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thesis entitled

Relation of Neuroendocrine System to Loss of Reproductive Function in Aging Female Rats

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

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Date September 8, 1977

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RELATION OF NEUROENDOCRINE SYSTEM TO LOSS OF REPRODUCTIVE FUNCTION IN AGING FEMALE RATS

By

Hive-Ho Huang

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Physiology

1977

ABSTRACT

RELATION OF NEUROENDOCRINE SYSTEM TO LOSS OF REPRODUCTIVE FUNCTION IN AGING FEMALE RATS

By

Hive-Ho Huang

1. Examination of daily vaginal smears revealed that beginning at about 10-12 months of age, or even earlier, rats gradually change from regular to irregular cycles and then to constant estrus; this is followed by pseudopregnancies of irregular length and finally to an anestrous state in the oldest rats.

2. Ovulation and cycling were induced in old constant estrous rats by daily injections of progesterone, ACTH, L-DOPA or by subjection to ether stress. Progesterone and ACTH were the most effective agents used for re-establishing estrous cycles in these rats. Most of the progesterone or ACTH treated rats showed regular cycles and the ovaries had many corpora lutea; they also showed proestrous serum LH surges. Ether stress and L-DOPA mostly induced irregular cycles and fewer corpora lutea in the ovaries; a small number of these rats showed proestrous LH surges. After treatment with each of these agents was discontinued, most of the rats returned to constant estrus or irregular cycling.

3. Serum LH, FSH and prolactin levels were measured before and after ovariectomy and during and after estradiol benzoate (EB) treatment in young mature female rats, in old constant estrous,

pseudopregnant and anestrous rats. It was found that the hypothalamus-pituitary system of old female rats has less capacity to secrete FSH and LH and more capacity to secrete prolactin than young female rats.

4. The positive feedback action of ovarian steroids on LH and FSH release was studied after ovariectomy of 16-20 month old constant estrous rats and 4 month old cycling female rats. Treatment with EB alone induced surges of LH and FSH in young rats 3 days after EB injection. No LH surges occurred in old rats, but a small surge of FSH was observed in old rats. When EB was followed 3 days later by a single injection of progesterone, both young and old rats exhibited surges of LH and FSH. However, serum LH and FSH rose to significantly higher levels in young than in old rats. These results suggest that failure of ovulation and cessation of estrous cycles in old constant estrous rats is due mainly to deficiencies in the hypothalamo-pituitary LH response to positive feedback by ovarian hormones.

5. Ovulation and formation of corpora lutea were induced in old constant estrous rats by injections of LHRH. The ability of pituitary to release LH and FSH in response to LHRH was tested in old constant estrous and young cycling rats. The capacity of the pituitary to release LH and FSH in old constant estrous rats was similar to that of young cycling rats. The cessation of estrous cycle in aging rats appears to be due mainly to changes in hypothalamic function.

6. Steady state concentration and turnover of NE and DA were measured in the anterior and posterior hypothalamus of 20-22 month old and 4-5 month old ovariectomized rats treated with estrogen or

with estrogen followed by progesterone. In old rats, concentration and turnover of NE were significantly lower in the anterior hypothalamus as compared with corresponding young rats, but there were no difference in DA concentration. Four hours after progesterone injection (critical period preceding the gonadotropin and prolactin surge), there was a decrease in DA turnover in the anterior hypothalamus of young rats, but this did not occur in old rats. Concentration and turnover of NE and DA were not different in the posterior hypothalamus between old and young rats, and NE and DA turnover were unaltered by a single injection of progesterone. These results provide additional evidence that loss of cycling and decreased stimulation by ovarian steroids on gonadotropin release in old rats are due largely to depressed catecholamine activity.

By 10 days after ovariectomy, serum LH and FSH in old rats were only about half as much as in young rats, whereas prolactin levels were 2-fold higher than in young rats. These results indicate that in old constant estrous rats, the decrease in NE and DA activities in the anterior hypothalamus was associated with a reduction in LH and FSH release and loss of cycling, and with an increase in prolactin release.

7. Sixteen 18 month old constant estrous rats were treated with progesterone and mated with fertile male rats. It was found that mating of old constant estrous rats, whether treated with progesterone to induce regularly estrous cycles, or not, resulted in pseudopregnancies in most of the rats and pregnancies in the remainder, but none of the fetuses survived or underwent parturition. The reasons for the pregnancy failures in the old rats are not entirely clear, but may be

due to faults in the ova or reproductive tract, decreased uterine sensitivity to ovarian hormones, deficient hormone secretion by the ovaries and placenta, or to infection of the reproductive tract.

DEDICATION

I dedicate this thesis to my mother
Pi
and to my wife,
Siang Lin.

ACKNOWLEDGEMENTS

I wish to acknowledge thanks to Dr. Joseph Meites for providing me with the opportunity and the support while undertaking these studies. It was indeed a honor and a pleasure for me to have been his student. I also wish to thank the members of my Guidance Committee: Drs. G. D. Riegle, E. Convey, C. Welsch, W. Frantz for their advice and encouragement. Appreciation is expressed to all my colleagues especially to Steve Marshall, John Bruni, James Simpkins and Clare Twohy.

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INTRODUCTION

In mammalian species, reproductive functions decline with aging and the capacity to produce live offspring by the female usually ceases well before death of the individual. Many neuroendocrine and other mechanisms involved in these changes remain to be investigated.

For many years, I have used the rat as a model for the study of reproductive changes with aging. Rats have a lifespan of about 2.5 to 3.5 years. Beginning about 10 months of age or earlier, the cycles of some rats become irregular and show a gradual reduction in the number of eggs ovulated. After normal cycling ends in female rats, they may exhibit patterns of constant estrus, pseudopregnancies of irregular length, or an anestrous state, as determined by examination of the ovaries and by daily vaginal smears (Aschheim 1961, 1965; Clemens and Meites 1971; Peng and Huang 1972).

My approach has been to try to study changes in the hypothalamus, pituitary, ovaries and reproductive tract that may contribute to the reproductive decline in old female rats. I, therefore, have observed the progression of estrous cycle in rats with advance of age and have tried to determine how these progressive changes are related to the neuroendocrine system. In this thesis, I have measured hypothalamic catecholamines, serum gonadotropins and prolactin, and have tested the ability of pituitary and ovaries to respond to synthetic GnRH. I have measured the response of hypothalamo-pituitary system to many stimuli in aged rats. In addition, I have attempted to reinitiate

cycling and pregnancies in old rats by treatments that attempted to correct neuroendocrine deficiencies.

LITERATURE REVIEW

I. Current Theories of Biological Aging

Theories on the nature of the aging process are numerous, and no other field of biological inquiry is underpinned by so many theories as the science of gerontology. Indeed, the aging process remains poorly understood. It is not a simple, discrete phenomenon, but rather encompasses a whole series of complex, interrelated changes occuring with time. Until now there is still a lack of sufficient fundamental data on the changes that with time affect almost all biological systems from the molecular level to the whole organism. Of the numerous theories that have been and continue to be advanced on the causative factors in aging, the following may be taken as representative: (1) genetic hypotheses regarding accumulation of cellular mutations and loss of biological information; (2) concept of progressive changes in biochemical molecular structure with age; (3) concept of changing immunological system with age; (4) general developmental theories relating to morphogensis and to cessation of somatic growth; (5) relation of reproductive function to senescence; (6) mechanical-chemical explanation of histological and pathological changes in nervous, endocrine, vascular, muscular, and connective tissues. Because of the wide diversity of aging theories, I would like to review only the most important and fundamental hypotheses that have been proposed by different investigators.

A. Genetic Programmed Theory

This theory proposes that the life-span of certain species of animal is controlled by a unique genetically determined aging program or a specific biological clock. For example, rats or mice rarely live more than four years, whereas many humans are still active at seventy. In man there is a positive correlation between the life duration of parents and their offspring. Monozygotic twins usually have almost identical life durations; however, dizygotic twins exhibit a greater difference in longevity (Everitt and Burgess, 1976). Hayflick and Moorhead (1961) observed that normal diploid human fibroblasts have a finite ability to replicate in vitro. Hayflick (1965) also reported the population-doubling potential of these cells is inversely related to the age of the donor. He proposed that these findings are a manifestation of aging at the cellular level. These observations have been confirmed and extended by many other workers in subsequent years. There is now some evidence supporting the notion that the numbers of population doublings of cultured embryonic fibroblasts are directly proportional to the mean life-span of the species (Hayflick, 1975). For example, the maximum life-span of the Galapagos Tortoise is about 175 years, and its fibroblasts show 90-125 generations of cell division before the cessation of mitotic capability. The oldest age of human subjects is approximately 110 years, and human fibroblasts exhibit 40-60 generations of cell division. Rats live no more than 4 years, and its fibroblasts can divide only 14-28 times. It also has been known that in vivo transplantation of normal tissues to young donor animals, followed after host aging by transplantation again to young donors, also reveals a finite capacity for cell replication. Thus in vitro and in vivo findings appear to be compatible with the notion

that normal human and animal cells are capable of only a finite number of mitotic events. However, some investigators do not agree with Hayflick's views and claim that non-fibroblast cells do not show the reported limitations on mitotic divisions.

Wilson (1974) interpreted that the genetic programmed theory as one involving wear-and-tear. The cause of aging is due to a 'running-out-of' program. The genetic program which orchestrates the development of an individual is incapable of maintaining the individual indefinitely. Thus senescence is not the result of programmed death, but due to lack of a genetic program to maintain the life of the organism.

B. Somatic Mutation Theory

Aging may result from random mutations in the DNA of somatic cells (Curtis and Gebhard, 1958; Szilard, 1959). The mutations are cumulative and cause errors in the coded information needed for protein synthesis. The postulate is that normally functioning cells cease to function, or function only in a somewhat impaired or abnormal fashion because of the gradual aquisition of mutations in the prime genetic material of the cells. Species with high mutation rates have short life-spans. The incidence of atypical chromosomes increases with age in rat liver (Curtis et al., 1966). The mutagenic agents may be radiation or substances which can cause chromosomal breaks or interfere with cell division. It has been reported that animals surviving the acute effects of exposure to ionizing radiation of various types have a shortened life-span, and it has also been noted that mice which have been subjected to radiation show premature onset of non-neoplastic disease of senescence and shortened life-spans. Nitrogen mustard, myleran, chlorambucil, and triethylene (all are

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alkylating agents) do have a life-shortening effect, but less than that produced by radiation (Welch, 1969). However, on the basis of the experimental evidence presently available, radiation does not seem to play a major role in accelerating the aging process, nor is the mutation role itself a major cause of aging. It is still possible that certain cell types may play a role in aging, but this kind of restricted hypothesis is much more difficult to prove or disprove and will have to await further experimental developments.

C. Error Theory of Protein Synthesis

Orgel (1963) proposed a model for biological aging based on a decrease in the fidelity of protein synthesis. The continual production of proteins within each cell involves correct reading of the language of the genetic code. This process includes a number of components: among them are the messenger RNA molecules produced from the DNA template, the ribosomes on which the actual assembly of protein takes place, and the transfer RNA molecules that bring the amino acids to the ribosomes. In addition, certain enzymes are themselves involved in the production of protein. Among the most important are RNA polymerase which is needed to produce the RNA molecules from the DNA and tRNA synthetases which are necessary to attach the amino acid to the tRNAs. A unique amino acid must be selected by each activating enzyme and this must be attached to the appropriate tRNA. A codon of messenger RNA must then pair with the anticode of an appropriate tRNA. Occasionally, in the course of protein production, some of the components of this process will not function properly and will make a mistake, and as a result an incorrect amino acid will be incorporated into a protein. An error in a tRNA synthetase can accumulate more

errors and lead to a cytoplasmic "error catastrophe" in protein synthesis and cause cell death. This "error catastrophe" hypothesis is supported by evidence obtained by Holliday (1969) on the fungus "podospora." He demonstrated that incorporation of amino acid analogue shortened its life-span.

D. Immunological Theory

The ability of individuals to synthesize antibody in response to antigen stimulation reaches a peak level of activity during adolescence and then begins to decrease gradually, long before any lesions associated with a declining immune system can be detected in the lymphatic tissues. The peak level of activity was detected in mice 16 weeks old, and it began to decrease soon after 20 weeks. When the mice were 120 weeks old, the level of activity was only 5% that of the peak level (Makinodan et al., 1971). Reduction in the ability to synthesize antibody among aged animals may be due to a decrease in the number of immunocompetent cell population and a deficiency in the differentiation process of antigen-triggered precursors or antibody-forming cell (Kishimoto and Yamamura, 1973). As the immune system is depressed with age, the defective cells and abnormal cells can not completely be eliminated. The faulty cells accumulate thereby increasing the numbers of tumors and non-functional cells eventually causes in the death of the organism (Everitt and Burgess, 1976).

Another immunological theory involved in the aging process is the autoimmune phenomenon. In 1962 Walford reported that mutated cells stimulate immunological reactions, that would eventually destroy the individual's own tissues. Both the titer of auto-antibodies and incidence of autoimmune disease rise progressively with age (Walford,

1966). Mice treated with imuran, an immunosuppressive agent, live only a little longer than controls. This suggests that if the immunological theory is valid it is concerned only with part of the aging process.

