease in average litter size in mice from 9.5 at 3-5 mo to 7.6 at 10-12 mo of age.

Cyclic patterns of vaginal cytology in laboratory rodents are also altered with age (Mandl and Shelton, 1958; Ingram, 1959; Clemens and Meites, 1971; Peng and Huang, 1972). Aschheim (1976) has been characterizing these changes in the rat for the past several years. Although some aged rats remain cyclic throughout their lifespan, Aschheim reports 2 changes in the estrous cycles of rats which become evident in his colony at about 12 to 15 mo of age. Beyond 10 months of age increased percentages of his rats show either constant estrous or repetitive pseudopregnant vaginal smears. Aschheim reported that rats showing constant estrous vaginal smears predominate in the second years of rat life (12 to 14 mo) with the repetitive pseudopregnant state becoming dominant in the third year (24 to 36 mo).

Ingram (1959) also reported a change in the sexual behavior of aged rats with age, i.e., sexual acceptance of the male was less strongly associated with vaginal estrous-type cornification.

Female--Gonadal Function

There are many possible explanations for the alterations that occur in the outward signs of reproductive activity with age. Several hypotheses have been suggested to explain the effect of aging on litter size. The most characteristic effect of aging on the ovary is the decline in the number of oocytes (Krohn, 1967). Although the number of oocytes are exhausted soon after menopause in women (Jones, 1970), other species experience reproductive failure with substantial numbers of oocytes remaining in the ovaries. In most rodents the rate of loss of oocytes is linear with increasing age and rarely reaches zero before death. Studies by Adams (1970) showed that litter size decreases more rapidly than ovulation rate with increasing age, and Mandl and Shelton (1959) reported that in rats, reduced fertility precedes depletion of ovarian oocytes. Thorneycroft and Soderwall (1969a) found a 7 fold increase in preimplantation deaths and a 2 fold increase in postnidation resorption in aged hamsters as another explanation for the decrease in litter size with age.

Several structural changes in the ovary have been reported to be associated with aging. Takacs and Verzar (1968) reported increased ovarian collagen content with age. Thorneycroft and Soderwall (1969b) found that senescent hamsters had fewer ovarian follicles than young female hamsters, while Aschheim (1976) reported that the number of

eggs ovulated are normal in aged rats which retain normal estrous cycles. Harmon and Tolbert (1967) reported a higher incidence of degenerate-looking corpora lutea in the ovaries of old pregnant mice and concluded that there was a reduction in luteal function. In addition, the production of corpora lutea decreases before there is any evidence of failure by the follicles to respond to FSH stimulation (Jones and Krohn, 1961). This observation coupled with the appearance of "deficiency" cells in ovarian interstitial tissue of aging rats (Wolfe, 1943) suggested that the ability of the pituitary to supply ovarian stimulation may be impaired with increasing age.

Ovarian steroid production in the human female changes dramatically during menopause in spite of increases in gonadotropic hormone secretion. Pincus et al. (1954) reported a progressive decrease in total urinary estrogens in women between the ages of 40 and 60 years. The relative decline in estradiol is greater in this interval than the decreases in estrone or estriol (Procope, 1969). Mattingly and Huang (1969) found increased ovarian production of androgens during the interval of declining estrogen secretion in women. In addition, Dilman (1976) has shown an increased production of noncalssical phenolsteroids in the aging female. Adamapoulos et al. (1971) has reported decreased pregnandiol excretion in women approaching menopause, which suggests that ovarian progesterone secretion may also be effected by aging. The effect

of aging on ovarian sex steroid secretion in laboratory rodent species remains almost totally unknown. Most estimates of aging effects on rat ovarian steroid secretion have been derived indirectly, using vaginal cytology as an index of sex steroid secretion.

Alterations in vaginal cyclicity with age have also been correlated with ovulatory and luteinizing activity of the ovary. Although the ovaries of constant estrous aged rats are anovulatory, atrophic and contain no corpora lutea (Aschheim, 1976; Clemens and Meites, 1971), aged repetitive pseudopregnant rats show normal ovulation at intervals of 12 to 30 days. The presence of freshly formed corpora lutea and the ability of the uterus to form deciduoma after endometrical trauma suggest that aged pseudopregnant rats are secreting considerable ovarian progesterone.

Female--Pituitary Function

Pituitary gonadotropin secretion is markedly altered in aged females. Everitt (1976) points out evidence for overstimulation of the ovary by excessive pituitary gonadotropin secretion in aged women that could lead to premature exhaustion of the ovary. However, Baranov et al. (1972) found that the excretion of estrogen, pregnandiol, and gonadotropins was not altered in women until after the first missed menstrual period. Llewellyn-Jones (1971) proposed that the decrease in the FSH:LH ratio found with advanced age may be responsible for the changes

in follicular development and sex steroid secretion associated with menopause.

In contrast, there is no evidence for increased LH secretion in aged rats. Aschheim (1976) cited the existence of ovarian deficiency cells in the interstitial tissue beginning at 13 months of age as evidence of inadequate LH stimulation. Aschheim also found that LH injection or pituitary implants would make the deficiency cell undetectable in the aged rats, and injections of purified LH in aged constant estrous rats will result in the resumption of normal estrous cycles. This suggestion of inadequate LH stimulation of aged rat gonads is supported by reports from our laboratory in both male and female rats (Shaar et al., 1975; Riegle and Meites, 1976). These studies have demonstrated that serum LH is diminished and serum prolactin is elevated in aged rats. Accordingly, Pecile et al. (1966) has reported proportionally higher numbers of acidophils present in aged rat pituitaries than in those of young rats.

Several workers have shown that pituitaries of young rats can maintain ovarian function in ovaries transplanted from aged donors to young recipients (Peng and Huang, 1972; Aschheim, 1976). Similarly, Pecile et al. (1966) found that gonadal function in young hypophysectomized female rats, as assessed by vaginal estrous cyclicity and by uterine and ovarian weight, could be restored by transplanting pituitary tissue from young donor rats. Transplantation from older donors did not restore

gonadotropic function. However, Peng and Huang (1972) also showed that pituitaries from aged donors transplanted under the median eminence region of hypophysectomized young adults could sometimes restore estrous cycling and fertility.

The data summarized thus far indicate important age differences in gonadal steroid and pituitary gonadotropin secretion in aged women and rats. These observations support the view that the ovary is not the primary site of malfunction in reproductive control systems in the aging rat. Although functional alterations at the hypophysial level are implicated, they may not be totally responsible for senescent impairment of reproduction.

Pituitary gonadotropin content and pituitary responsiveness to hypothalamic stimulation is also effected by aging. Clemens and Meites (1971) found increased prolactin content in aged constant estrous female rats. Although Matsyama (1966) reported increased gonadotropin content in 12 mo old constant estrous rat pituitaries, Clemens and Meites (1971) have reported decreased pituitary LH content. Watkins et al. (1975) and Riegle and Meites (1976) recently reported lower serum LH concentrations in aged rats in response to acute LHRH injections than in similarly treated young rats.

Female--Hypothalamic Function

Many investigators now believe that a primary site of age related alteration in the reproductive control system

is the hypothalamus. Dilman (1976) suggests that aging of the hypothalamus reduces its sensitivity to estrogen feedback, possibly due to impaired neurotransmitter synthesis (Frolkis, 1966). This theory is supported by reports of reduced inhibition of ACTH secretion by glucocorticoids in aged rats (Riegle and Hess, 1972; Riegle, 1973). Shaar et al. (1975) also demonstrated a smaller increase in serum LH after castration in aged rats as compared to young rats and a diminished response to negative feedback by administered gonadal steroids. However, Odell and Swerdloff (1968) and Wise et al. (1973) found similar hypothalamic-pituitary sensitivity to estrogen feedback in both pre- and post-menopausal women.

Alterations in hypothalamic function have not been correlated with anatomical changes of the hypothalamus with age. Andrew (1956) showed no cellular destruction in the supraoptic and paraventricular nuclei in the senile human hypothalamus. Finch (1973) also reported that brain weight and cellularity do not vary with age.

Several studies suggest that differences in the concentration of biogenic amines and the ability of the hypothalamus to secrete its releasing hormones is of great significance in the effect of aging on reproductive control systems. Finch (1973) found that catabolism of norepinephrine and dopamine is decreased in aged mice. Clemens and Meites (1971) reported elevated hypothalamic follice stimulating hormone releasing factor (FRF) activity in

aged constant estrous rats compared to that of young ones on the day of estrous.

Attempts have been made to experimentally alter hypothalamic control mechanisms. Systemic progesterone treatment of several days duration to aged rats in constant estrus is capable of inducing ovulation in association with at least one apparently normal estrous cycle (Clemens et al., 1969). Clemens et al. (1969) also demonstrated that direct electrical stimulation of the hypothalamic preoptic area of old constant estrous rats can cause ovulation. Their report further showed that prolonged systemic epinephrine administration could induce ovulation in old rats, the effect possibly mediated through alteration of certain central nervous system neuroendocrine functions. Similarly, chronic administration of L-dopa or iproniazid, drugs assumed to increase hypothalamic catecholamine availability, restored regular cycling patterns in old constant estrous rats (Quadri et al., 1973). Watkins et al. (1975) reported a decreased response to acute hypothalamic stimulation with L-dopa in aged rats as compared to young rats. It was found that the resulting increase in LH and decrease in prolactin was less pronounced in aged rats.

Riegle and Meites (1975) demonstrated that young rats respond to acute stress with increased serum LH, while aged rats show no such increase with stress, suggesting some alterations in the CNS mediated hypothalamic-pituitary mechanism with age.