E. Cross-Linkage Theory

A cross-linkage is a chemical bond joining together two or more macromolecules. These macromolecules may be DNA, RNA, protein, or lipids. The bonds formed are generally covalent bonds which are characteristically stronger than the ionic or hydrogen bond formed by eletrostatic interaction. Cross-linkage, when it occurs, is usually permant and seldom is broken. Cross-linking between molecules is capable of inducing alterations that render the molecules non-functional. The configuration of an enzyme can be changed in such a way that it no longer performs catalysis. A site on a molecule of messenger RNA may become "tacked on" to a site on a ribosome, thereby inactivating both ribosome and RNA. A base in a DNA molecule may change sufficiently to render inactive one ellele of a gene on a chromosome, or a histone molecule may react with the DNA molecule and inactivate the DNA. These and similar alterations are capable of significantly impairing cellular efficiency, as reflected in the reduction of the information potential of the genome or in disturbance at the organismic level, and so lead to shortened life-span and death (Bjorksten, 1968). There is considerable evidence to support the cross-linkage theory. The cross-linkage of collagen molecules satisfactorily explains the increased structural stability of collagen fiber with advancing age. Increasing cross-linkage of the connective tissue network between a capillary and a tissue cell would impede the transport of nutrients

and waste products. This could explain the decline with age in the defficiency of most physiological processes (Everitt and Burgess, 1976). Cross-linkage is brought about by the presence of reactive groups, either found normally on the macromolecules or produced by various agents, such as free radicals, aldehydes, peroxides, quinones and sulfhydryl groups. Although the evidence supporting the cross-linkage theory is strong some experimental results still are not conclusive. Inhibition of collagen cross-linkage with the lathyrogen B-amino propionitrile has not consistently increased the life-span of rats. Also increasing the cross-linkage of collagen with gold salts failed to increase the mortality of rats.

F. Free Radical Theory

Free radicals are highly reactive cellular components derived from atoms or molecules in which an electron pair has been transiently separated into two electrons that exhibit independence of motion. Because the magnetic moments of these electrons are no longer complementary, such radicals exhibit a large increment of free energy and can oxidatively attack adjacent molecules, especially if these molecules are partially activated. Free radicals may be generated to a small extent by radiation or simply by the statistical probability that compounds may dissociate in such a way so as to form a free radical; more importantly they form a key component of the oxidative processes that occur within the mitochondria. The interaction of metal ions with cell components also may generate free radicals. Not only are free radicals very likely to undergo spontaneous chemical reactions, but they do so in such a way they tend to be self-propagating, that is, one free radical will generate others, which, in turn, will

generate others, producing a chain reaction. Only a few free radicals are needed at any given time to wreak a considerable amount of havoc (Spiegle, 1972).

The accumulation of free radicals has been regarded as basic to accelerated aging as observed in several species (Harman, 1956). Free radicals may act on such irreplaceable vital molecules as nuclear DNA, membranal lipid, and collagen (Harman, 1968). Experimental evidence supporting the free radical hypothesis of aging as well as the life prolonging role of radioprotective and antioxidant compounds has derived in large part from the long-term studies conducted in mice by Harman. Among the many compounds tested for this purpose, cysteine, cysteamine, hydroxylamine, and butylated hydroxytoluene were shown to increase average longevity by 7 to 29% (Harman, 1956, 1957, 1961, 1968). Vitamin E, a natural antioxidant, also has been investigated with respect to its role in decreasing the free radical activity in body tissues. A deficiency of vitamin E accelerated the accumulation of the intracellular age pigment, lipofuscin, and thus may bring about the impairment of cellular function (Sulkin and Srivanij, 1960). Since lipofuscin contains peroxidated lipid, it has been postulated that vitamin E may prolong life by preventing peroxidation and other free radical-initiated damage to the lipoidal membranes with which this lipid soluble vitamin is intimately associated within the tissues. However, the mode of action by which radioprotectors and antioxidants exert a protective effect on general health and longevity remains controversial and is little understood.

G. Hypothalamic-Pituitary Hormone Theory

Normal aging is usually accompanied by the gradually developing imbalance of the internal environment of the body and disturbances in the homeostatic mechanism. The neuroendocrine system is of prime importance in the integration of internal and external environmental factors. Thus, the constancy of the milieu interieur must be maintained for normal physiological function. The functions of the hypothalamus and endocrine system are extremely complex and diverse, and the relation between hormone secretion and the aging process are very inconclusive and inconsistant. To simplify these complexities, some investigators proposed several aging theories involving the hypothalamus.

1. Hypothalamic Threshold Feedback Theory

Dilman (1971) proposed that intrinsic aging of the hypothalamus is the cause of aging in other organs and systems of the body. He believes that the process of normal aging involves an elevation of hypothalamic threshold to systems which control energy, adaptation and reproduction systems. That is, with increasing age the hypothalamus becomes less sensitive to feedback inhibition by glucose, adrenocortical steroids and sex steroids. He insists that elevation of hypothalamic threshold to feedback inhibition leads to alteration of the rhythmic function of the main homeostatic systems, and the resultant imbalance of the internal environment, causes age-related pathology.

2. Hypothalamic Disregulation Theory

According to a series of studies by the noted Russian gerontologist, Vladimir V. Frolkis, individual components of the hypothalamus age in different ways and at different rates. A lack of uniformity in hypothalamic aging would thus create irregularities in

hypothalamic function, which could initiate the development of age-related pathology and particularly arterial hypertension, atherosclerosis, myocardial infarction, diabetes and a pathological climacteric syndrone (Frolkis et al., 1972).

II. Functional Neuroanatomy of the Hypothalamic-Pituitary System

A. General Anatomy of the Hypothalamus

Many books and reviews have been written concerning the neuroanatomy of hypothalamus (DeGroot, 1959; Daniel, 1966; Knigge, 1974). The role of the hypothalamus in pituitary function is depicted in the colored illustrations of the nervous system by Netter (1968).

The hypothalamus is the ventral-most portion of the diencephalon and is exposed on the central surface of the brain. It is bordered anteriorly by the lamina terminalis, posteriorly by the interpeduncular fossa, dorsally by the hypothalamic sulcus in the third ventricle and ventrally by the tuber cinereum. Three distinct regions can be identified when the hypothalamus is analyzed in a rostral to caudal sequence: the supraoptic, tuberal and mammillary areas. Within these rather distinct areas there are specific groups of nuclei. The supraoptic area lies above the optic chiasm and fuses rostrally with the preoptic area. The supraoptic region contains the supraoptic and paraventricular nuclei concerned primarily with the secretion of oxytocin and antidiuretic hormone (ADH) respectively (Bargmann and Scharrer, 1951). The tuberal region of the hypothalamus refers to the area just dorsal to the tuber cinereum located on the central surface of the brain between the optic chiasm and mammillary bodies. The infundibular area attaches to the median emminence of tuber

cinereum. Near the infundibular attachment is located the periventricular nucleus or arcuate nucleus. Both names are used to describe the gray area enveloping the base of the third ventricle. Immediately lateral to the periventricular nuclear borders of the third ventricle are the dorsomedial and ventromedial nuclei. The mammillary or posterior hypothalamic region is named for the mammillary body, the most prominent structures in the region.

The hypothalamus receives projections from many fiber tracts coming in from broad areas of the brain. The afferent pathways include the fornix, medial forebrain bundle, thalamohypothalamic fibers, mammillary peduncle and stria terminalis. In addition, the hypothalamo-hypophysial tract, periventricular fibers and mammillary efferents are the three major efferent fiber tracts of the hypothalamus.

B. Hypothalamo-Hypophysial Portal System

The pituitary gland is attached to the brain by the infundibulum and tuber cinereum, an extension of the third ventricle which is prolonged downward at the pituitary stalk. Part of the tuber cinereum and the uppermost part of the neurophypophysis is called the median eminence. It is in this region, the infundibulum and median eminence, that the hypophysial portal blood vessel originate. There are no direct neural connections between the hypothalamus and anterior pituitary; rather, the connection is by way of the hypophysial portal blood vessels.

Popa and Fielding (1930) first observed that the vessels transcending the human pituitary stalk are true portal vessels connecting a capillary bed in the median eminence region of the basal hypothalamus to sinusoids in the anterior pituitary. The anatomy of portal vessels

was confirmed by Wislocki and King (1936) who (contrary to Popa and Fielding) concluded on the basis of morphological evidence that blood flow was directed from the hypothalamus into the pituitary. The presence of this vascular connection, termed the hypophysial portal vessels, was observed to be a common feature among vertebrates (Green and Harris, 1947, 1949; Harris, 1972). Direct microscopic observation demonstrated blood flow was downward in the amphibian (Houssay et al., 1935), in the rat (Green and Harris, 1949; Barrnett and Greep, 1951), in the dog by Torok (1954) and in mice by Worthington (1955).

The hypophysial portal vessels deliver the blood to the anterior pituitary tissue from long and short portal vessels. The long portal vessels run down the pituitary stalk and the short vessels lie at the lower level (Adams et al., 1965; Daniel, 1966). Upon entering the second capillary bed, the sinusoids of the anterior lobe, the blood is carried past the parenchymal cells and is discharged through venules sinuses which lie around the pituitary gland. The capillary bed in the median eminence and stalk feeding the long portal vessels is supplied mainly by the inferior hypophysial arteries and by the arteries of the trabecula, which is part of the superior hypophysial arteries. Each group of portal vessels, the long and short, supplies a specific area in the anterior pituitary. These specific areas and effects of vessel ablation are discussed in detail for the rat and the sheep (Daniel and Prichard, 1956, 1957) and in man (Xuereb et al., 1954).

C. Chemotransmission and Hypothalamic Releasing Hormones

The classic investigations of the midbrain of fishes by E. Scharer (1928, 1930, 1932) are generally accepted as having led to establishment of the concept of neurosecretion. Bargmann and Scharrer

(1951, Scharrer (1952), and Scharrer and Scharrer (1963) provided the basic evidence to support the contention that neurohypophysial hormones are produced by or in the cell bodies of supraoptic and paraventricular nuclei of the hypothalamus and are transported by axoplasmic flow to the neural lobe where they are stored and from which they are released. The hypothalamus regulates secretion of the adenohypophysial hormones by producing specific hypophysiotropic hormones. Taubenhaus and Soskin (1941) suggested that the rat hypothalamus secretes an acetylcholine-like substance into the portal vessels to stimulate pituitary release. Later, Markee et al. (1948) and McDermott et al. (1951) proposed that epinephrine, acetycholine, and histamine might be involved in the release of gonadotropins, ACTH and other pituitary hormones. The direct influence of the hypothalamus in anterior pituitary function was first demonstrated by Harris and Jacobson (1952). They observed the return of estrous cycles in hypophysectomized rats when the anterior pituitary was removed and transplanted under the median eminence. However, when anterior pituitary was transplanted under the temporal lobe of the brain in hypophysectomized rats, normal cycles were not resumed. Harris (1949, 1955) proposed the "chemotransmitter hypothesis." He suggested that neurohormones released by the hypothalamus are transported via the hypophysial portal vessels to the anterior pituitary, where they either stimulate or inhibit hormone secretion. Subsequent demonstrations that extracts of hypothalamic tissue contained substances which act directly on the pituitary to alter hormone release supported his hypothesis.

D. Location of Hypothalamic Hypophysiotropic Hormones

By transplanting fragments of anterior pituitary tissue into different areas of hypothalamus, it was observed that pituitary grafts showed some histological maintenance and functional activity, particularly when they were placed in certain areas of the medial basal hypothalamus (Halasz et al., Knigge, 1962; Desclin and Flament-Durand, 1963; Flament-Durand, 1964). But better functional activity was found when the pituitary was transplanted under the median eminence (Halasz et al., 1965; Flament-Durand, 1965). Halasz and Szentagothai (1962) introduced the term "hypophysiotropic area" to refer to the need for connection with that portion of the hypothalamus for maintenance of normal anterior pituitary function and histology. This medial basal area included the arcuate nucleus and the medial parvicellular region of the retrochiasmatic area. Relatively little information is presently available concerning the intrahypothalamic pathways and intrinsic cytoachitectonics of the medial hypothalamus. Szentagothai and associates (1968, 1969) used Golgi-Cox preparation to present brief descriptions of the neuron histology and terminal arborization patterns in some of the major regions of the rat hypothalamus. In the ventromedial nucleus the pattern of terminal ramification of presynaptic afferent fiber suggested that both marked convergence and divergence of information must occur here. It is hypothesized that the hypophysiotropic hormones are synthesized or present in the cell bodies within the hypophysiotropic area and are normally released at the nerve ending in the median eminence (Halasz et al., 1962; Szentagothai and Halasz, 1964).

E. General Physiology of Hypothalamic Regulatory Hormones

The existence of at least nine hypothalamic regulators of the pituitary gland is now reasonably well established. For three pituitary hormones there is a dual system of hypothalamic control, one system being inhibitory and one being stimulatory. The need for hypothalamic inhibitors, as well as stimulators of growth hormone, prolactin, and melanocyte-stimulating hormone, can be explained by the absence of negative feedback from their target tissues. In the case of corticotropin, thyrotropin, luteinizing hormones, and follicle-stimulating hormone, hormones (corticosteroids, thyroxine, and sex steroids) from the target glands inhibit secretion of these anterior pituitary hormones by negative feedback action exert on the pituitary, hypothalamus, or both (Schally et al., 1973). Synthetic releasing hormones as well as hypothalamic extracts do not appear to be species specific in their actions on pituitary hormone secretion. However, a single hypothalamic hormone can influence the release of more than one pituitary hormone (McCann and Dhariwal, 1966; Schally et al., 1973; Convey et al., 1975). The isolation and synthesis of LH-RH was followed by many demonstrations that this agent stimulates the release of both LH and FSH (Schally, 1973; Convey et al., 1975; Yen et al., 1975). Separate hypothalamic hormone which selectively stimulate LH and FSH release have not been isolated. Because both natural LH-RH and the synthetic decapeptide corresponding to its structure possessed major LH-RH as well as FSH-RH activity in human beings and rats. Schally et al., (1973) suggested that one hypothalamic hormone, designated LH-RH/FSH-RH, could be responsible for stimulating release of both FSH and LH from the anterior pituitary gland. The occasional divergence of FSH and LH release in the human mentrual cycle, the estrous cycle

in other mammals probably is due in part to interaction with sex steroid or different biological half lifes of LH and FSH.

Corticotropin-releasing hormone (CRH) was the first hypothalamic hormone to be demonstrated by Saffran and co-workers (1955; and Guillemin et al., 1957), but its instability and the difficulty with its assays have delayed the isolation of adequate amounts for elucidation of its structure. Little or no progress has been made on its chemistry, although the existence of CRH, different from vasopressin, is well accepted (Anderson, 1966). It is not known whether the physiological CRH from the hypothalamus is related to the proposed neurohyphysial CRF, but since hypothalamic CRH is destroyed by some proteolytic enzymes (Schally et al., 1968), it is probably a polypeptide.

The existence of thyrotropin-releasing hormone (TRH) was demonstrated in 1961 by Schreider and co-workers. In 1966 Schally et al., reported that TRH isolated from porcine hypothalami contained three amino acids, histidine, proline, and glutamic acid, in equimolar ratio. The structure of ovine TRH which was also (pyro) glutamine-histidine-proamide has been reported by Burgus et al., in 1970. Synthetic TRH has the same activity as natural TRH (Bowers et al., 1970). Natural and synthetic TRH, in addition to causing the release of TSH has been shown to stimulate prolactin release in vitro (Hill-Samli and MacLeod, 1974; Dibbet et al., 1974; Smith and Convey, 1975) and in vivo (Bowers et al., Mueller et al., 1973; Takahara et al., 1974), in several species including man, and to stimulate release of growth hormone in vivo in rats (Takahara et al., 1974).

The chemical structure of growth hormone releasing factor (GRF) is unknown, and assays are far from satisfactory. The existence of a

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growth hormone release-inhibiting hormone (GIF or somatostatin) was suggested by Krulick et al. (1968). Somatostatin is a tetradecapetide (Brazeau et al, 1973; Brazeau et al., 1974). Somatostatin was reported to inhibit growth hormone release in vitro in rats (Takahara et al., 1974) in vivo in rats (Chen et al., 1974). Somatostatin was also reported to inhibit secretion of both insulin and glucagon (Fujimoto et al., 1974; Vate et al., 1975). It was conceivable that somatostatin could be used in the control of some types of diabetes in man.

The chemical structure of prolactin inhibiting factor (PIF) and prolactin releasing factor (PRF) remains unknown. From their behavior during gel filtration on sephadex and other characteristics (Meites and Nicoll, 1966), it can be surmised that both PIF and PRF have small molecular weights. PIF is probably a small polypeptide.

F. Mechanism of Action of Hypothalamic Regulatory Hormones

Two principal theories account for the mechanism of action of the hormone releasing factors (McCann, 1971). The first of these is the stimulus-secretion coupling hypothesis of Douglas and Poisner (1964). In this hypothesis the releasing factors would induce a change in the permeability of cell membrane of the pituitary gland which lead to depolarization of cell membrane and to uptake of calcium ions. The calcium ions would then activate the release process by some unknown mechanism. The other hypothesis for the mechanism of action postulates that the andenyl cyclase system is involved. According to this hypothesis, releasing factors would combine with specific receptors on the cell membrane, which would in turn cause an activation of adenyl cyclase leading to generation of cyclic AMP from ATP. The

cyclic AMP would in some manner bring about release of hormone.

III. Hypothalamic Neurotransmitters

A. General

The hypothalamus receives many neural afferent fibers from different levels of the central nervous system. Transmission of signals from one neuron to another, or from a neuron to a cell that is innervates, is mediated by a familiar process: the neurotransmitter, a specific substance stored within characteristic subcellular vesicles, is released and liberated near the receptor or synapse. The neurotransmitter then diffuses a short distance to reach a special zone (the postsynaptic membrane) on the receptor cell where it alters the permeability of specific ions. This causes a change in electrical potential within the postsynaptic cell. If the cell is an neuroendocrine transducer cell, hypothalamic regulatory hormones are released to control the secretion of the pituitary gland. The brains of mammals are able to synthesize at least three monoamines that appear to be neurotransmitters and to have a specific role in the control of anterior pituitary function. These substances are norepinephrine (NE), dopamine (DA), and serotonin (5-HT). The first two are catecholamines and have their origin in circulating tyrosine; 5-HT is an indolamine and is synthesized from circulating tryptophan. The brain probably synthesizes other neurotransmitters in addition to NE, DA, and 5-HT. Eninephrine, acetylcholine, gamma amino butyric acid (GABA), histamine, octopamine and tryptamine have also been found in the brain of several species. The evidence that NE, DA, and 5-HT function as central neurotransmitters and have an important role in control of anterior

pituitary function is best known and hence these compounds are described in detail below.

B. Catecholamines

1. Distribution of Catecholamines in the Hypothalamus

Vogt (1954) first described the gross distribution of norepinephrine in the central nervous system. Further study on regional analysis revealed that unusually high concentrations of norepinephrine, dopamine, and serotonin were present in the hypothalamus. The discovery and use of the histochemical technique by Hillarp and Falck (1962) provided a singular advancement for further anatomical localization of aminergic systems. The development of the "punch" technique by Brownstein et al. (1976), and sensitive enzymatic isotopic assays for catecholamine by Coyle and Henry (1973), have made possible the precise mapping of hypothalamic catecholamines.

a. Norepinephrine

Neurons of the paraventricular, supraoptic dorsomedial, and periventricular nuclei, and those of the retrochiasmatic area and the internal layer of the median eminence receive a strong supply of noradrenergic terminals from fiber systems that ascend in the medial forebrain bundle from nuclei of origin in the pons, medulla, and the locus ceruleus of the midbraid (Ungerstedt, 1971). Measurement of norepinephrine of hypothalamic nuclei (Palkovits et al., 1974) demonstrated that the norepinephrine content was highest in the retrochiasmatic area of the anterior hypothalamus and high in the periventricular nucleus, dorsomedial nucleus and in the median eminence.

b. Dopamine

Dopamine cell bodies originate in the substantia nigra in the midbrain (Ungerstedt, 1971), and the axons course through the lateral hypothalamus and terminate in the caudate nucleus and putamen of the corpus striatum. The medial hypothalamus receives relatively few dopaminergic afferents. However, a few dopamine cell bodies are located in the arcuate nucleus of the hypothalamus and give rise to short axons which innervate the external layer of the median eminence (Fux, 1963; Fux and Hökfelt, 1966, 1970). A very high concentration of hypothalamic dopamine was found in the median eminence and arcuate nucleus, and high concentration in the suprachiasmatic, paraventricular, and medial portion of the ventromedial and dorsal medial nuclei (Palkovits et al., 1974).

2. Metabolism of Catecholamines

The biosynthesis of brain catecholamine is initiated by the uptake of tyrosine from the circulation. The uptake of tyrosine into brain is mediated by an active transport process (Chirigos et al., 1960). The first biochemical transformation involves the meta-hydroxylation of tyrosine. This step is catalyzed by L-tyrosine hydroxylase. The hydroxylation of tyrosine results in the formation of a catechol amino acid, L-dihydroxylphenylalane (L-DOPA). L-DOPA is metabolized to dopamine (DA) by dopa decarboxylase almost immediately after it is formed. The conversion of DA to norepinephrine is catalyzed by dopamine-B-oxidase. Epinephrine is mainly present in the adrenal medulla and is synthesized from norepinephrine by phenylethanolamine-*N*-methyltransferase (PNMT). The rate-limiting step in catecholamine biosynthesis is the hydroxylation of tyrosine to DOPA by tyrosine hydroxylase (Nagatsu et al., 1964) and its activity is regulated by

feedback inhibition of its end products (DOPA, dopamine, and norepinephrine) (Levitt et al., 1965, 1967). The catecholamines within any neuron are concentrated within synaptic vesicles. Nerve stimulation causes the catecholamine molecules to be released into the synaptic cleft. Only a small fraction of these molecules presumably interact with "receptors" on the postsynaptic membrane.

The physiological actions of NE within the synaptic cleft are terminated largely by the process of reuptake into the presynaptic terminals. Within the synaptic cleft catecholamines can be inactivated by enzymatic mechanisms; they are O-methylated through the action of the enzyme catechol-O-methyl transferase (COMT). They can also be destroyed enzymatically through the action of monoamine oxidase (MAO). The end-products are their respective aldehydes.

C. Serotonin

1. Distribution of Serotonin in the Hypothalamus

From fluoresence histochemical studies, it is clear that most of the cell bodies of the central serotonin neurons are localized mainly in the raphe nuclei of the lower midbrain that send afferent fibers via the medial forebrain bundle to the hypothalamus (Dahlstrom and Fuxe, 1964; Fuxe, 1965). Serotonin is high in the suprachiasmatic and arcuate nuclei, and moderately high in the median eminence, preoptic area, premammillary nucleus and the posterior hypothalamic area (Brownstein et al., 1976).

2. Metabolism of Serotonin

The biosynthesis of brain serotonin is initiated by the uptake of tryptophan from the blood. The first biochemical transformation in serotonin biosynthesis involves the 5-hydroxylation of tryptophan by

hydroxylase to form 5-hydroxy-tryptophan (5-HTP). This amino acid is rapidly decarboxylated through the action of aromatic-L-amino acid decarboxylase to the biogenic amine, serotonin (Wurtman, 1970). Serotonin does not exert feedback control over its own synthesis similar to the end product inhibition of tyrosine hydroxylase ascribed to norepinephrine (Jequier et al., 1969). The rate of serotonin synthesis is thought to be regulated by tryptophan availability, since under normal physiological conditions tryptophan hydroxylase is not saturated with substrate. Thus, the amount of dietary tryptophan can alter the synthesis of brain serotonin (Moir and Eccleston, 1968; Wurtman and Fernstrom, 1971).

The main pathway for serotonin metabolism appears to involve oxidative deamination by monoamine oxidase to 5-hydroxyindole acetaldehyde, and can then be oxidized to 5-hydroxylindole acetic acid (5-HIAA) by aldehyde dehydrogenase, or reduced to 5-hydroxytrptophol by alcohol dehydrogenase (Wurtman, 1971). The physiological action of central serotonin appears to be terminated mainly by active reuptake of serotonin by the presynaptic neuron (Glowinski et al., 1965; Glowinski and Axelord, 1966; Wurtman, 1972; Wilson, 1974).

IV. Hypothalamic Regulation of Pituitary Gonadotropin and Prolactin Release

A. General

The earliest evidence for hypothalamic control over the functions of andenohypophysis came from classical technique of lesions and electric stimulations. As early as 1921, Bailey and Bremer reported atrophy of the testis following lesions restricted to the hypothalamus of the dog. A series of paper from Dey (1941, 1943) indicated that

lesions in the median eminence region disrupted gonadal function. It is now clear that median eminence lesions result in impairment in the secretion of all anterior pituitary hormones with the exception of prolactin, the secretion of which is enhanced (McCann and Porter, 1969). By placing a paper plate or other obstacle between the cut ends of the pituitary stalk, or by pituitary grafs to distant sites, pituitary function were permanently disrupted. But taking out the paper plate between pituitary grafts placed under the median eminence which were revascularized by portal vessels, pituitary functions were fully restored (Harris, 1950; Harris and Jacobsohn, 1952).

Central acting drugs have long been known to influence the secretion of the pituitary. Systemic administration of cholinergic drugs and adrenergic drugs were reported to induce ovulation in rats and rabbits, whereas blocking agents inhibited ovulation (Sawyer et al., 1974; Markee et al., 1948; Sawyer et al., 1949; Everett et al., 1949; Markee et al., 1952). Adrenergic blocking agents of the dibenamine type as well as the anticholinergic agent, atropine, were found to block ovulation induced by coital stimulation if administered within second after mating. Therefore Sawyer et al. (1949) postulated a cholinergic-adrenergic sequence for the control of the release of gonadotropins, and they suggested three possible pathways for this control: (1) a direct innervation of anterior pituitary by adrenergic nerve fibers; (2) release of an adrenergic neurotransmitter from nerve endings into the hypothalamo-hypophyseal portal circulation; (3) release of an adrenergic neurohumor from cells in the median eminence into the portal circulation in response to cholinergic nerve stimulation. However, none of these possibility was demonstrated to be valid. When sterotaxic techniques were perfected, and pure

synthetic epinephrine and norepinephrine became available, Sawyer (1952) administered the drug intraventricularly and found they induced ovulation. This ovulation could be prevented by prior administration adrenergic blocking drugs. Other drugs thought to act only on neurons, such as atropine and pentobarbital, also were effective in blocking norepinephrine-induced ovulation, suggesting that this catecholamine acted as a central stimulatant rather than as a mediator carried to the pituitary by the portal vascular system (Sawyer, 1964). This led to subsequent studies attempting to localize the site of the central action of biogenic amines and their relation with the secretion of pituitary hormones. Later many pituitary hormone releasing factors or inhibiting factors were extracted from the hypothalamus or synthesized. Most or all of them are small polypeptides (Schally et al., 1973; Reichlin et al., 1976). This further indicates that biogenic amines do not directly control the secretion of pituitary hormones.