Male--General Considerations

While the male reproductive system is often regarded as less complex than that of females, it also seems that investigators have a more incomplete understanding of the details concerning male reproductive control mechanisms, and particularly the alterations occurring in them with age. In male reproduction, advanced aging is characterized by the progressive decrease in spermatogenesis, hormonal secretion, secondary sex characteristics, and libido.

Male--Gonadal Function

The testes of senescent rats have been found to be significantly smaller than those of young adults (Peng et al., 1973) and similar findings have been reported in men (Stearns et al., 1974). A reduction in size and activity of the seminiferous tubules has also been reported in aged males(Adams, 1972). Although sperm production has been found to decrease, abundant sperm have been found in the testis and epididymis of sexually inactive aged males (Bishop, 1970; Peng et al., 1973). This observation that the loss of gametogenesis is not the primary lesion associated with decreasing fertility in aging males is in agreement with data from the female. The most common degenerative change in the testis with age is fibrosis in the seminiferous tubules. The numbers and histological appearance of Leydig cells of the testis have also been found to decline with advancing age (Albert,

1961, Peng et al., 1973). On the other hand, Lynch and Scott (1950) reported increased structural evidence for activity of sertoli cells associated with atrophied Leydig cells in the aged human testis. Here it was postulated that increased sertoli cell secretions of estrogens could inhibit LH secretion and contribute to the atrophy of the Leydig cells. Peng et al. (1973) also reported decreased seminal vescicle weight in aged rats.

There appears to be significant species differences in hormonal changes occurring with age. Eleftheriou and Lucas (1974) reported no decrease in serum testosterone concentrations in aging mice. Reports generally agree that plasma testosterone decreases in men over 70 years of age (Vermeulen et al., 1972; Persky et al., 1971; Rubens et al., 1974; Stearns et al., 1974), although Vermeulen et al. (1972) observed changes in testosterone to be highly variable. Ghanadian et al. (1975) found serum testosterone in aged male rats decreased as compared to young adults.

There have also been some reports of decreased testicular ability to respond to acute stimulation. Longscope (1973) showed that testosterone increased more in young than in aged men after administration of HCG. Similarly Rubens et al. (1974) reported decreased Leydig cell response to HCG in aged men, and suggested that the decreased testosterone secretion with age has a primary testicular origin.

Male--Pituitary Function

In support of the theory of primary testicular degeneration in men, Stearns et al. (1974) reported that mean LH concentrations rise after 40 yr of age, with gonadotropin concentrations being inversely related to testicular size. Stearns et al. (1974) hypothesize that in men there is a primary decline in testicular function beginning at 45-50 yr of age, with a resulting increase in pituitary LH secretion. However, alterations in the reproductive system of male rats appear to be somewhat different. Riegle and Meites (1976) reported decreased serum LH in the aged male rat as compared to the young adult. Several reports have also shown decreased pituitary LH with age (Debeljuk et al., 1972; Peng et al., 1973; Riegle et al., 1976) in the rat. Debeljuk et al. (1972) has also demonstrated that the pituitary response to acute LHRH stimulation is decreased in aged male rats. These reports indicate that testicular degeneration is not the primary source of dysfunction in the reproductive control system in the rat.

Male--Hypothalamic Function

There are conflicting reports concerning changes occurring in the hypothalamic sensitivity to negative feedback with age. Peng et al. (1973) reported a larger increase in plasma LH after castration in aged males than in young male rats. Shaar et al. (1975) found the opposite

result, accompanied by an increase in pituitary sensitivity to testosterone negative feedback in aged male rats.

In summary, observations indicate that reproductive control mechanisms are altered in aging mammals. It appears, however, that the specific changes are not coincident in humans and in the rat. Data from men and women imply primary degeneration of the gonad in both sexes may result in the increased serum LH concentrations demonstrated with advancing age. The rat, on the other hand, shows no such increase in pituitary secretion and other sources of degeneration besides the gonad may be implicated. There is considerable evidence which suggests age alterations in the function of the ovary, pituitary, and hypothalamus in the female rat. To date, much less is known concerning aging effects of gonadal control systems in the male. The experiments to be presented were intended to further characterize the alterations occurring in the reproductive control system of the male rat with age and to try to localize the primary site of these alterations.

## METHODS

### Experimental Animals

Young adult and aged male Long Evans rats (Blue Spruce Farms, Altamont, New York) were used in these studies. Rats included in the young group were three to six months of age, while those classified as aged ranged from twenty to thirty months. The studies included in this report have considered the effects of age on these two age groups only, with no consideration of intermediate ages. Rats to be used in aged groups were obtained either as retired breeders or surplus rats raised in our colony. All rats were housed in the Endocrine Research Unit's rat colony under conditions of controlled light (12 hr light cycle) and temperature (21°-22°C) and given free access to Wayne Lab-Blox (Allied Mills, Chicago, Ill.) and water. Mean body weights for young and aged rats were 436 and 502 grams respectively. In some studies, rats were subjected to more than one experimental treatment. In these instances, all rats were allowed a period of recovery of at least three weeks following previous experimental use to assure adequate hematocrit and blood volume recovery before they were considered suitable for further experimentation.

## Blood Collection

Rats were removed from their cages and transported to a surgery room before experimentation. All blood samples were taken under light ether anesthesia by orbital sinus puncture using heparinized capillary tubes. The volume of each blood sample ranged from 1 to 1.5 ml. Pretreatment blood samples were obtained within 30 to 60 seconds after the rats were first disturbed. This method of blood sampling has been shown to keep alterations of hormone concentrations due to stress effects at a minimum (Euker et al., 1975).

Blood samples were allowed to clot at room temperature for at least 30 min and then refrigerated overnight. Serum was then separated by centrifugation, decanted, and stored at 20°C until the day of radioimmunoassay for LH or testosterone.

## Hormone and Drug Treatment

Several experiments were performed involving treatment with synthetic luteinizing hormone releasing hormone (LHRH). Each rat was under light ether anesthesia when given 500 ng LHRH (Eli Lily Co., Indianapolis, Ind.) in 0.5 ml of physiological saline into an exposed jugular vein. In other experiments, human chorionic gonadotropin (HCG) at doses ranging from 1 to 20 IU was administered intraveneously in 0.5 ml of saline. When L-dopa was given,

15 mg dosages of the drug suspended in 0.5 ml of saline were given twice daily (∿8 am and ∿5 pm) for 10 consecutive days either subcutaneously or intraperitoneally.

## Hypothalami Collection and Preparation

The procedure for handling hypothalamic tissue was a modification of that of Shaar and Clemens (1974). Sixteen young and 16 aged male rats were decapitated as rapidly as possible after removal from their cages. Hypothalamic tissue (3 x 3 x 2 mm) was then quickly collected from each rat, weighed, and placed in a tissue homogenizer containing 0.2 ml cold 0.4 N perchloric acid. The tissue was manually homogenized, the homogenate decanted, and the homogenizer rinsed with another 0.2 ml of cold 0.4 N perchloric acid. The combined homogenate for each hypothalamus was centrifuged at 20,000X g for 30 min at 3°C. The supernatant was then decanted into a clean tube containing 0.1 ml of 190 EDTA (Sigma Chemical Co., St. Louis, Mo.) and stored at -20°C until assayed.

## Catecholamine Assay

Hypothalamic catecholamine assays were conducted in Dr. James Clemens' laboratory at the Eli Lily Company in Indianapolis, Indiana, under the technical direction of Dr. Carl Shaar. Norepinephrine and dopamine were measured by a modification of the microflourescent technique of Laverty and Taylor (1968). Eight hypothalamic extracts from each age group were used for dopamine quantification,

with the remaining 8 extracts used for measurement of norepinephrine. Each individual frozen preparation was thawed, transferred to a beaker and 2 ml of 1 M sodium acetate buffer and 1 ml of 1% disodium EDTa were added; then 240 mg of heat activated, neutral, grade 1 alumina were added and the mixture swirled for 5 minutes. After mixing, the supernatant was decanted and discarded. The alumina with the adsorbed catecholamines was then washed into a micro column containing 140 mg of alumina. The column was washed 3 times with triple distilled water, after which catecholamines were eluted into evaporating tubes with 5 ml of .2 N acetic acid. After evaporation to dryness on an evapormix, the residue was resuspended in .4 ml of triple distilled water and catecholamine determinations were made by acid fluorescence using a system of reverse blanks and an internal standard. Ten µl of phosphate buffer (PH 6.5), 10 µl of .02 N iodine and 50 µl of alkaline sulfite solution were added to 100 µl of the resuspended extract. After 5 minutes, 30 µl of glacial acetic acid were added to develop the fluorescence. Fluorescence of norepinephrine was read at excitation wave length of 392 mµ and emission of 490 mµ. After 40 min of heating at 100°C, dopamine fluorescence was measured at 320 m  excitation and 380 m  emission. Analysis of these data was performed using Student's t-test and a probability less than 0.05 was considered significant.

## Radioimmunoassay for Testosterone

The radioimmunoassay procedure for testosterone determination was that described and validated by Mongkonpunya et al. in 1975.

Duplicate aliquots (100 µl) from the unknown serum samples to be assayed were dispensed into 16 x 100 mm disposable culture tubes (Scientific Products, McGaw Park, Ill.). In order to account for losses occurring during the extraction procedure, 3000 dpm of $^{3}H$-1,2 testosterone (New England Nuclear, Boston, Mass.) was added to a third group of a representative number of tubes (10-20 per assay) containing a standard serum (100 µl). All tubes then received 2 ml of benzene: hexane (1:2), were vortexed for 30 seconds each, and were stored at -20°C for at least 1 hr in order to freeze the aqueous phase. The organic solvent from the tubes containing unknown serum samples were then decanted into 12 x 75 mm disposable culture tubes, and those with $^{3}H$- testosterone were decanted into scintillation vials.