B. Lutenizing Hormone (LH) and Follicle-Stimulation Hormone (FSH)

In normal physiological condition the secretion of LH and FSH is usually parallel. Only one gonadotropin releasing hormone (GnRH) has been isolated from the hypothalamus and shown to release both LH and FSH (Schally et al., 1973). The first direct evidence for the presence of GnRH (LRF) in the hypothalamus was reported by McCann et al. (1960) and was based on a bioassay method. The distribution of GnRH activity was subsequently carefully assessed by a number of workers. A major development arising from the preparation of specific antibodies to LRH has been a radioimmunoassay to measure LRH in specific locations, and to study distribution of this neurohormone. By microdissection, GnRH concentration was found highest in the median

eminence, with lesser amounts in the arcuate nucleus. Small amounts are detectable in the preoptic area (Palkovits et al., 1974). Barraclough (1967) proposed dual hypothalamic control of GnRH secretion mediated by two separate "GnRH producing areas." The tonic secretion of LH is stimulated by the ventromedial and arcuated nuclei, and regulation of the preovulatory LH surge by the preoptic suprachiasmatic nuclei. GnRH is believed to be liberated at nerve endings in the median eminence and upper pituitary stalk in relationship to the primary portal vessel capillaries. Some portion of releasing hormones reach the primary portal plexus by trans-median eminence transport, and he postulated that the releasing hormones are secreted into the ventricular system, taken up by the lumenal processes of the tanycytes of the median eminence, and then actively transported for release at the capillary end of the cell. It appears that release from nerve ending is probably the major route of hypophysiotropic hormone release; the tanycytes may also play a role in maintaining basal function (Reichlin et al., 1976).

The function of catecholamines in the control of gonadotropin secretion remains unclear and controversial, although the anatomical distributions of norepinephrine and dopamine in the hypothalamus have been well established. Many workers now agree that the norepinephrine is the main stimulant to "the transducer cells" controlling gonadotropin secretion (Meites et al., 1977). Fuxe and Hökfelt (1969) reported that tuberoinfundibular DA inhibited the release of LRH, and this view was confirmed by the experiments of Uemura and Kobayashi (1971). By contrast, Schneider and McCann (1969) reported that dopamine stimulated LH and FSH release from pituitary incubated with hypothalamus fragments. Dopamine injected in the third ventricle caused an increase

of GnRH both in the portal and periphereal blood as well as in serum LH and FSH (Kamberi, 1976; McCann and Moss, 1976). Sawyer (1952) found that intraventricular injection of norepinephrine stimulated ovulation in the rabbit. Recently Sawyer et al., (1974) reported that dopamine not only failed to stimulated LH release, but dopamine inhibited the stimulatory effect of norepinephrine.

Serotonin and melatonin are generally believed to inhibit gonadotropin release. Systemic injection of 5-HT, treatment with drugs that increase brain levels of 5-HT, or direct infusion of 5-HT into the third ventricle, resulted in inhibition of LH release (Meites et al., 1977). Serotonin and melatonin had no effect on pituitary gonadotropin release when infused into the portal vessel (Kamberi et al., 1970 a, 1971 a). This indicated that the inhibition may occur in the hypothalamus.

C. Prolactin

Under most conditions, the mammalian hypothalamus exerts a chronic inhibitory action on prolactin release. Removal of hypothalamic influence by placement of lesions in the median eminence or section of the pituitary stalk, results in enhanced release of prolactin (Meites et al., 1977). However, some experiments showed that the hypothalamus can stimulate prolactin secretion. Nicoll et al. (1970) observed both prolactin inhibition and prolactin stimulating activity in rat hypothalamic extract when incubated with rat pituitary. The chemical structure of prolactin inhibiting factor (PIF) and prolactin releasing factor (PRF) are unknown at present. The hypothalamic tripeptide, TRH, has been shown to stimulate release of prolactin as well as TSH in human (Bowers et al., 1971; Jacobs et al., 1971), in rats (Jacobs et al.,

1971; Mueller et al., 1973).

PIF activity in the hypothalamus can be altered by several means, including use of central acting drugs, hormones, and physiological stimuli. Reserpine (Ratner et al., 1965), haloperidol (Dickerman et al., 1972), sodium pentobarbital (Wuttke et al., 1971 a), estrogen (Ratner and Meites, 1964) and suckling stimulus (Ratner and Meites, 1964; Minaguchi and Meites, 1967) were found to decrease hypothalamic PIF activity and stimulate prolactin secretion. Monoamine oxidase inhibitors (pargyline, iproniazid), L-dopa (Lu and Meites, 1971), ergocornine (Wuttke et al., 1971 b) and prolactin itself (Chen et al., 1967; Clemens and Meites, 1968; Voogt and Meites, 1971) were demonstrated to increase hypothalamic PIF activity and inhibit prolactin secretion. This observation provided evidence that catecholamines are involved in the control of prolactin secretion. Drugs or hormones which reduced brain catecholamines depressed hypothalamic PIF activity and stimulated prolactin secretion (Meites, 1972).

In contrast to mammals, the avian hypothalamus appears to exert a stimulatory influence on prolactin secretion and apparently contains a prolactin releasing factor. Pituitary tissue removed from many mammalian species and cultured in vitro continued to release prolactin at a good rate, but pigeons failed to do so (Nicoll and Meites, 1962). Kragt and Meites (1965) demonstrated that an extract of pigeon hypothalamus stimulated prolactin release by pigeon pituitary in vitro. Hypothalamic extract from the chicken, quail, tricolored blackbird, duck and turkey (Meites, 1967; Nicoll, 1965; Gourdji, and Tizier-Vidal, 1966; Chen et al., 1968) also induced release of prolactin when incubated with pituitary from these species. Thus the predominant influence of avian hypothalamus on prolactin release is stimulatory.

However, the existence of a PRF in the mammalian hypothalamus still requires confirmation.

Dopamine, serotonin and norepinephrine are the three more important hypothalamic biogenic amines which participate in the control of prolactin. There is a considerable evidence that an increase in hypothalamic dopamine elevates PIF release and depresses prolactin secretion. Kamberi et al. (1971 b) reported that an injection of dopamine directly into the third ventricle increased PIF in the portal circulation and reduced serum prolactin concentration. A single injection of L-dopa, precusor of dopamine, also reduced serum prolactin (Lu and Meites, 1972). Apomorphine, a potent dopamine receptor stimulating drug (Anden et al., 1967) was reported to inhibit prolactin release in vitro (MacLeod and Leymeyer, 1974; Smalstig and Clemens, 1974) and in vivo (Mueller, et al., 1976). This effect was antagonized by a dopamine receptor blocking drug, pimozide (Janssen et al., 1968; Smalstig and Clemens, 1974; Smalstig et al., 1974). Some investigators have proposed that dopamine is a PIF. Shaar and Clemens (1974) reported that removal of catecholamines from rat hypothalamic by aluminum oxide absorption or treatment with monoamine oxidase resulted in complete loss of PIF activity in pituitary-hypothalamus co-incubation. This indicated that endogenous catecholamines accounted for all the PIF activity in crude hypothalamic extract. However, Meites et al. (1977) argued that these treatments also may have resulted in destruction of the putative polypeptide PIF, and of all the prolactin releasing substances. No increase in prolactin release was observed following the removal of catecholamines, as might have been expected if all inhibition had been eliminated. Although it has been shown that dopamine can directly inhibit pituitary prolactin release in vitro,

Meites et al. (1977) still believe that dopamine may act on the hypothalamus by inducing release of a polypeptide PIF into the portal vessels.

The role of norepinephrine in the control of prolactin is not clearly established as with dopamine, norepinephrine acted directly on the pituitary to inhibit prolactin release in vivo (Takahara et al., 1974; Schally et al., Blake, 1976) and in vitro (MacLeod, 1969; Shaar and Clemens, 1974) in rats. However, Kamberi et al. (1971 b) reported that norepinephrine injected into the third ventricle induced a small increase in serum prolactin. Clonidine, a norepinephrine agonist, was reported to increase prolactin release in estrogen-primed ovariectomized rats (Lawson and Gala, 1975). Administration of 1.4-dihydroxyphenylserine (DOPS), a precusor of norepinephrine, increased serum prolactin concentration (Donoso et al., 1971), whereas disulfiram inhibited dopamine-B-hydroxylase, and reduced brain norepinephrine, and decreased prolactin release (Meites and Clemens, 1972). These findings suggest that norepinephrine may stimulate prolactin release.

Kamberi et al. (1971 b) reported that injection of serotonin into the third ventricle of male rats stimulated prolactin release. Systemic injection of serotonin precusors, tryptophan or 5-hydroxytroptophan, also increased blood prolactin concentration in rats (Lu et al., 1973) and human (MacIncoe and Turkington, 1973). Blockade of 5-hydroxytryptophan synthesis with parachlorophenylalanine (PCPA) prevented suckling-induced prolactin release in the rats (Kordon et al., 1973). In addition PCPA, methysergide, a serotonin receptor blocker, also blocked suckling-induced prolactin release (Gallo et al., 1975) and stress-induced prolactin release (Marchlewska-Koj and Krulich, 1975).

Electric stimulation of the raphe nucleus markedly elevated serum prolactin concentration in rats and forebrain serotonin turnover, whereas placement of lesions in the raphe nucleus significantly reduced serum prolactin concentration and decreased forebrain serotonin turnover (Meites et al., 1977). Taken together, these observations suggest that serotonergic neurons stimulate prolactin release.

Acetylcholine, gama-amino-butyric acid, histamine and other putative neurotransmitters also may be involved in control of gonadotropin and prolactin secretion. But at present the data are unclear and highly controversial.

V. Estrous Cycle and Hormone Feedback

A. Female Reproductive Cycle

Lataste of Bordeux was one of the first to draw attention to the cyclic nature of the sexual process on a variety of rodents. Next came the work of Heape, who defined and named the various phases of the female cycle in mammals. Heape also drew attention to the fact that the ovulation in the rabbit was induced by coitus. As a result of Heape's work, Marshall was able to differentiate between the physiological activities of the Graafian follicle and the corpus luteum in their relation to accessory organs (see Cole and Cupps, 1969). Stockard and Papanicolaou (1917) reported their work on the estrous cycle of the guinea pig, and similar studies on the mouse were reported by Allen in 1922 and on the rat by Long and Evans in the same year. Moore and Price (1932) first proposed that the rhythmicity of estrous cycle is the consequence of a reciprocal interrelationship between the gonads and pituitary gland. In the same year, Hohlweg and Junkman

(1932) suggested the existence of a "sex centrum" within the central nervous system, because they found that anterior pituitary transplants into the anterior chamber of the eye failed to result in development of castration cells. In 1937 Harris demonstrated that electrical stimulation of the hypothalamic region of the brain caused the female rabbit to ovulate. His series of reports in late 1940's and early 1950's (1948, 1955, 1961) really established the neurovascular concept. This field soon became very active, and it is impossible to single out individuals whose contributions have led to the greatest developments. It is sufficient to say that the hypothalamus is now recognized as the center that controls the secretion of the anterior pituitary and regulates the estrous cycle in non-primate mammals and mentroual cycles in primates.

The estrous cycle in the female mammal is characterized by cycle changes in the morphology of the reproductive organs and in the behavior of the animal. In rats or in some domestic animals the estrous cycle is divided into various phases which are referred to as proestrus, estrus, metestrus and diestrus. Proestrus pertains to that phase of the cycle when secretory activity of corpus luteum is declining and the reproductive organs are coming under dominant influence of the intermediate-sized definitive follicles and gonadal hormones produced by them. Estrus is defined as that phase of the cycle when the female accepts coitus. Metestrus is the short transitional stage following ovulation in which the effects of the estrogen are declining and the secretion of progesterone is rising. Diestrus is that phase of cycle during which progesterone exerts a dominant influence on the accessory organs. Anestrus designates a period of variable length in which the reproductive organs are relatively quiescent. The patterns of estrous

cycles in female mammals are quite diverse, ranging from the constant estrus patterns exhibited by the rabbit to long cycles including a period of anestrus, exhibited by the bitch. This wide diversity reflects the difference in the inherent patterns of reproduction in different species, but the cycles, when compared on the basis of the physiological mechanisms involved, are quite similar.

Rats are the most commonly used laboratory mammals. A number of ecological or environmental factors can alter their estrous cycles and reproductive function. For example: the light-dark period, temperature, food, humidity, stress, pheromones...etc. Under constant laboratory conditions, they show 4 or 5 day estrous cycles. Since the estrous cycle and life-span of rats are short, they are often used as a model for studying female reproductive function, hormone regulation and aging process.

B. Control of Estrous Cycle

The estrous cycles of rats, if pregnancy or pseudopregnancy do not intervene, can be broken down into three stages: (1) follicular growth accompanied by estrogen secretion; (2) ovulation, which includes the final maturation of the egg and its release into the reproductive tract; and (3) corpus luteum development with estrogen and progesterone secretion.