Culture tubes containing various known amounts of purified testosterone (Sigma Chemical Co.) were pipetted from a stock solution having a concentration of 10 ng/ml. At least three sets of standards containing 0.0, 0.02, 0.05, 0.10, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 ng were included in each assay to serve as reference standards.

All serum extracts and testosterone standards were dried by air. Testosterone antibody (Niswender antiserum to testosterone - 3 - oxime - bovine serum albumin, #666)

was diluted 1:3000 in 0.1% gelation in 0.1 M phosphate buffered saline and 200 µl of this solution was added to each tube. Tubes were vortexed briefly and allowed to incubate at room temperature for 30 min. At this time approximately 30,000 dpm $^{3}$H-1,2,6,7-testosterone (New England Nuclear, Boston, Mass.) diluted in 200 µl gel PBS was added to each tube, vortexed, and refrigerated at 4°C for 24 hr to allow the complexing reaction between antigen and antibody to attain equilibrium.

To separate free from bound testosterone, a dextran-coated charcoal solution was made with 0.025 gm of dextran 150 and 0.25 gm of carbon decolorizing neutral Norit suspended in 100 ml distilled water, and 0.5 ml of this solution was added to each tube. Each tube was then vortexed briefly, chilled in an ice bath for 10 min and centrifuged in a refrigerated centrifuge at 2500 x g for 10 min. A 0.5 ml aliquot of the supernatant fluid of each tube was then diluted with 5 ml of liquid scintillation cocktail (Research Products International Corp., Elk Grove Village, Ill.) in scintillation vials for quantification of radioactivity in a liquid scintillation spectrometer (Nuclear Chicago, Des Plaines, Ill.). Vials made to check extraction recovery also received 5 ml of scintillation fluid and were counted. For comparison among assays, standard rat serum and blank extraction tubes are also assayed with each set of unknown serum samples.

All tubes were counted for four minutes. Standard curves were drawn on semilogarithmic paper correlating CPM with the log of the reference standard doses. Values for all unknown serum samples derived from the standard curves were then transformed into ng/ml concentration units. Duplicate values were averaged and statistical analysis was performed using Student's t-test. A critical alpha probability value of 0.05 was selected for these analyses.

## Radioimmunoassay for LH

The radioimmunoassay procedure for LH determination was that described and validated by Monroe et al. in 1968 and routinely used in the laboratory of Dr. J. Meites at Michigan State University.

Radio-iodination of purified rat LH (LER 1056 LH) with $^{125}I$ was performed at Dr. Meites' facilities. Labeled hormone was eluted through a 1 x 15 cm Bio-Gel P60 column and then diluted to a concentration of approximately 30,000 counts per minute (CPM) as counted by an automatic well counter (Nuclear-Chicago, model 1085L; Des Plaines, Ill.) using 0.1% gelation phosphate buffered saline (PBS). Anti-ovine antiserum (supplied by Dr. Niswender, Fort Collins, Colo.) was made by immunization of rabbits with purified hormone and was diluted to a concentration of 1:28,000 for use in the assay. The antigen-antibody complex was precipitated using a second antiserum resulting from specific immunization of sheep against rabbit gamma globulin. This

ovine-rabbit gamma globulin serum was diluted to a working concentration which from various bleedings ranged from 1:35 to 1:60.

Duplicate aliquots from the unknown serum samples to be assayed were dispensed into 12 x 75 mm disposable culture tubes (Scientific Products, McGaw Park, Ill.), and then diluted to a volume of 0.5 ml with gel PBS. A volume of 200 µl of the working rabbit antiserum (anti-LH) was added to each tube and the tubes vortexed briefly. Equilibration of the complexing reaction between available hormone antigen and antibody was accomplished during 24 hours of incubation at 4°C. At the end of the incubation, 100 µl of the labeled LH solution was added to each tube, the tubes vortexed, and incubated for another 24 hours at 4°C, allowing the competitive reaction between the unknown amount of endogenous hormone in the serum sample and the radio-labeled hormone to equilibrate.

Following this incubation, 200 µl of the ovine anti-rabbit gamma globulin antibody solution was added to each tube. The tubes were then briefly vortexed and refrigerated at 4°C for 72 hours to allow maximum antigen-antibody complexing and precipitation. At the end of 72 hours 3 ml of cold PBS were added to each tube and the tubes were centrifuged at 2200 RPMs for 30 min. The supernatant was poured off and each tube placed in a plastic holding jacket for counting in the automatic gamma well counter.

Culture tubes containing known amounts of purified hormone were also included in the assay to serve as reference standards. Five identical samples of 11 different doses ranging from 0.8 to 40.0 ng of purified NIH LH RP-1 was used for this purpose. Total count tubes, normal rabbit serum tubes (NRS) and total antibody binding tubes were also included in the assay procedure in order to determine general binding characteristics of each assay. Only radio-labeled hormone was added to the total count tubes and these tubes were a reflection of the total efficiency of count recovery. Normal rabbit serum (NRS) tubes received 200 µl of a standard rabbit serum diluted in gel PBS in place of hormone specific antibody, in order to monitor non-specific binding activity. Total antibody binding tubes were equivalent to "zero hormone" standards. The counting time for all tubes in a given assay was determined by the time necessary for the "zero hormone" standards to equal 10,000 CPM. Also, the non-specific activity represented by the NRS tubes was subtracted from all sample tubes by appropriate adjustment of the background setting on the gamma counter.

Standard curves were drawn on 3-cycle semi-logarithmic graph paper correlating CPM with the log of the dose of reference standard hormone. These standards curves showed 50% cold hormone binding at 8-15 ng. Quantitative serum hormone data for all unknown serum samples derived from the standard curves were then transformed into ng/ml

concentration units. Duplicate values were averaged and statistical analysis was performed on the experimental data using Student's t-test. A critical alpha probability of less than 0.05 was considered significant.

# EXPERIMENTAL

## Experiment 1. The Effects of HCG on Serum Testosterone

Introduction:

Relatively little is known about testicular function in aged male mammals. Although aged men have been reported to have lower blood concentrations of testosterone than young men (Persky et al., 1971; Vermeulen, 1976), other studies have shown this age-related decline in blood testosterone levels in men to be variable (Vermeulen et al., 1972). Other reports have suggested that the metabolism of pregnenolone is slower in older men and that the aged human testis may have less $C_{17}$-$C_{20}$ hydroxylase activity (Axelrod, 1972). The decrease in blood testosterone of the human male is in conflict with a recent report showing no change in plasma testosterone in aging mice (Eleftheriou and Lucas, 1974). Although our laboratory has reported decreased serum LH in aged male rats (Riegle and Meites, 1976), little information is available concerning blood testosterone concentrations or testicular responsiveness in the aged male rat. This study was undertaken to compare serum testosterone levels and testicular responsiveness to gonadotropin stimulation in the aged male rat.

Procedures:

The effect of HCG on serum testosterone was studied in two trials. In the first trial, 23 young and 27 aged male rats were assigned to one of three treatment groups to be given either 0.5 ml of physiological saline or 0.5 ml of saline containing 1 IU or 5 IU of HCG via jugular injection. Serial blood samples were taken from each rate before the intravenous injections and at 15, 30, and 60 minutes after injection.

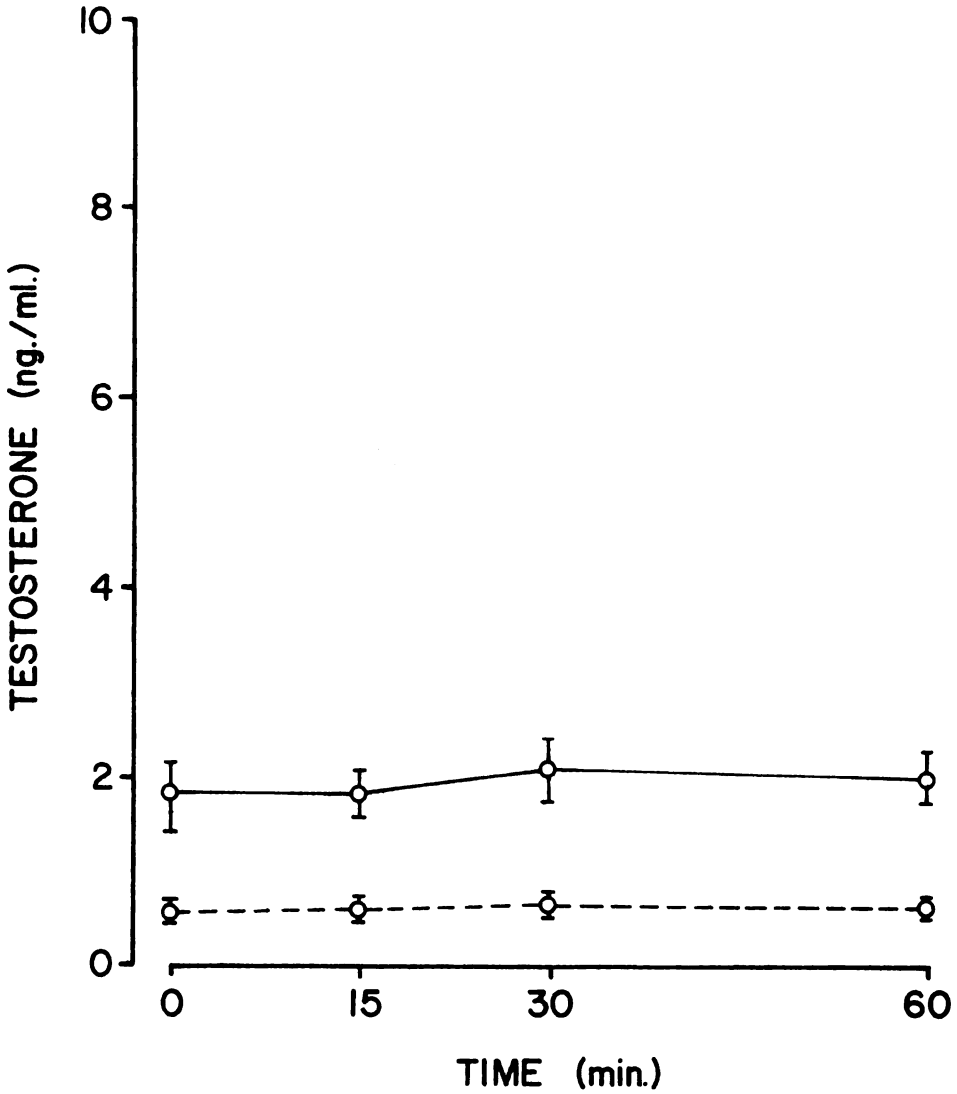
In a second trial, groups of 32 young and 31 aged male rats were assigned to one of 4 experimental groups and were given either intravenous injections of 0.5 ml of physiological saline or 0.5 ml of saline containing 1, 5, or 20 IU of HCG. Serial blood samples were taken from each rat before the intravenous injection and at 45, 90, and 150 minutes after the HCG treatments.