The first stages of oogenesis and folliculogenesis are independent of hormones from the pituitary gland. Smith (1930) reported that 4 days after hypophysectomy of rats, no normal follicles of even medium size are present, but primordial follicles are undergoing development continually, and atresia invariably occurs not later than the stage of cavity formation. Eight days after hypophysectomy of immature rats,

follicles larger than 330u virtually disappear but that there is an increase in the number of smallest follicles (23-32u) (Paesi, 1949). Apparently the development of growing follicles beyond the four layered granulosa stage in the rat depends on pituitary hormone. The final stages of oogenesis and follicular growth need the stimulation of FSH and LH. In immature rats the injection of FSH increases the total number of growing follicles, but there is no increase in the percentage of vesicular follicles. Injection of LH does not change the total follicular count, but it does lead to a significant increase in the number of vesicular follicles (Lane and Greep, 1935). Soon after the growing follicle of the rat has become a bilaminar structure, the theca begins to appear as an aggregation of stromal cells surrounding the granulosa layer (Deane, 1952). The significant function of the theca is that it marks the point at which the follicles develop their own blood supply (Bassett, 1943), and it eventually becomes the principle source of estrogen or other steroids from the follicles (Dempsey and Bassett, 1943; McKay and Robinson, 1947; Deane, 1952). Granulosa and theca cells separated from human ovarian follicles have the capacity for formation of cholesterol and for all steroid intermediates through the estrogens. However, when incubated with labeled acetate, estradiol-17B only was isolated from the theca cells and recombined tissue types (Ryan et al., 1968). Porcine granulosa cells can transform pregnenolone into progesterone, but estrogens are not formed from C21 precusors (Bjersing, 1967). Estrogen has a direct effect on the growing follicles. The daily injection of 0.5ug of estradiol benzoate into hypophysectomized rats increase the number of small and medium size follicles (Croes-Buth et al., 1959), and in conjunction with exogenous FSH, estrogen produces larger vesicular follicles than with FSH alone (Paesi, 1952). The

transformation of a growing follicle to a vesicular follicle and to subsequent estrogen secretion by the theca evidently requires both FSH and LH. Daily injection of 25ug of FSH into hypophysectomized rats cause vesicular follicles to grow; however, they become atretic after several days of treatment. No estrogen is produced by these follicles. But when FSH and LH were given concurrently, vaginal cornification occurred and there was an increase in uterine weight (Greep et al., 1942).

The diestrus of the rat estrous cycle may be two or three days in length. During this time, follicular development occurs at all stages, from the primordial through the large antrum containing follicles. Thus at the time of an ovulatory surge of gonadotropins, large Graafian follicles are contained within the ovaries. It is these large follicles that ovulate during a particular cycle. Late on the last day of diestrus and during the morning of proestrus, a significant rise in estrogen secretion can be detected in blood. Although the stimulus for this rise in estrogen secretion appears to be LH, no major changes in LH levels are observed at this time. The rise in estrogen secretion continues to a peak on the afternoon of proestrus and then, as the plasma levels of LH, FSH, prolactin, and progesterone begin their abrupt and major surge of secretion, estrogen levels begin to decline, reaching basal values on the morning of estrus. Increase in estrogen secretion from diestrus 2 to proestrus is directly or indirectly responsible for the major manifestation and hormone events of the estrous cycle: (a) the nucleated and cornified vaginal smears on the day of proestrus and estrus, respectively; (b) uterine balloning on proestrus; (c) the surge of LH secretion occurring on the afternoon of proestrus induces ovulation and initiation of corpus luteum formation

several hours later on the morning of estrus; (d) the surge of FSH secretion shown during the proestrus-estrus interval, which may participate in the ovulatory process and initiate follicular growth during the next cycle; (e) the major rise in prolactin secretion occurring at proestrus which, in turn, stimulates morphological regress of the previous crop of corpra lutea; (g) the surge of LH stimulates a rapid rise in progesterone secretion from the follicles which, along with estrogens, initiates sexual receptivity and also causes relaxation of the cervical sphincter allowing the accumulated uterine fluids to escape.

C. Feedback Effects of Gonadal Steroids on Secretion of Gonadotropins

1. Negative Feedback

In the intact rats the secretion of LH and FSH by the anterior pituitary is held in check by the feedback action of gonadal steroids. The inhibitory effects that gonadal secretions exert on the secretion of gonadotropins are most easily shown by removing the gonadal steroids. When the ovaries or testes are removed in rats, both pituitary and blood levels of LH and FSH show marked increase. In every case investigated, blood levels of LH and FSH rise more slowly following gonadectomy in female than in the male (Gay and Midgley, 1969; Yamamoto et al., 1970; Blackwell and Amoss, 1971; Yapper et al., 1972; McCann, 1974). In the male the rise in blood LH occurs within the first 6 hours (McCann et al., 1973), and with a gradual additional increment continuing for month. However, in the female the rise is delayed and occurs only after several days. It takes about 5 to 6 weeks to arrive at maximal levels (Huang et al., 1976 b). Both Yamamoto et al. (1970) and Tapper et al. (1972) reported that blood levels are elevated within 24 hours

following ovariectomy at diestrus, whereas when the ovaries were removed at other days of cycles, the first detectable increase occurs much later.

The ovarian steroid most potent in inhibiting gonadotropin secretion by pituitary is estrogen (Schwartz and McCormack, 1972). The elevated blood levels of LH and FSH in long-term ovariectomized rats are lowered significantly within 24 hours after injection of a single dose of estradiol benzoate, ranging from 0.2 to 5.0 ug (Ajika et al., 1972). At 48 hours following a single injection of estrogen, FSH and LH levels were lowered even further. Injection of progesterone alone does not suppress the elevated LH and FSH in ovariectomized rats (Tapper et al., 1972). However, if low doses of estrogen are combined with progesterone, then small doses of progesterone can further suppress LH levels (McCann, 1962; McCann and Ramirez, 1964). High doses of progesterone injected on the day prior to proestrus in rats showing 4 days estrous cycles, can delay ovulation (Everett and Sawyer, 1949; Zeilmaker, 1966; Brown-Grant, 1967). Since progesterone by itself is practically inert in inhibiting gonadotropin secretion, the most important inhibitor is estrogen. The effect of progesterone is to synergize with estrogen to inhibit gonadotropin secretion.

The feedback of ovarian hormones has been examined in great depth, and reviews that include discussion of the anatomical sites of their action have been presented by Flerko (1974), Davidson (1969) and Halasz (1969). By implanting minute amounts of steroids into either the hypothalamus or anterior pituitary, Rose and Nelson (1957) first reported that a direct inhibitory effect of estrogen on the pituitary. Flerko and Bardos (1961) found that the negative feedback of daily injections of estrogen can be reduced or abolished by lesions

in an anterior region of the hypothalamus extending between the optic chiasm and the paraventricular nucleus. Moreover, implantation of ovarian tissue or estrogenic substance in the anterior hypothalamus results in ovarian atrophy. Lisk (1960, 1962), and Sawyer and co-workers (1961, 1964) reported effects from placement of estrogen and testosterone in the hypothalamus. They observed little effect from pituitary implantation and concluded that the principal site of negative feedback of gonadal steroids was on the hypothalamus. This conclusion has been considered in detail and rejected by Bogdanove (1963, 1964). He has presented strong evidence for a direct effect of estrogen on the anterior pituitary, and the inhibition was inversely related to the distance from the implant in the gland. The implants were capable of blocking the formation of castration cells in the pituitaries of castrated rats. Halasz and Gorski (1967) found that complete differentation of the medial basal hypothalamus did not prevent pituitary castration cell formation. Furthermore, Antunes-Rodrigues and McCann (1967) showed that castration still increased pituitary and plasma LH in rats with anterior hypothalamic lesions. Subsequent studies demonstrated that implants of estrogen in either the median eminence region or in the pituitary gland itself can inhibit LH secretion, as evidenced by decrease in blood and pituitary concentrations of LH in castrated rats (Ramirez et al., 1964), and can increase the secretion of prolactin (Ramirez and McCann, 1964). In summary, these studies are generally in favor of the medial basal hypothalamus as the main site for negative feedback inhibition of gonadotropin secretion by estrogen in females, while the pituitary may be a secondary site of estrogen sensitivity.

2. Positive Feedback

The stimulatory effects of gonadal steroids on gonadotropin secretion was first demonstrated by Hohlweg in 1934. He found that estrogen administered to prepuberal rats led to formation of corpora lutea. Later, Everett (1964) observed that estrogen or progesterone administered under certain special conditions could advance ovulation during normal estrous cycle in the adult rats. The stimulatory effect of estrogen in prepuberal rats could be demonstrated only in the female (Dorner and Doke, 1967; Wagner, 1968). The ability to respond in this fashion apparently develops in the absence of testosterone during the neonatal "critical period." Thus, estrogen or progesterone-induced luteinization did not occur in androgenized constant estrous rats (Huang and Meites, unpublished observation). The phenomenon of positive feedback presents some problems. How can one steroid both stimulate and inhibit gonadotropin release? Barraclough (1973) proposed that large doses may be inhibitory, whereas small doses may be stimulatory. The length of exposure to a steroid and the age of the animal also may influence the type of response obtained. However, estrogen can have stimulatory effects even when administered in large dose (Hohlweg, 1934), and inhibitory effects can be revealed with chronic administration of low doses (Byrnes and Meyer, 1951). It has also been suggested that time is the important factor-stimulatory effects tend to result from acute exposure to steroids, and negative feedback from chronic exposure (Flerko, 1966). The situation is more complex than this, however. Callantine et al., (1966 a) reported that chronic administration of small doses of estrogen can also stimulate LH secretion; certain combinations of time and dose may provide the determining factor. The ratio between blood estrogen and progesterone

concentrations in the present or immediate past may be the key to understanding why it is that a given ovarian steroid sometimes has stimulatory and sometimes inhibitory effects. There is evidence for estrogen-progesterone interactions in three of the stimulatory feedback situations: precocious stimulation of gonadotropic function in prepuberal rats, induction of ovulation in pregnancy, and advancement of ovulation in mature cycling rats by injection of estrogen.

The idea that progesterone might be the ovarian signal for the ovulating surge of LH, originally arose from the early observation of Everett. In 1948 he reported that progesterone injection on diestrus 3 of a 5 day cycle would cause ovulation to occur 24 hours earlier than it would have spontaneously. Later, it was shown that progesterone injections could advance LH release by a few hours in rats showing 4 day estrous cycles if administered on proestrus when the effects of estrogen are already apparent. However, when given a few hours earlier, or on diestrus 2, progesterone delays ovulation by 24 hours (Schwartz, 1969). Progesterone also has been shown to facilitate the induction of ovulation by estrogen in immature rats (Docke and Dorner, 1966). Progesterone also facilitates the superovulation effects of PMS or FSH in prepuberal rats (Meyer and McCormack, 1967). Success in inducing ovulation in pregnant rats depends on precise timing of the estrogen injection during the early days of pregnancy (Everett, 1947). The possibility exists that success in this experimental situation depends upon a precise level of progesterone at the time of estrogen action.

Since the circulating ovarian steroid levels in the intact rats show cyclic fluctuation patterns, to avoid the complexity of gonadal-pituitary hormone relation, ovariectomized rats were used as model for

studying the stimulatory effect of ovarian steroids on gonadotropin release. Estrogen administration to long-term ovariectomized rats results in surges of LH secretion (Caligaris et al., 1971 a; Kalra et al., 1972; Swerdloff et al., 1972; Jackson, 1972). Neil (1972) reported that rats had been ovariectomized 2 weeks previously, and then treated with a small dose of estrogen on day 1 and a large dose on day 2, exhibited a major surge of LH at 1900 hours on day 3. Caligaris et al (1971 a) observed that in long-term ovariectomized rats, following a regimen of large dose of estrogen, daily surges of LH occurred in the afternoon for 3 to 4 days. This confirms Everett's (1961) original hypothesis that a timing signal for LH surge occurs daily in the rat but can be expressed only when estrogen levels are elevated.

In long-term ovariectomized estrogen primed rats, a major surge of LH and FSH was induced a few hours later after an injection of progesterone (Caligaris et al., 1971 b). The release of LH induced by progesterone always occurred on the afternoon regardless of when progesterone was injected; i.e., progesterone injected at midnight between days 2 and 3 after the estrogen injection displayed a surge of LH secretion during the afternoon of day 3 but not before, and progesterone injection at noon on day 3 induced a surge of LH within a few hours at the same time as the rats injected at midnight. This study confirmed the earlier work of Everett and Sawyer (1949) using progesterone injections in 5 day cyclic rats to investigate the ovulation response. They concluded that LH release was related to the time of day and not to the time progesterone was injected. Caligaris et al. (1971 b) also found progesterone alone to be ineffective in stimulating long-term ovariectomized rats to release a surge of LH. A response to

progesterone was observed only when it was preceded by injection of estrogen. Later, many other investigators also reported the surge of LH in response to progesterone injection. Jackson (1972) observed that injection of progesterone at 1100 hours on day 3 following an injection of estrogen on day 1, caused an increase of LH 5 hours later. Kalra et al. (1972) reported that progesterone stimulated a major surge of LH and FSH which peaked at 6-7 hours following progesterone treatment in estrogen-primed rats.