Results:

Figure 1 illustrates serum testosterone concentrations in the control groups of young and aged rats which received only the saline injections in the first experiment. Serum testosterone concentrations were significantly lower in aged male rats than in young male rats before treatment and throughout the sampling period. Testosterone concentrations remained stable throughout the sampling period in both groups.

Figure 1. Effects of intravenous administration of 0.5 ml saline on serum testosterone concentrations in young and aged male rats.

These data illustrate serum testosterone levels in saline injected control rats. Serum testosterone concentration expressed in ng/ml appears on the ordinate, with interval of time in minutes after injection represented on the abscissa. Blood samples were taken prior to injection of saline, and 15, 30, and 60 minutes afterwards. The solid line represents the means for 8 young rats and the dashed line signifies those for 6 aged animals. Standard errors are indicated with bars above and below each mean.


10
8
6
4
2
0
TESTOSTERONE (ng./ml.)
0
15
30
60
TIME (min.)

The response of young and aged male rats to 1 and 5 IU of HCG is plotted in Figure 2. Pretreatment concentrations of serum testosterone were higher in young than in the aged rats. After intravenous injection of 1 or 5 IU of HCG, rats in both age groups had sharp, steady increases in serum testosterone. This increase in testosterone, however, was approximately twice as great in young as in aged males. Also, both young and aged rats showed a greater increase in serum testosterone following injection of 5 IU of HCG than did the groups injected with 1 IU of HCG.

Figure 3 shows the results of the second trial which considered the effects of HCG stimulation over an extended sampling period. As in the first experiment, serum testosterone concentrations in the control groups which received only the saline injections were significantly lower in the aged male rats than in young male rats. Serum testosterone was increased in both young and aged rats following all 3 doses of HCG. The increase in testosterone was smaller in the aged rats compared to the young rats at each dose and at all sampling intervals. The increase in serum testosterone levels following HCG injection was sustained throughout the sampling period in rats of both age groups. The increase after 20 IU was not significantly greater than after 5 IU except for the initial sample at 45 minutes in the aged group.

Figure 2. Effects of intravenous administration of 1 or 5 IU HCG on serum testosterone concentrations in young and aged rats.

These data illustrate the effects of injection of 1 or 5 IU HCG on serum testosterone levels in groups of 16 young and 14 aged male rats. Serum testosterone concentration expressed in ng/ml appears on the ordinate, with interval of time in minutes after treatment represented on the abscissa. Blood samples were taken prior to injection of HCG and 15, 30, and 60 minutes afterwards. Open circles represent the means of rats given the 1 IU treatment, and open squares signify those of the rats given the 5 IU dose.

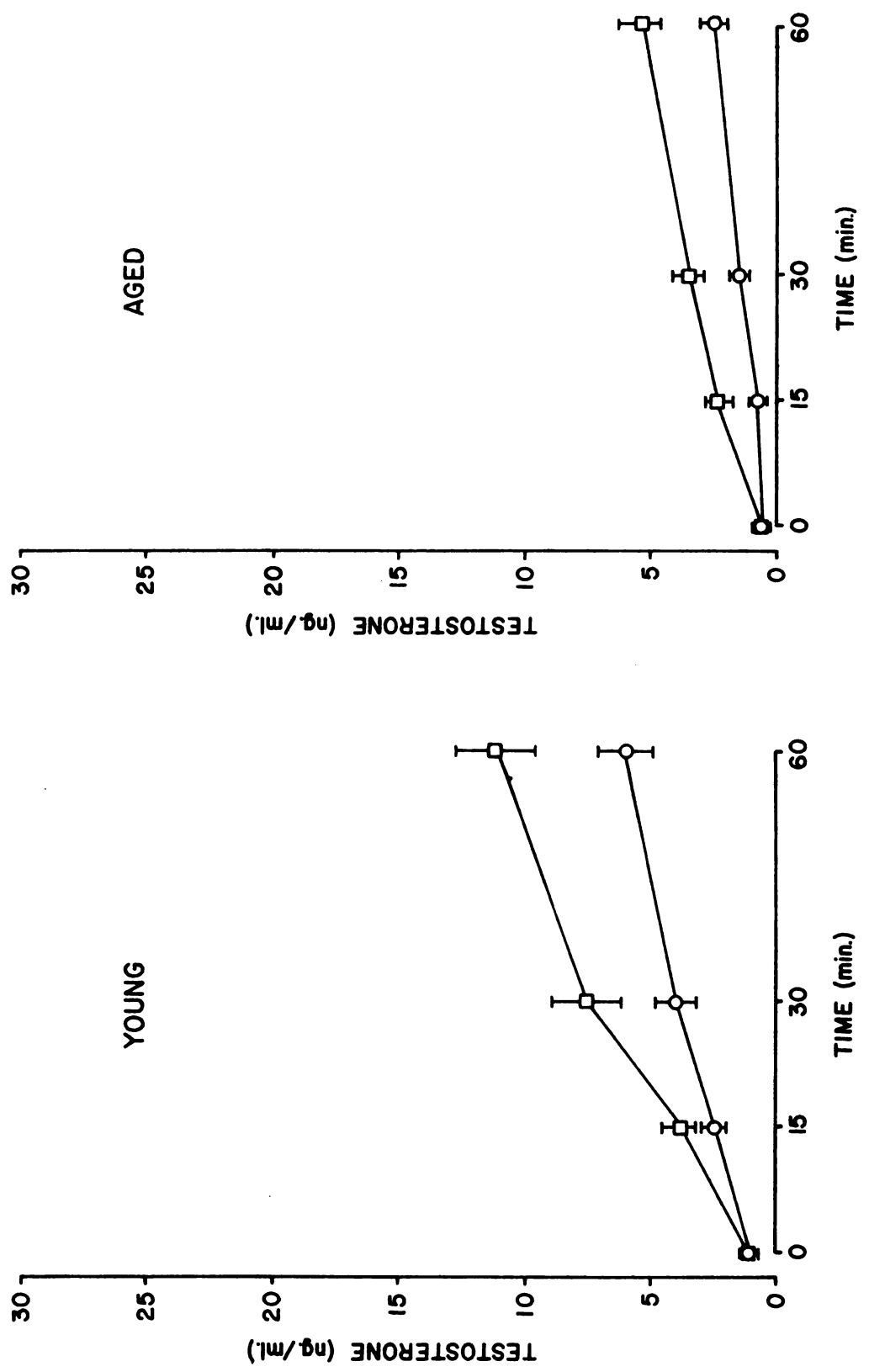
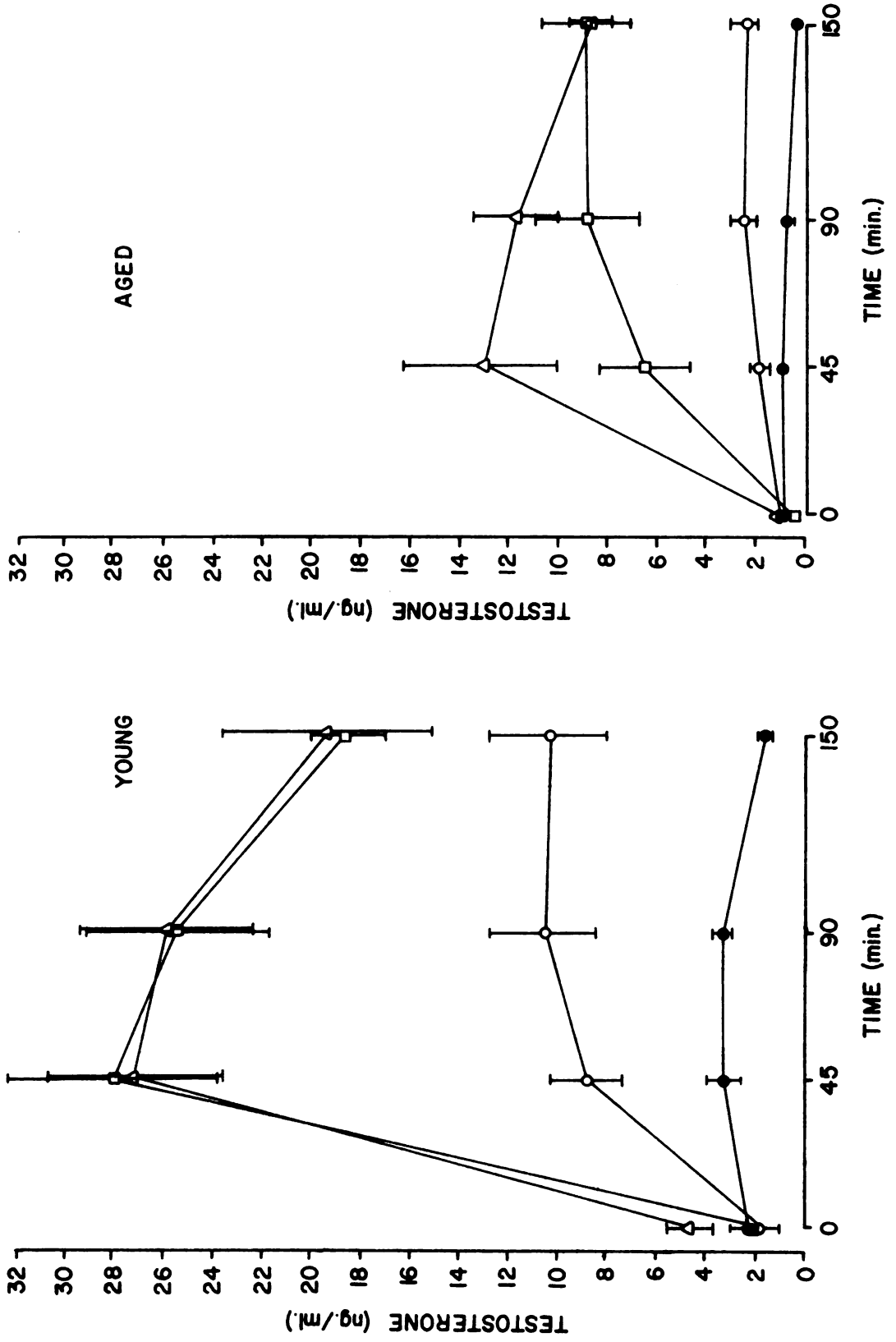

AGED
YOUNG
TESTOSTERONE (ng./ml.)
TIME (min.)
0
5
10
15
20
25
30
0
15
30
60

Figure 3. Effects of intravenous administration of 1, 5, or 20 IU HCG on serum testosterone concentrations in young and aged rats.

These data illustrate the effect of injection of 1, 5, or 20 IU HCG on serum testosterone levels in groups of 24 young and 23 aged male rats. Serum testosterone concentration expressed in ng/ml appear on the ordinate, with interval of time in minutes after injection represented on the abscissa. Blood samples were taken prior to injection of HCG and 45, 90, and 150 minutes afterwards. Solid circles represent means of saline injected control rats. Open circles denote means of the 1 IU dose rats, open squares correspond to means of the 5 IU dose rats, and open triangles signify those of the 20 IU treatment rats. Standard errors are indicated by bars above and below each mean.


AGED
YOUNG
TESTOSTERONE (ng./ml.)
TIME (min.)
0
45
90
150
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32

Discussion:

These data indicate that there are substantial alterations in testosterone secretion in aged male rats both in terms of resting serum testosterone concentrations and testicular responsiveness to acute gonadtropin stimulation. The decreased testosterone concentrations in blood samples from the control groups of aged rats are in general agreement with data from aged human males (Persky et al., 1971; Vermeulen, 1976), but are quite different from the level of plasma testosterone reported in aged mice (Eleftheriou and Lucas, 1974).

The increased serum testosterone concentrations in both age groups following HCG stimulation in the initial HCG experiment demonstrated that the testes of the aged male is capable of responding to stimulation by gonadotropin. However, this experiment did not indicate whether maximal testicular steroid secretion had been stimulated or what the time course of increased serum testosterone levels following HCG administration was in either the young or the aged groups. Since testosterone concentrations had not peaked prior to 60 minutes, it was possible that given sufficient time after HCG injections, aged rats would attain serum testosterone concentrations similar to the levels achieved following HCG injections in the young groups. However, in the second trial, serum testosterone concentrations were also higher in the young males than in aged male groups at all sampling intervals. The longer experimental sampling

interval, and the increased dose of HCG used in the second HCG experiment, suggests that 5 IU of HCG was sufficient to show maximal testicular effect. The 20 IU of HCG injection was more effective than the 5 IU HCG injection in stimulating serum testosterone only at the 45-minute sampling interval in the aged male group. Serum testosterone concentrations in the aged male 5 and 20 IU treatment groups were similar at both the 90- and the 150-minute sampling intervals. These data also suggest that the peak in serum testosterone concentrations occurs between 45 and 60 minutes following HCG injection in both age groups and that these elevated testosterone levels are sustained for at least 150 minutes after the injection of gonadotropin.

## Experiment 2. The Effects of Multiple LHRH Injections on Serum LH

Introduction:

Several laboratories have hypothesized that significant changes occur in hypothalamic-pituitary control of anterior pituitary hormone secretions during aging. Our laboratory has recently reported that single LHRH injections stimulate smaller increases in serum LH in aged compared to young adult rats of both sexes (Watkins et al., 1975; Riegle and Meites, 1976). Since these aged rats had lower pretreatment serum LH concentrations, it is possible that they are receiving less endogenous hypothalamic releasing hormone stimulation than are young rats. This experiment was designed to determine the effect of a longer duration of LHRH stimulation on pituitary LH secretion in young and aged rats.

Procedure:

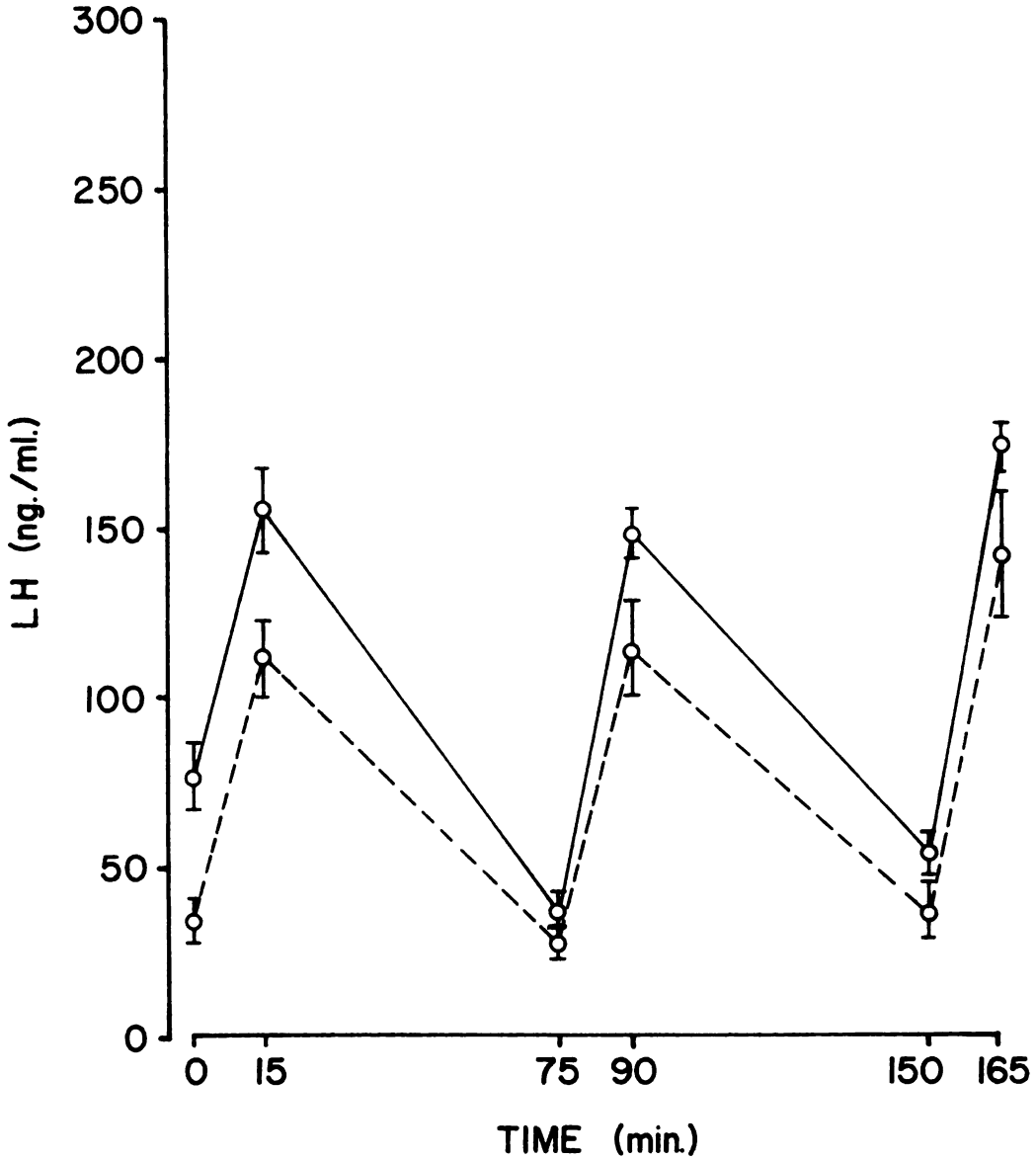
Groups of 17 aged and 24 young males received 3 intravenous injections of 500 ng of LHRH at 75-minute intervals. Serum LH was measured in serial blood samples taken before each LHRH injection and 15 minutes following each drug treatment.