Various types of approaches have been used to investigate the sites of ovarian steroid positive feedback in the central nervous system. These include electrical and electrochemical stimulation, electrolytic lesions, implantation of ovarian tissue or ovarian steroid pellets, microinjection of hormones and autoradiography. Electrical activity in the brain after injection of H^3 ovarian steroids have successfully demonstrated the sites of ovarian steroid stimulation. Stimulation anywhere within a broad band of tissue extending from the preoptic region rostrally to the arcuate-median eminence region caudally is capable of evoking ovulation accompanied by a marked rise of blood LH in the proestrous rats (Cramer and Barraclough, 1972; Kalra et al., 1971). Stimulations outside of this medial basal region were ineffective. Blood FSH levels were raised by stimulation, which were located slightly more caudally in the region extending from the anterior hypothalamic area to the arcuate-median eminence region (Kalra et al., 1971).

Electrolytic lesions in the anterior hypothalamus, interrupting neural connections between preoptic and hypophysiotropic areas, produced constant vaginal cornification. The animals appear poised on the brink of ovulation, but never go beyond this point. The ovaries are filled

with large follicles and no corpus luteum. Although the cyclic discharge of gonadotrophins required for ovulation is absent in such animals, the negative feedback actions of gonadal steroids are still operative. Isolation of the hypothalamus from the rest of the brain by using the Halasz knife in rats, causes blockage of ovulation and constant vaginal cornification (Ramaley and Gorski, 1967). A front cut, in which the connections coming from the preoptic region are severed, again produces constant vaginal cornification. However, if one makes a cut around the medial basal hypothalamus to sever its connections with structures lying lateral, caudal, and dorsal to it, then gonadotropin secretion and estrous cycle are not interfered with (Halasz and Gorski, 1967; Halasz, 1969). These studies indicate that the preoptic region is involved in the ovulatory surge of gonadotropin.

Lisk (1965) reported that the implantation of estrogen in the suprachiasmatic region could induce behavioral estrus in rats. Smith and Davidson (1968) found that implants of estradiol benzoate in the anterior hypothalamic and preoptic region resulted in precocious vaginal opening, whereas implants in other areas of the brain, including the median eminence, were ineffective. Kalra et al. (1975) recently have reported that an evelation of LH in estrogen primed rats can be induced by implantation of estrogen into the preoptic area. Some other workers have found a rise in blood LH after median eminence implantation of estrogen (Kannwischer et al., 1967; Motta et al., 1968), and advancement of ovulation occurred after implantation of steroid into the pituitary gland (Davidson et al., 1970). Most people favor the idea that the stimulatory feedback effect of estrogen in female rats is exerted specifically on the anterior hypothalamic-medial preoptic region.

Using the techniques of autoradiography and uptake of labeled

estrogen in hypothalamus and pituitary, estrogen was found to be selectively concentrated in the anterior pituitary and in several regions of the hypothalamus (Kato and Vellee, 1967; Stumpf, 1968). In the hypothalamus the steroid is concentrated particularly in the arcuate nucleus, but also in the lateral portion of the ventromedial nucleus. In the preoptic region there is considerable concentration of steroids in the medial preoptic and suprachiasmatic nuclei and in the nucleus interstitialis striae terminalis. Besides the hypothalamus, probably the limbic system (amygdala, hoppocampus, midbrain reticular formation) also influences the estrous cycle mechanism, but it is still too early for a definitive conclusion.

VI. Female Reproductive Function in Aging Rats

The regulation of gonadotropin secretion and estrous cycle is controlled by the neuroendocrine system. The causes and origins of fundamental changes and decline of reproductive function associated with advanced age occur not only in particular reproductive organs but in the whole reproductive system.

A. Age Changes in Central Nervous System and Hypothalamus

It is almost universally accepted that when neurons are lost in mammals, they are not replaced by division of the remaining cells. The loss of nerve cells is one of the most consistent changes in the brain of aging mammals. Ellis (1920) found a consistent decrease with age in the number of purkinje cells per unit area of the cerebellum of adult humans. The cell density in the cerebral cortex of humans ranging in age from newborn to 95 years was studied by Brody (1955), who

also reported a reduction in cell number with age in all cortical layers, with the greatest decrease occurring in the superior temporal gyrus, precentral gyrus and the area striata. The postcentral gyrus shows the least change in cell number. Similar decreases have been described in rodents. In the guinea pig, the decrease in Purkinje cells is as high as 40 per cent in senility. In rat, the number of Purkinje cells in the 1000 day old animal is about 20 per cent less than in the 200-day-old rat (Sulkin, 1961). The degeneration of the nerve cell body is reflected not only in cellular loss with age but in changes in cell size. The cells of ganglia, both autonomic and sensory, undergo a decrease in size with age. Increase in cell size may also occur with age in cells where accumulation of relative inert material such as lipofuscin pigment and fat accompanies the degenerative process.

In contrast to nerve cells, glial cells increase in number with age by a process of amitotic division (Brizze et al., 1969). The increased glial density in the aged cortices is greatest in the internal granular and outer half of the internal pyramidal layer. The increase in glial cells with age is postulated to represent a compensatory process of the brain to overcome morphologic and functional neuron loss or neuronal changes occurring with age.

The nucleus of nerve cell is smaller in the older than in the younger animal. The alteration in the shape of nucleus in the senile nerve cell includes elongation of the nucleus, irregularity of nuclear membrane and lobulation. The nucleolus in the nucleus of the nerve cell is generally paler in the older animal. Also a decrease in the amount of Nissl substance is seen in the older animal (Sulkin, 1961).

Systemic studies of the changes occurring in brain deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from infancy to advanced old age

are lacking. However, DNA content of the human brain from approximately 21 years to 90 years of age has been reported. From the third decade, when DNA content is low, it increases steadily and in the 90 to 93-year old group DNA is higher than at any other time in the life-span. In the mouse brain, DNA content changes only slightly with increasing age. This increase in DNA is accompanied by a decrease in protein and lipids, and may partially reflect a proliferation of glial cells. The increase of DNA in old age may be due to two factors: an increase in pyknosis of the neurons and an increase in the number of glial cells. RNA content in the human brain increases up to the age of 40, when it reaches a certain average level that remains for the next two decades and then falls rapidly. This life-cycle change is superimposed upon the short-lasting, reversible fluctuation in RNA contents that result from increased functional demands at various age periods. In view of the significance that has been attributed to RNA in learning and memory, changes RNA per cell may account for the characteristic changes that take place in memory with senescence. Age-associated differences also have been observed in total RNA content and synthesis in the rat brain. The total amount of RNA increases sharply between the first and second week and the total RNA content per cell increases from birth to adulthood. In old rats, the RNA content per cell decreases significantly (Timiras, 1972).

Recently several investigators have reported that age-associated alterations in the activities of biogenic amines and other neurotransmitters of central nervous system may underlie the changes of neuroendocrine function. Clemens et al., in 1969 suggested that the reduction of reproductive function in old rats may contribute to the depression of hypothalamic catecholamine activity. Subsequently, Finch

(1973) found that dopamine and norepinephrine concentrations in the hypothalamus of old mice were not different from those in young mature male mice, but turnover of these amines was decreased in the old mice. Riegle and Miller (unpublished) made preliminary observations that hypothalamic dopamine and norepinephrine concentrations are lower in old as compared to young male rats. Recently, we (Simpkins et al., 1976) have measured the concentrations and turnover of dopamine, norepinephrine, and serotonin in several brain regions of old and young male rats. We found that dopamine and norepinephrine concentrations in the medial basal hypothalamus and the remaining hypothalamus were significantly lower in old than in young rats. The turnover of dopamine in the medial basal hypothalamus was significantly greater in young than in old rats, but no differences in the remaining hypothalamus or in olfactory tubercles were found. However, the norepinephrine turnover rate of the remaining hypothalamus in young rats was about two-fold greater than in old rats, but no difference in the medial basal hypothalamus was seen. Hypothalamic and brain concentration of serotonin did not differ between old and young rats, but turnover of serotonin in the hypothalamus but not in the rest of the brain was greater in old than in young rats.

Other putative neurotransmitters may be also affected by aging. Himwich (1973) reported that there was a marked decrease in glutamic acid, and glutamine in the brain of old rats and a fall in gamma amino butyric acid (GABA) and aspartic acid contents in old hamsters. Timiras (1972) found that the activity of cholinergic neuron was reduced in the aged rat. In contrast to this decrease in neurotransmitters in old rats, some investigators have found no significant changes in the contents of acetylcholine (Hollander and Barrows, 1968; McGeer

et al., 1971), GABA, glycine, and glutamic acid (Fonda, Acree, and Auerbach, 1973) in aging rodents. At present there are not enough data to make a clear-cut conclusion in this field.

Changes in the morphology and neurochemistry of central nervous system with age are associated with alteration of CNS function in old animals. In electrophysiologic terms, the CNS can be viewed as a series of circuts transmitting and receiving signals from sense organs to the brain and from one part of the brain to another, as well as from the brain to the periphery. In this sense, it is evident that the efficacy of signals may be disturbed not only by irregularity in the action of the cells carrying the signals, but also by the amount of random ground activity "neural noise," which is characteristic of this tissue; i.e., the signal-to-noise ratio may be impaired either by a reduction in signal strength or by an increase in noise level. Of the several electrophysiologic characteristics of the aged CNS, the most likely to affect signal strength and noise level would seem to be the reduction in number of functional cells, the increase in random activity, and the longer after effects of neural activity (Timiras, 1972).

The hypothalamus is the most important vital CNS structure participating in the control of the autonomic nervous system (ANS). The hypothalamus play an enormous role in the regulation of homeostasis, emotion, body temperature, endocrine secretion, metabolism, eating and sexual behavior. Since the functions of the hypothalamus are extremely broad, diverse, and complex, the Russian gerontologists strongly believe that the changes in the structures and functions of the hypothalamus with age cause the disturbance of homeostatic control system and develop imbalances of the internal environment of the body,

and eventually lead to the age-related pathology and death. As briefly mentioned in a previous section, Dilman (1976) claimed that theoretically, it is possible to distinguish three main types of disturbances that may lead to hormonal homeostatic failures: type A- elevation of hypothalamic threshold to homeostatic suppression (the central type of homeostatic failure); type B- decreased hormone action at the hypothalamus level (peripheral type of homeostatic failure); type C- impaired hormonal secretion or qualitative shifts in the spectrum of secreted hormones (dysfunctional type of homeostatic failure). A higher organism exercised a great many functions and thus possesses many homeostatic control systems. But in the final analysis the main parameters of organismal function are determined by the three basic homeostatic control systems: energetic, adaptional and reproductive. These three biological phenomena are indispensable to the survival of an animal and its species.

According to the experimental data of the several investigators, the central type of homeostatic failure is the key factor causing imbalance of the internal environment inherent to normal aging. The inhibition of growth hormone secretion (GH) after glucose intake is less in middle-aged man than in young adult. A standard glucose load fails to reduce the level of GH and free fatty acids. This indicated that in middle-aged people the hypothalamic threshold to energy homeostatic suppress is elevated (Dilman, 1976). A raised threshold of the hypothalamic eating center to the inhibitory effect of glucose may cause an increased appetite in many persons with aging. The elevated blood-GH level with age lowers the glucose utilization in muscle tissue and induces compensatory hyperinsulinemia. As a result, excessive glucose is mainly metabolized to fat. Thus leads to an

increase of body weight and probably obesity. The disturbance in the metabolism of fat and carbohydrate, and in energy homeostasis causes the elevated blood glucose and accumulation of cholesterol. Thus results in the development of age-specific pathology; i.e., prediabetes, adult onset diabetes and atherosclerosis.

The age-associated changes in the adaptation control system were demonstrated in a series of experiments by Riegle and Hess (1970, 1972, 1973). They found that the most important age-related alteration in adrenocortical function is the decreased sensitivity of the hypothalamic pituitary adrenocorticotropin control mechanism in the aged rat. Since the threshold of the hypothalamus to the feedback suppression of corticoids rises with age, the homeostatic regulation is disturbed and may lead to imbalance in the diurnal rhythm of glucorticoid secretion.

Another important consequence of elevation of hypothalamic elevation to suppression in the adaptation system is, presumably, the damaging effect of nonclassical phenolsteroid excess, produced by the adrenal glands. This substances are likely to suppress cellular immunity and thus lower resistence to infection (Dilman, 1976).

In reproductive homeostasis, Dilman (1976) believes that menopause in woman and the cessation of estrous cycle in old rat may be largely due to the elevated hypothalamic threshold to the inhibitory effect of estrogen. In contrast Aschheim (1976) claims that the hypothalamus of old rat shows hypersensitivity estrogen feedback suppression, and thus causes deviations of the estrous cycle. However, from the work I have done, both of them appear to be only partially correct, since they did not consider that the mentrual cycle or the estrous cycle are regulated by both a negative and positive feedback by ovarian

steroids. The details of this work will be presented later.

Frolkis et al. (1972, 1976) found that age changes in the hypothalamic structures and functions are not uniform, and show considerable irregularity. For example, the electric excitability of the anterior and posterior areas of the hypothalamus increases, whereas the electric excitability of the lateral area decreases. Along with this, the sensitivity of all these structures to catecholamines and acetylcholine increased with age. They proposed that these irregular changes in various structures and functions of the hypothalamus may cause the heterochronic age changes in the organs or systems of organism. Thus, the functional disregulation of the hypothalamus and hypothalamic misinformation of the organism causes the collapse of homeostasis and a decrease in adaptive capacities, finally leading to age-related disease and death.

B. Age Changes in Pituitary Gland

Cooper (1925) studied the human pituitary from foetal life to old age. She described the vascularity of the anterior pituitary to be at its maximum at puberty. Little change was seen until the age of 40 or 50, after which a decrease occurred. In the case of the posterior pituitary, which does not have a rich blood supply, there was no change with increasing age.

The individual cells of the anterior pituitary in very young subjects seemed well-spaced, while later they became more densely packed. The basophils were present in greatest numbers in older persons. Cooper also found an increase in amount of connective tissue in old age. In the posterior pituitary she noted an increase in golden pigment with age which may have been ceroid pigment. Another paper of

significance on the human pituitary is that by Parson (1935) which is based on a study of 107 human male and female glands ranging from childhood to 78 years. He found little change in size and weight between 20 and 80 years. There was, however, a slight decrease in weight of the pituitary in females over 60 years age. Parson also described an invasion of posterior pituitary by basophil cells which seemed to come from the anterior pituitary or the pars intermedia. Tessauro (1952) referred to the invasion by basophils, stating that it began at about 10 years of age and reached a peak at about 40 years.

A number of studies have been made on aging pituitaries of rats. Wolfe et al. (1938) found that in old rats there was a tendency for atypical areas of hypertrophic cells to occur and that these cells were similar in many ways to those found in adenomata of the pituitary. These groups of atypical cells varied from small areas which contained only a few cells to much larger areas which measured to 1 mm across, and in two of the animals they examined these atypical areas occupied practically one lateral half of the gland. They differed from the cells normally found in the pituitary in that they were considerably hypertrophied, and although most of them appeared similar in staining reaction to chromophobes, some were found large acidophils. In these hypertrophied chromophobes the nucleus appeared to swollen but there appeared to be very little chromatin present, the nucleoli were also very large and there was a negative image of the Golgi apparatus which is normally present in pituitary cells and which was greatly increased in size in these enlarged cells. A number of cells contained clear vacuoles which were quite small in size and when enlarged acidophils were present, there was a reduced number of granules present. When adenomata occurred, they appeared to be made up largely or in some

cases exclusively of chromophobe-like cells. Apart from these changes, they also demonstrated that there was very little influence of age on the relative proportions of cell type in the anterior pituitary of the rat, but they found an increase in amount of colloid in the pituitary. In virgin female old rats, Wolfe (1943) found a decrease in numbers of acidophils and an increase in chromophobes. But these changes were most pronounced at a relatively early part of the life-span. In animals over 17 months of age nearly a third of the anterior pituitaries showed adenomata. He also found very little alteration in the granulation of the basophils with age and thought that the appearance of these cells in old animals suggested a decrease of functional activity. It is of interest also that he found a continuous decrease in mitosis of pituitary cells, so that by 6 months it was difficult to find even one.

The presence of adenomata in old rats also has been referred to by Saxton (1941). He indicated, as have other workers, that the adenoma-like lesions in albino rats were composed of chromophobe cells. He emphasized that a number of them frequently showed multicentric origin of nodules and that the chromophobe cells of which they were composed contained abundant lipoidal vacuoles.

The reticular tissue of the anterior pituitary of the rat, according to Lansing and Wolfe (1942), showed an increase in number and thickness of fibers but with no apparent transformation of these fibers into collagenous ones.

In the mouse pituitary, Blumenthal (1955) found only a small increase of connective tissue in old age but a decrease in size of the gland cells. He also found a decrease in mitosis with age, and after 21 months no mitosis could be found at all in the male mouse

pituitary. The number of mitosis were demonstrated to decrease progressively in female mice, but it took a little longer for them to disappear completely. They were almost absent by 22-26 months, but curiously enough, there was a slight increase of mitosis again, between 27-31 months.

Weiss and Lansing (1953) made an electron microscopic study of the aging pituitary gland. They used Swiss albino mice and found that in the pituitary of normal young animals the chromophobes, acidophils and basophils contained granules, an endoplasmic reticular system and mitochondria in varying amounts, and that the nuclear membrane appeared to be double. With the onset of aging, however, the nuclear membrane was found to become irregular in outline, and the cortex of the nucleus became dense and the mitochondria enlarged and vacuolated, and endoplasmic reticular system became fragmented.

In the golden hamster, Spagnoli and Charipper (1955), found an increase in numbers of basophils in older females and found some decrease in numbers of acidophils in older males. Some increase in density was found, with loss of regularity of the reticular network. Degranulation of many cells seemed to have occurred, vacuolation was more common in the basophils, and some nuclei were pyknotic.

The assessment of pituitary function with aging requires knowledge of the factors regulating pituitary hormone secretion and techniques for measurement of pituitary and target gland hormones in response to provocative stimuli. Some synthetic hypothalamic releasing and inhibiting factors are available now. Along with this, the application sensitive and precise radioimmunoassay techniques for measurement of changes in pituitary and target gland hormones in peripheral serum in response to pharmacological and physiological stimuli, makes the study

of pituitary function in old age more accurate and meaningful than earlier studies.

The release of pituitary hormones is dependent upon a dynamic balance between many agents, including the circulating levels of pituitary and targed gland hormoned, the hypothalamic releasing hormone, the biogenic amines in the hypothalamus, and the cerebral input to the hypothalamus. Ideally, assessment of pituitary function in old age requires the measurement of all these factors before making a valid conclusion. However, because of the limitation of methodology in the past, the data and conclusions are only of limited value.

In the rat, evidence on pituitary FSH and LH level are not very convincing. Lausen et al. (1939) studied pituitaries of rats of different ages and measured gonadotropic activity by bioassay. Throughout sexual life the activity remained at the puberal level; however in three females, 2.5 year of age a remarkable increase in total gonadotropic content was found. Similar assays were carried out by Solomon and Shock (1950), Duncan et al. (1952), Blumental (1955), and Ceresa and Lacroix (1951). All pointed out that there were no difference between the gonadotropin content of the pituitary glands of young mature and old animals. On the contrary, by ovarian bioassays (method of Steelman and Pohley for FSH, ascorbic acid depletion method of Parlow for LH) and pigeon-crop sac assay of Lyons for prolactin, Clemens and Meites (1971) found that pituitary FSH and prolactin concentrations were significantly higher in the old constant estrous rats than in the young rats, but pituitary LH concentration was significantly lower in the old than in the young rats. However, by the same methods, Aschheim (1968) found that in the same neuroendocrinological situation, adult and senile female rats showed about the same pituitary

content of LH and the pituitary of old pseudopregnant rat had 2 to 3 times more FSH than that of younger rats. Labsetwar (1969) reported an increased LH content in the pituitary of irregularly cycling rats aged 9 months.

To assess the functional changes in pituitary function in old men by giving hypothalamic releasing hormones, Lazarus and Eastman (1976) found that elderly men show similar gonadotropin responses as younger men following administration of synthetic luteinizing hormone releasing hormone (LHRH). In female rats, Watkins et al. (1975) reported that old constant estrous and pseudopregnant rats had smaller increases in serum LH after LHRH injection than young cycling female rats. However, some of my data do not agree with these results, and will be presented elsewhere in this thesis. In male rats, we found that the pituitary of old rats showed less response to both single and multiple injections of LHRH than in young rats (Bruni et al., 1976).

C. Age Changes in Ovaries

The first prerequisite for the maintenance of reproductive ability is the continued presence of oocytes. It is clear that in all species which have been studied, the number of oocytes in the ovary declines with increasing age. The relationship between the exhaustion of oocytes and the termination of reproductive capacity varies in different species and even in different strains of the same species.

The most comprehensive quantitative study of this problem was conducted by Jones and Krohn (1961 a) on several strains of inbred and hybrid mice. Their investigation showed that there is considerable interstrain difference in the number of oocytes in the ovaries at birth, the stage of development that these oocytes have reached at

birth, and the rate of loss of these cells after birth. No relationship was found between the total number of oocytes present at birth, or their stage of development at this time and the age at which the ovary became depleted of germ cells. There was, however, a correlation between the rate of depletion after birth and the age at which the oocytes were exhausted. Another important conclusion which Jones and Krohn (1961 a) drew from their data is that there is not a constant relationship between the number of oocytes remaining in the ovaries and the average age at which the last litter was born. In this respect animals of the CBA strain appeared to be far more efficient than any of the other strains included in the study, since the number of oocytes had declined to about 100 when last litter was born but in other three strains over 1000 oocytes were found in each pair of ovaries when reproduction ceased.

The only other rodent species in which a detailed study of the decline in oocytes associated with aging has been carried out is the rat. A detailed investigation was conducted by Mandl and Shelton (1959) which showed that, as in the mouse, there is a regular decline in the number of oocytes in the ovaries throughout life although there is still a considerable number present at death. It is therefore obvious that the final termination of reproductive capacity in this species is not due to exhaustion of oocytes.

Exhaustion of the oocytes from the ovary and cessation of menstruation have traditionally been closely associated in women; however, numerous studies of postmenopausal ovaries clearly demonstrated that a few oocytes are commonly still present in follicles of varying size, although they usually are degenerate. In addition to manopause, there are other events that accompany the gradual downhill slope of

ovarian involution. An early symptom is the occurence of anovulatory cycles. Follicular cysts are frequently encountered in the ovaries of women during the few years just prior to menopause and active corpora lutea are very rare at this time. The breakdown of the formation of the functional corpora lutea begins to take place in the premenopausal years and partially accounts for the lower incidence of pregnancy and increasing frequency of miscarriage in menopausal women (Talbert, 1968).

It also has been found that ova recovered from the uterus of menopausal women and old animals appear grossly abnormal. Studies on the transplantation of ova from old female animal to the uterus of the young have indicated that the ability of these ova to develop normally is significantly impaired; however, the extent of such a functional loss varies with the species studied (Talbert, 1968).

It is apparent from estimates of the number of oocytes in the ovaries of the rat, mouse, dog, and human at puberty, that a relatively small percentage of these cells is lost by ovulation. This has been demonstrated experimentally in the mouse by Jones and Krohn (1961 a) and in the rat by Shelton (1959), who observed that the rate of decline of oocytes in breeding and virgin animals of these species is not noticeably different. A large percentage of ova is lost by atresia. Ingram (1953), working with rats, and Jones and Krohn (1961 b) with mice, found that hypophysectomy reduced the rate of decline of oocytes in the ovaries of these two species, and pointed out that the preservation of oocytes was due to a reduction in the rate of atresia.

Estrogen levels in women are relatively constant between the ages of 20 and 40 years, decline significantly in the following two decades, and stabilize at low levels thereafter. The latter has been shown to

be closely associated with the cessation of menstruation (Paulsen et al., 1958). The decline in estrogen levels has been related to the loss of follicles characteristic of the aging ovary, inasmuch as these follicles represent the major site of estrogen production. This interpretation is supported by the finding that the rate of catabolism and excretion of estrogens does not change appreciably with age, indicating that the titers of estrogens in the urine is a reasonable reflection of steroid secretion (Pincus, 1956). All three classical estrogens (estrone, estradiol and estriol) are excreted, but the most potent estrogen, estradiol, is excreted in the least amount (McBride, 1957; Procope, 1969). The pathway of steroid biosynthesis appears to change at time of the menopause, leading to increased production of adrogens (Mattingly and Huang, 1969) and nonclassical phenolsteroids (Dilman, 1976).

The urinary excretion of pregnanediol, a product of progesterone breakdown, is decreased in women approaching the menopause (Adamopoulos et al., 1971). This indicates that the secretion of progesterone also declines. The reason for the decline in production of progesterone seems to be the decline in number of functional corpora, which in turn, is due to the decrease in number of follicles and increased incidence of anovulation.

The influence of age on ovarian steroid secretion in laboratory rodents has not been systemically studied. From the changes of vaginal smears and histology of the reproductive tract, Aschheim (1976) inferred that the blood estrogen levels of old constant estrous rats may be not different from young cyclic rats. However, old pseudopregnant rats seems to have higher progesterone and lower estrogen levels than young cyclic rats.

There is general agreement that the loss of responsiveness of the ovary to gonadotropic hormone stimulation is a change of paramount importance in the cessation of reproductive capacity in women. This is clearly shown by the abrupt fall in estrogen secretion and the virtual disappearance of normal growing follicles which occur in the presence of increasing levels of plasma gonadotropins at the menopause.

Histological examination of the postmenopausal human ovary often gives the impression that it is a "lifeless organ" with little evidence that it is still capable of responding to gonadotropic stimulation. Direct evidence of its insensitivity has been shown by injection of pregnant mare serum gonadotropin (PMS) into postmenopausal women with little or no effect on the ovary. However, most of the evidence indicates that the ovaries of old rodent species are not as responsive to gonadotropic stimulation as the ovaries of young adults, but this change is neither as sudden nor as great as that which takes place in women. Twelve to 14 month old hamsters and mice both showed a reduced response to PMS when compared with young adults of the same species. Since this is the age that reproductive capacity is rapidly diminishing in these species, this change may be a factor in the reduction in litter size. Rats ovaries have also been shown to decline in sensitivity to gonadotropins with age. This was demonstrated by the failure of 18 month old hypophysectomized rats to show an estrous smear following treatment with chorionic gonadotropin, although treated young adults all responded (Talbert, 1968). However, we found that the ovary of the aged rat still retains the capacity to bind gonadotrophin in a manner qualitatively similar to that of the ovary from young cyclic rats. Only cystic follicles showed limited gonadotropin-binding activity (Steger et al., 1976 a). These findings are in accord with

the observations that the ovary of aged rat can ovulate and maintain regular estrous cycles with the proper hormonal stimulus (Aschheim, 1965; Clemens and Meites, 1969; Peng and Huang, 1972). Old constant estrous rats with cystic follicles do not readily ovulate (Huang and Meites, 1975).