Results:

The data in Figure 4 show that the young male rats had higher pretreatment serum LH concentrations and higher serum LH 15 minutes after the first LHRH injection. On the

Figure 4. Effects of three consecutive injections of 500 ng LHRH on serum LH concentrations in young and aged male rats.

These data illustrate the effects of three LHRH injections given 75 minutes apart on serum LH levels in groups of 24 young and 17 aged male rats. Serum LH concentration expressed in ng/ml appears on the ordinate, with interval of time in minutes after the first injection represented on the abscissa. Blood samples were taken prior to and 15 minutes following each LHRH injection. Solid line represents means of young rats and the dashed line signifies those of aged rats. Standard errors are indicated by bars above and below each mean.


300
250
200
150
100
50
0
LH (ng./ml.)
0
15
75
90
150
165
TIME (min.)

other hand, serum LH concentrations were similar in rats of both ages before and after the second and third LHRH injections.

Discussion:

The lowered pretreatment serum LH concentrations and reduced responsiveness following the first LHRH injection in the aged rats are in agreement with the report of Riegle and Meites (1976). The overall similarity of serum LH concentration between rats in the two aged groups in this experiment clearly indicates that the pituitary of aged male rat's pituitary is capable of a higher level of activity than it normally maintains, at least for the experimental interval tested. The ability of the pituitary to increase LH secretion after orchidectomy in aged rats (Peng et al., 1973; Shaar et al., 1975) also seems to indicate that the ability of the hypothalamic-pituitary control system to increase LH secretion remains functional. These data lend support to the hypothesis (Dilman, 1971) that the hypothalamic-pituitary control system becomes less sensitive to feedback control in aged male rats. Thus, the decrease in tonic function at the testicular and hypophysial level with age may be only secondarily due to a degeneration of function at those sites in response to a primary chronic decrease in the stimulatory activity of higher centers, most likely the hypothalamus and CNS.

## Experiment 3. The Effects of LHRH Injections on Serum Testosterone

Introduction:

The previous experiments have shown that serum LH concentrations are increased in aged male rats following LHRH injections and serum testosterone concentrations are increased after HCG stimulation. This study considered the effect of LHRH treatment on serum testosterone concentrations.

Procedure:

Groups of 8 aged and 8 young male rats received intravenous injection of 500 ng of LHRH. Serum testosterone was measured in serial blood samples taken before and 15, 30, and 60 minutes after LHRH injection.

Results:

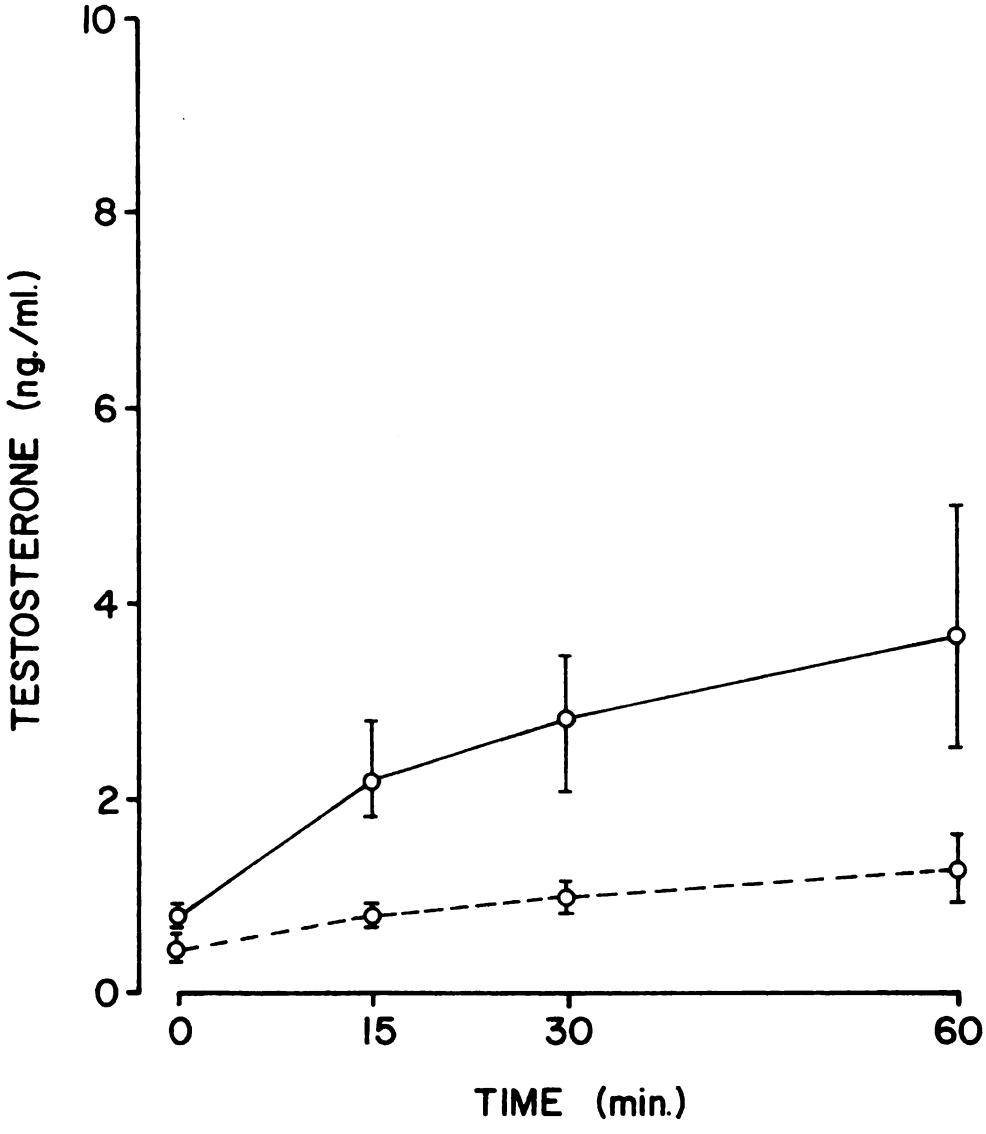
The data in Figure 5 show that the pretreatment concentration of testosterone was higher in young than in aged rats. Serum testosterone concentrations were progressively increased following LHRH injections in young rats, while serum testosterone in aged rats was not significantly increased over saline injected control levels.

Discussion:

Although the previous experiments have shown that the aged male rats' testes and pituitaries are capable of responding to LHRH and HCG stimulation, serum testosterone

Figure 5. Effects of intravenous administration of 500 ng LHRH on serum testosterone concentrations in young and aged male rats.

These data illustrate the effects of LHRH injection on serum testosterone concentrations in groups of 8 young and 7 aged male rats. Serum testosterone concentration expressed in ng/ml appears on the ordinate, with interval of time in minutes after injection represented on the abscissa. Blood samples were taken prior to injection of LHRH and 15, 30, and 60 minutes afterwards. The solid line represents means for young rats and the dashed line signifies those for aged animals. Standard errors are indicated by bars above and below each mean.


10
8
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2
0
TESTOSTERONE (ng./ml.)
0
15
30
60
TIME (min.)

did not increase in this experiment following LHRH. There are several factors which could be involved with this discrepancy. The increase in serum LH following LHRH may be less than the LH activity associated with the HCG injections, or the testes may not respond to the LH endogenously secreted by the aged rat pituitary. Both hypotheses will require additional experimentation before either can be applied to these data.

## Experiment 4. Hypothalamic Norepinephrine and Dopamine Content

Introduction:

The hypothalamus contains large amounts of norepinephrine and dopamine (Palkovits et al., 1974) which have been shown to be involved in control of release of anterior pituitary regulatory-hypothalamic hormones. Experiments from our laboratory and others have suggested fundamental changes in hypothalamic control of endocrine function with aging. It is conceivable that these changes in hypothalamic catecholamine content are related to the effects of aging on this control tissue.

Procedure:

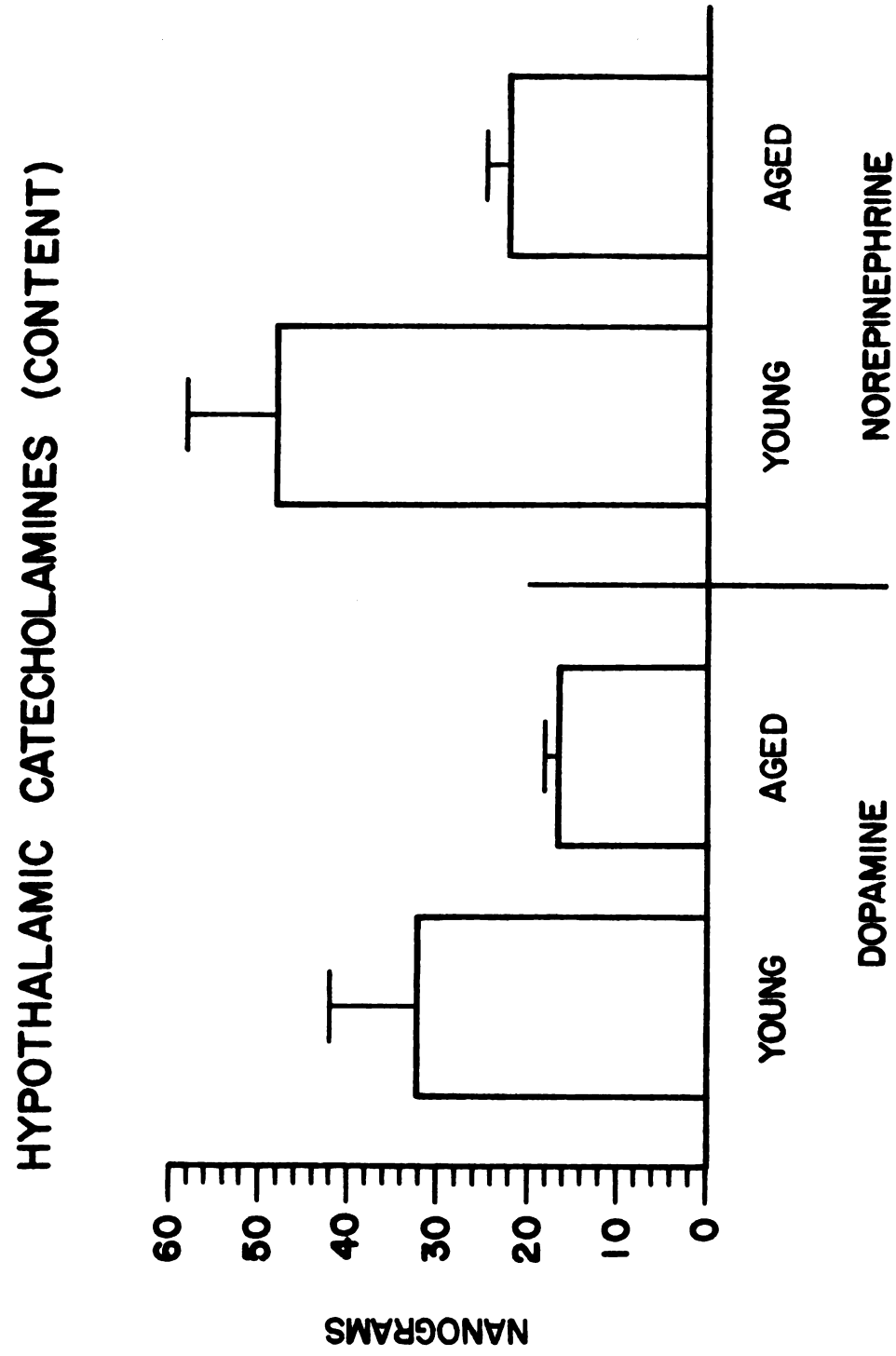
Groups of 16 young and 16 aged male rats were decapitated as rapidly as possible after removing them from their cages. Each hypothalamus was weighed and homogenized as previously described. Hypothalamic norepinephrine and dopamine was determined separately from 8 hypothalamic extracts from each age group.

Results:

Dopamine, and norepinephrine content in hypothalami of young and aged male rats are shown in Figure 6. In both instances, the hypothalamic content of catecholamines of young rats was about twice that found in the aged group. Average hypothalamic dopamine and norepinephrine content of young rats was 32.5 $\pm$ 9.3 mg per hypothalamus and

Figure 6. Hypothalamic catecholamine content in young and aged male rats.

These data represent the catecholamine content of hypothalami from groups of 8 young and 8 aged male rats. The height of each bar represents mean hypothalamic dopamine or norepinephrine content expressed as nanograms/hypothalamus, with brackets corresponding to the standard error of the mean.


HYPOTHALAMIC CATECHOLAMINES (CONTENT)
NANOGRAMS
60
50
40
30
20
10
0
YOUNG
AGED
DOPAMINE
YOUNG
AGED
NOREPINEPHRINE

47.6 ± 10.7 mg per hypothalamus, respectively. Hypothalamic content of dopamine and norepinephrine in aged rats averaged only 15.6 ± 2.5 mg and 22.8 ± 1.8 mg per hypothalamus, respectively. Hypothalamic weights averaged approximately 20 mg with no significant difference in size between the two age groups.

Discussion:

The results of this study suggest that important changes in hypothalamic catecholamine function may accompany aging. Although it is agreed that both adrenergic and dopaminergic pathways are involved in regulation of hypothalamic endocrine secretions, the precise mechanism by which these amines regulate hypothalamic secretion remains to be determined. Techniques such as those used in this study measure only the catecholamine content of the whole hypothalamus. Current concepts of the mechanism of amine control of hypothalamic function suggest that differences in hypothalamic catecholamine content in specific areas of the hypothalamus and turnover in individual hypothalamic nuclei may be more important in understanding the mechanisms of hypothalamic control (Palkovits et al., 1975).

Although the present data do not indicate differences in hypothalamic function, in particular anatomical regions, they indicate that there are major decreases in catecholamine function in our aged male rats which is consistent with our earlier reports of alterations in adrenocortical (Riegle, 1973) testicular and pituitary secretions.

## Experiment 5. The Effects of Chronic L-dopa Treatment on Pituitary Responsiveness to LHRH

Introduction:

We and others have hypothesized the changes in hypothalamic function may be involved in age-related alterations in adrenocortical and gonadal function. Quadri et al. (1973) demonstrated that daily administration of epinephrine, iproniazid or L-dopa could cause the resumption of cycling in aged constant estrous rats, suggesting that a deficiency of hypothalamic catecholamines may be responsible for the reduced ability of the pituitary to release LH and an increased pituitary release of prolactin in aged rats. In addition, Watkins et al. (1975) found that a single systemic injection of L-dopa could increase serum LH concentrations and reduce serum prolactin concentrations in both young and aged female rats. The present study considered the effects of chronic L-dopa treatment on serum LH following LHRH injection.

Procedure:

Groups of 19 aged and 30 young male rats received an intravenous injection of LHRH. Serum LH concentrations were measured in blood samples from all rats taken before and at 15 and 45 minutes after hormone injection. These rats were then randomly divided into subgroups. Aged male rats received intraperitoneal injections of 0.5 ml of saline or 15 mg of L-dopa suspended in 0.5 ml of saline, twice

daily for ten days. The young rats were assigned to one of 2 experimental groups. The young control rats received 0.5 ml of saline alone, twice daily. The L-dopa treated group was subdivided with half of the group receiving 15 mg of L-dopa suspended in saline by intraperitoneal injection twice daily with the other treated group receiving a similar L-dopa suspension subcutaneously twice daily for the 10-day period. On the 11th day, 15 hours after the last L-dopa injection all the rats received a second LHRH injection. Serum LH concentrations were measured in blood samples before and at 15 and 45 minutes after LHRH injection.

Results:

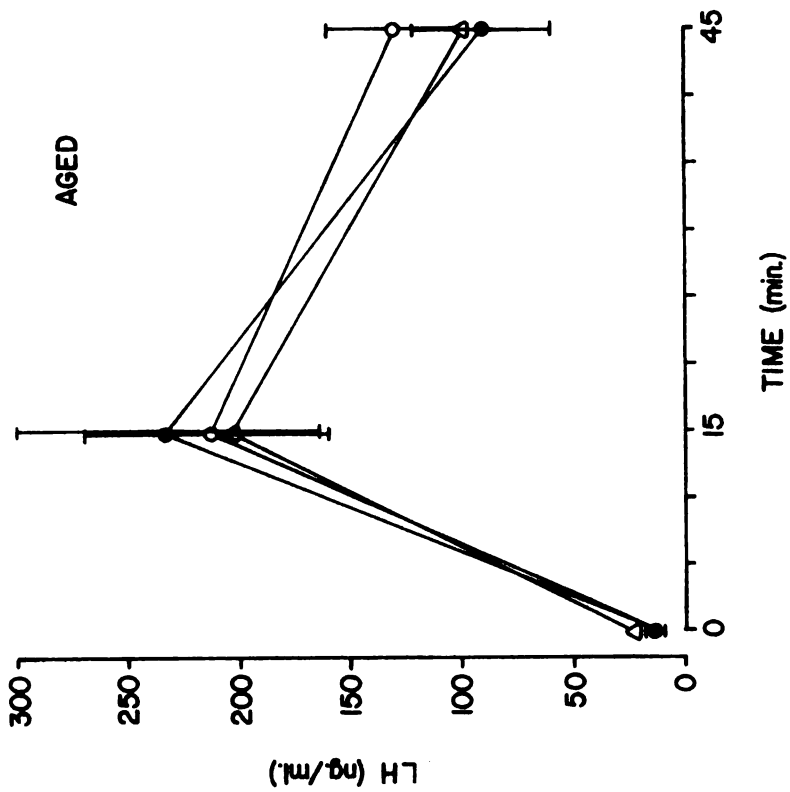
Although the pretreatment LHRH response showed that serum LH concentrations were higher 15 minutes after LHRH injection in the young than in the aged rats, serum LH 15 minutes after LHRH treatment at the end of the L-dopa injection regime was similar for both ages (Figure 7). Intraperitoneal L-dopa injection did not affect serum LH concentrations in either age groups. On the other hand, serum LH concentrations in young rats receiving L-dopa by subcutaneous injection were lower 15 minutes following LHRH than serum LH in either the control or intraperitoneal injected groups.

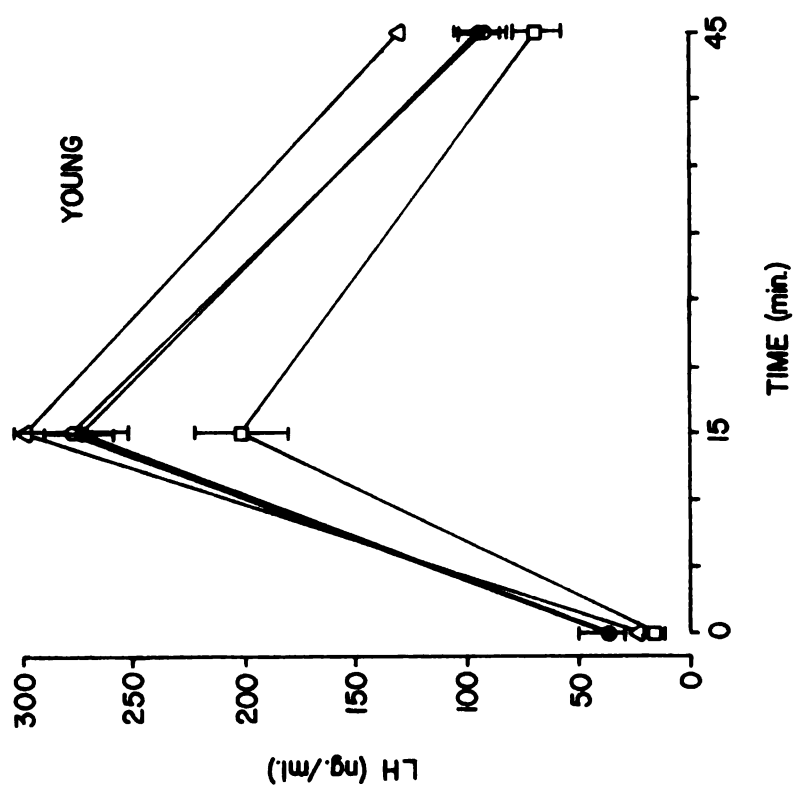
Discussion:

Although acute administration of L-dopa by intraventricular (Kamberi et al., 1970b and 1971a) or systemic

Figure 7. Effects of intravenous administration of 500 ng LHRH on serum LH concentrations in young and aged male rats prior to and following 10 days of L-dopa treatment.

These data illustrate the effects of LHRH injection on serum LH levels in groups of 30 young and 19 aged male rats before and after treatment with L-dopa. Serum LH concentration expressed in ng/ml appears on the ordinate, with interval of time in minutes after LHRH injection represented on the abscissa. Blood samples were taken prior to LHRH injection and 15 and 45 minutes after LHRH injection. Open triangles denote means of the response prior to the 10-day treatments. Open circles signify means of rats given L-dopa via intraperitoneal injections, and open squares correspond to means of those given L-dopa via subcutaneous injections. Solid circles represent means of control rats after they were treated with saline for 10 days. Standard errors are indicated by bars above and below each mean.


AGED
LH (ng./ml.)
300
250
200
150
100
50
0
0
15
45
TIME (min.)


YOUNG
LH (ng./ml.)
300
250
200
150
100
50
0
0
15
45
TIME (min.)

injection (Watkins et al., 1975) has been shown to affect pituitary release of LH and prolactin, interpretation of these experiments suggest that chronic L-dopa treatment does not significantly affect the concentration of these hormones in serum. Several factors could be involved with the inconsistency. The forementioned studies considered only the acute effects of this drug on serum hormone levels. In addition, Watkins et al. (1975) studied L-dopa effects in female rats. Riegle and Meites (1976) showed that similar L-dopa treatments could acutely affect serum prolactin, but not LH in male rats. On the other hand, Quadri (1973) found that catecholamine injections similar to those used in this study would reinitiate estrous cycles in aged constant estrous rats. These experiments do not indicate whether systemic L-dopa injections effect catecholamine function in the hypothalamus. The results of this study, as well as the effect of acute L-dopa treatment, suggest that any effect of L-dopa on hypothalamic function is short-lived and indicates that chronic administration of L-dopa may have little effect on neuroendocrine control systems.

# DISCUSSION

The data reported here indicates substantial alterations in several components of reproductive control systems in the male rat. There are, first of all, significant changes in testosterone secretion in aged male rats, both with respect to basal serum testosterone concentrations and in testicular responsiveness to acute gonadotropin stimulation. The decrease in resting testosterone concentrations in the serum of aged male rats is consistent with reports concerning senescent men (Persky et al., 1971; Stearns et al., 1974; Vermeulen et al., 1972), while data concerning the concentrations of plasma testosterone in aged male mice has been quite different (Eleftheriou and Lucas, 1974). The response of both young and aged rats to the various doses of HCG given in the first experiment were dose related and exhibited the same dynamics. However, the amplitude of the testicular response was proportionally less in the aged groups than in the young groups in all cases. Explanation for this decrease in testicular function and responsiveness with age may involve a decrease in the effectiveness of the systemic circulation carrying HCG to the gonad, a decrease in the numbers or specificity of

testicular binding sites for HCG, a decreased activation of the intracellular machinery to enhance testosterone synthesis and secretion, and most probably a decrease in the secretory capacity of the testes with age simply due to decreased numbers of functioning Leydig cells. While testicular function is somewhat impaired, it is to be emphasized that the testes of aged animals do respond to gonadotropin stimulation, and the control system at the level of the testis appears to remain intact.

Results from the second experiment verified an earlier report from our laboratory (Riegle and Meites, 1976) that pituitaries of aged rats secrete less LH after a single acute LHRH injection than that of young rats. The inconsistency here lies in the logic that if the hypothalamic-pituitary mechanism were responding to decreased negative feedback that should accompany low blood testosterone levels, serum LH and pituitary responsiveness to LHRH would be expected to be increased rather than showing the decrease reported.

A second experiment also demonstrated that serum LH concentrations after LHRH stimulation of a longer duration were not significantly different between the age groups. Thus, pituitaries of aged rats are capable of substantial secretory activity, certainly more than they normally maintain. Together, the first two experiments suggest that the decrease in serum testosterone and decreased testicular response to acute HCG stimulation may

first reflect a long-term lack of normal LH stimulation of the testes. This would account for the reported atrophy of Leydig cells in testes of aged males (Albert, 1961; Peng et al., 1973).

These experiments also indicate that the decrease in tonic function of the aged rat testis is not due to a degeneration of function originating at the level of the pituitary since it clearly retains a considerable capacity to respond. Instead, these data seem to support the hypothesis that the sensitivity of hypothalamic-pituitary control system to feedback control is altered with age (Dilman, 1971), resulting in a chronic decrease in the stimulatory activity of the CNS in general or more specifically the hypothalamus. The decrease in the stimulatory activity of these higher centers would then be the causative factor in the decrease in tonic functions at the testicular and hypophysial level.

This theory, while it does account for the decrease in the ability of the aged testis to function and respond to stimulation, it does not completely explain the virtual lack of testicular response to LHRH stimulation in aged males in the third experiment. It is possible that the complete lack of testicular response to LHRH may be due to an additive effect of decreased responsiveness at both the pituitary and testicular levels, resulting in a complete flattening of the response curve. However, analysis of acute HCG and LHRH experiments together might also suggest

the possibility that while the LHRH injections do stimulate secretion of adequate amounts of LH by the aged male rat pituitary, perhaps this endogenous LH secreted by the aged rat is an anahormone, with impaired biological activity, thus also explaining the subsequent lack of testicular response.

A great deal of experimental evidence suggests that hypothalamic releasing hormone secretion can be influenced by hypothalamic catecholamine function. The ability of the hypothalamic neurons to secrete releasing hormones could be related to changes occurring with age in the hypothalamic response to stimulatory or inhibitory input and the availability of neurotransmitters which have been shown to influence hypothalamic endocrine function. Interpretation of data presented herein suggests that important changes in hypothalamic catecholamine function may accompany old age. Although the methods used can recognize only gross changes in hypothalamic catecholamine content and cannot distinguish between individual nuclei, they do show that there are major decreases in catecholamine function in aged male rats which are consistent with the alterations in pituitary and testicular secretions. Perhaps the cause of the decline in function and responsiveness of the testes and pituitary originates with the decrease in hypothalamic catecholamine content. In any case, these preliminary results would suggest that aging differences in the ability of the hypothalamus to secrete its releasing hormone is of

great significance in the effect of aging on reproductive control systems and that hypothalamic catecholamine concentrations play an integral part. Clearly, much more experimentation in this area is necessary before the effects of aging on the hypothalamus and the resulting impact on the endocrine system as a whole is understood.

Recent studies suggest that systemically injected L-dopa may be converted to hypothalamic catecholamines with biological activity. Previous experiments have shown that acute treatment with L-dopa will result in a transient decline in serum prolactin and an increase in serum LH (Watkins et al., 1975) in young rats, and similar but less dramatic changes following acute L-dopa injection in aged rats. These reports, coupled with the present data concerning decreased hypothalamic catecholamines with age, stimulated an attempt to examine the effects of L-dopa treatment of a longer duration. However, in this study chronic treatment with L-dopa did not significantly change pituitary responsiveness to LHRH in the aged males as was anticipated.

Simultaneous assessment of all experiments presented generally suggest that with age, functional deterioration of gonadotropin control occurs at the level of the gonad, the pituitary and at the hypothalamus, with primary dysfunction occurring in the hypothalamus. The testes and pituitary of the aged rat may function in response to a chronic lack of stimulation originating at the hypothalamus,

which has become less sensitive to feedback control than young rats. More experimentation is necessary to understand more specifically the nature and degree of hypothalamic alterations.

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