D. Age Changes in the Reproductive Tract

After the menopause the human uterus normally undergoes a considerable degree of atrophy. Woessner (1963) found that the wet weight of the uterus reaches its maximum in women at about 30 years of age and then remain relatively stable until menopause. Beyond the age of 50 a 53% reduction in wet weight takes place. This decrease is accompanied by a 61% decline in collagen and a 44% decline in elastin. Also, collagen in the older uterus show evidence of increased cross-linkages. The uterine myometrium of very old women contains excessive fibrillar networks with complete disintegration of the normal reticular structure. The elastin fibers become fragmented and form coarse clumps. The changes occurring with age in the endometrium, depending on the age and degree of estrogenic activity in the plasma, may range from severe atrophy to hypertrophy. It seems certain that the general atrophy of the uterus after menopause is the result of a pronounced decrease in the amount of estrogen acting on the uterus (Timiras, 1972).

In contrast to the results in the human, there is considerable evidence that fibrous connective tissue does increase with age in the uterus of the rat, guinea pig and the mouse. This increase involves both the endometrium and the myometrium, and was particularly noticeable in the circular muscle layer (Talbert, 1968). In 1975 we reported

that there was no uterine atrophy in old constant estrous and pseudo-pregnant rats, but very old anestrous rats showed an infantile-like uterus and ovaries (Huang and Meites, 1975). There is no direct evidence that the changes in the quality and quantity of fibrous tissue in the uterus of aging rodents has any influence on the ability to maintain embryos or fetuses. Nevertheless, the suggestion of Sobel and Marmorston (1956) that an increase in fibrous tissue might impair the transfer of nutrients and the removal of metabolic products, is worthy of consideration.

Using scanning electron microscopy, it was found that the post-menopausal endometrium exhibited less cilia, microvilli and glandular activity than that shown in either the pre- or post-follicular phase in cycling women. The cilia that were present were shorter and had an abnormal appearance. In young estrous female rats, the endometrial cells were completely covered by densely packed microvilli and dispersed droplets of secretory material. However, endometrial cells of aged rats showed a marked reduction in the density of microvilli and in the amount of secretory material present (Steger et al., 1976 b). The alterations in the morphology of the uterus of postmenopausal women and old rats may result in an altered environment which would be inhospitable for implantation and development of the embryo.

In postmenopausal women, the fallopian tube also diminishes in size and motility as a result of deficient estrogen levels. The epithelium appears flat with little or no sign of secretory function (Timiras, 1972). No data are available on the morphology of the oviduct of aging laboratory animals.

Studies of the sensitivity of the human uterus to estrogen in women of different ages are not available, but the frequent occurence

of various degrees of hyperplasia of the endometrium of postmenopausal women with estrogen secreting tumors or hyperplasia of estrogen-producing cells, indicates that uterine responsiveness to estrogen does not disappear after the menopause. It has been reported, however, that estrogen withdrawal bleeding is less frequently noted in women past 60, which suggests that the uterus eventually become less sensitive (Talbert, 1968).

In rats, Ingram (1959) found no evidence of decreased sensitivity to estrogen. However, Chatterjee and Mukkerjee (1974) reported that none of the old rats they treated with steroids responded with decidual stimulation, whereas all the young rats given an identical estrogen and progesterone regimen showed satisfactory decidual responses.

E. Age Changes in Menstrual or Estrous Cycle

It is common knowledge that the human menstrual cycle tends to be irregular in length during the last few years of reproductive life. The earliest cytologic change detectable before menopause in vaginal smears is a decrease in the number of cornified cells seen in the preovulatory phase of the normal cycle. With menopause, only pre-cornified cells and tansitional epithelium from intermediate layers are noted in the smears. Basal layer cells are sometimes seen in smears from menopausal women and their appearance is a sign of estrogen deficiency (Paschkis et al., 1967). Cessation of cyclic ovarian activity at the menopause is still thought to be mainly caused by ovarian failure. That hypothalamic-pituitary mechanisms are still operating normally is indicated by the elevation in gonadotropin release, which occurs in the postmenopausal state in response to the lower-than normal estrogen levels (Timiras, 1972).

Aging female rats and mice show a gradual decline in number of ova, ovulations and litter size (Mandle and Zuckerman, 1951; Ingram et al., 1958; Mandl and Shelton, 1959) and finally cease to cycle and reproduce (Ingram, 1959; Thung et al., 1956). Examination of daily vaginal smears reveal that beginning at about 8-12 months of age, or even earlier, rats gradually change from regular to irregular cycles and then to constant estrus; this is followed by pseudopregnancies of irregular length and finally to an anestrous state in the oldest rats (Aschheim, 1961; Huang and Meites, 1975). Some rats may continue to cycle irregularly even up to 2 years of age, and some may revert temporarily from the constant estrous or pseudopregnant state to irregular cycles. We have never observed that old anestrous rats return to an earlier pattern.

Transplantation of ovaries from immature rats into old castrated rats and from old rats into young castrated rats, has indicated that the deviation of estrous cycle in rats are due mainly to aging changes in the hypothalamo-pituitary mechanism rather than to changes in the ovary (Aschheim, 1964, 1965; Peng and Huang, 1972). When we transplanted the pituitary of old rats into hypophysectomized young rats, the young rats showed restoration of the estrous cycle and some rats even showed the ability to become pregnant (Peng and Huang, 1972). This demonstrated that the irregularity of the estrous cycle in old rats is mainly due to aberrations of the central nervous system. The pituitary plays only a minor role in this process. In an elegant study by Clemens et al. (1969), it was found that old constant estrous rats could be induced to ovulate and to reinitiate estrous cycles by electric stimulation of the hypothalamus or systemic injection of central acting drugs. In mice, however, Krohn (1962) obtained quite different

results. When the ovaries from a strain of young mice which were transplanted into old mice (CBA), which were either cycling irregularly or were in constant estrus, this resulted in restoration of regular cycles in most of the hosts. This observation indicates that cessation of reproductive cycle in at least one strain of mice may be the result of ovarian aging rather than due to a primary change in the pituitary or hypothalamus. The CBA strain of mice may be more comparable to women in their post-reproductive characteristics than rats.

GENERAL MATERIALS AND METHODS

I. Animals and Blood Collection

Multiparous female Long-Evans rats, 10-12 months old, were obtained from Blue Spruce Farms, Altamont, N.Y. They were housed in steel cages in an air-conditioned and temperature-controlled ($24^{\circ} \pm 2^{\circ}$ C) room. Light was provided daily from 0600 to 2000 h by artificial fluorescent lamps. The animals were fed a diet of Wayne Lab Blox Pellets (Allied Mells, Chicago, Illinois) supplemented with carrots, and were given tap water ad libitum. When the animals reached 16-20 months of age, daily vaginal smears were taken on the morning of each day, using a small glass dropper and lukewarm tap water. Animals that showed vaginal cornification continuously for 20 days were considered to be in constant estrus; those that exhibited long diestrous phases for periods of 10-30 days interspersed with 1-2 days of estrus were considered to be pseudopregnant; rats that showed no cyclic activity and only vaginal leukocytes were considered to be anestrous. Animals that became diseased were not included in this study.

Blood samples were taken under light ether anesthesia by orbital sinus puncture. Serum was separated and frozen at -20°C until assayed.

II. Ovary Transplantation Technique

Ovaries were removed from inbred Long-Evans strain rats. The ovaries were immediately placed in a petri dish with 37°C physiological saline. The ovaries were cut into fine pieces on slide glass with a razor blade. A small slit was made through the anterior chamber of eye or the transparent renal capsule membrane, and two ovaries were put underneath the anterior chamber of the eye or the kidney capsule. All surgical procedures were performed with ether anethesia and surgically treated rats were given 0.2 ml of bicillin (Wyeth Lab, Inc., Philadelphia, Pa) by intramuscular injection to prevent infection.

III. Radioimmunoassays of LH, FSH and Prolactin

Prolactin (PRL), luteninizing hormone (LH) and follicle stimulating hormone (FSH) were measured in the serum of rats using the NIAMDD radioimmunoassay (RIA) kits for double antibody method of Niswender et al. (1968) and values were expressed in terms of the respective NIAMDD reference preparation's NIAMDD rat PRL-RP-1, rat LH-RP-1 and rat FSH-RP-1. Each serum sample was assayed at three dose levels and the average was obtained.

IV. Hypothalamic Catecholamine Assays

Hypothalamic norepinephrine and dopamine were assayed by the radioenzyme method of Coyle and Henry (1973) using catecholamine-O-methyl transferase (COMT) isolated from rat liver by a modification of the method of Nikodijevic et al. (1970). The assay was sensitive to

320 pg dopamine and 500 pg norepinephrine and linear to at least 4 ng for both catecholamines. Results are expressed as ng dopamine and norepinephrine per gram tissue wet weight.

V. Methods of Statistical Analysis

Analysis of variance coupled with Student-Newman-Keuls multiple range test was carried out as described by Sokal and Rohlf (1969). Differences between age and treatment means were considered significant only when the P value was less than 0.05.

EXPERIMENTAL

I. Reproductive Capacity of Aging Female Rats

A. Objectives

The purpose of this study was (1) to determine the progression of reproductive changes in old female rats, (2) to describe more adequately the gross and histological changes in the pituitary, ovaries and uterus of old female rats, and (3) to test the ability of L-dopa to reinitiate estrous cycles in the different categories of old rats.

B. Materials and Methods

Old constant estrous, pseudopregnant and anestrous rats were treated with L-dopa. L-dopa first was dissolved in warm 0.5 n HCl and then 0.5 n NaOH was added to achieve a pH of 6.5 - 7.0. The solution was injected s.c. at a dose of 30 mg twice daily at 0900 h and 1700 h to all 3 categories of rats for a period of 30 days. Control rats in the categories were given injections daily only with the solution used for L-dopa. Vaginal smears were taken daily during treatment and during a post-treatment period of 40 days. L-dopa initiated vaginal cycling only in the constant estrous rats. Most of these animals were laparotomized during the diestrous phase to determine whether corpora lutea were present. All animals were sacrificed at the end of the experimental periods, and the ovaries, uterus and pituitary from each rat were removed, weighed, fixed in

Bouin's fluids and sectioned and stained with eosin and hematoxylin for histological examination. Anterior pituitaries were stained with Masson's trichrome stain.

C. Results

The relationship between the vaginal smear patterns and the pituitary, ovaries, and uterus is shown in Table I. The ovaries of 9 control constant-estrous rats (Group 1) had mature follicles but no corpora lutea, whereas the ovaries of the other two rats had very large cystic follicles. The uteri of these rats appeared to be stimulated and were lined with columnar epithelium. Most of these control rats showed no change in vaginal pattern during the treatment and post-treatment periods. Of a total of 21 constant estrous rats treated with L-dopa, corpora lutea were found in the ovaries of 12 rats that exhibited cycling during treatment (Group 2). These 12 rats showed 4-6 estrous cycles each during treatment and 1-3 cycles early in the post-treatment period, followed by constant estrus. Their pituitary glands appeared to be normal under gross and histological examination and did not differ from those of the controls. The ovaries of the 4 L-dopa-treated rats (Group 3) which exhibited long periods of vaginal cornification interspersed with short diestrous phases, had mature follicles but no corpora lutea, their pituitary glands appeared to be normal in weight and histological appearance. Three constant estrous rats given L-dopa (Group 4) became pseudopregnant and had large ovaries with numerous corpora lutea, and tumorous areas in the pituitaries. The 2 constant estrous rats (Group 5) which showed no change in vaginal pattern during L-dopa treatment had heavy ovaries with very large, cystic follicles filled with fluid. Their pituitary

glands were normal in size and histological appearance.

Of 11 control pseudopregnant rats, 8 rats (Group 6) showed no change in vaginal smear patterns and had hypertrophic ovaries with numerous corpora lutea. Three of these rats had small tumorous areas in their pituitaries, 4 had hemorrhagic pituitary glands, and the pituitary of one rat appeared to be normal. The other 3 rats (Group 7) changed from irregular pseudopregnancies to the anestrous state during treatment, and showed atrophic ovaries and uteri, and large pituitary tumors at the time of sacrifice. Of 16 L-dopa-treated pseudopregnant rats, 13 rats (Group 8) showed no change in vaginal smear patterns and had hypertrophied ovaries with numerous corpora lutea. Six of these rats had small pituitary tumors and 5 had hemorrhagic pituitary glands. Two rats had apparently normal pituitaries. Three pseudopregnant rats (Group 9) became anestrous and exhibited atrophic ovaries and uteri, and large pituitary tumors.

The 10 control anestrous rats (Group 10) and 11 L-dopa-treated anestrous rats (Group 11) all had atrophic ovaries with no follicles or luteal elements, and small uteri with no epithelial lining. All anestrous rats had large pituitary tumors with many hemorrhagic and necrotic areas, and large vacuolated and giant cells. Most of these rats had hypertrophied mammary glands and some of these were tumorous.

D. Discussion

The present study shows that at least 3 major patterns are present in old female rats after cessation of estrous cycles, and indicates that the progression is from a constant estrous state to a series of irregular pseudopregnancies and finally to an anestrous state. It must be emphasized that not all aging rats necessarily follow the above

sequence nor is each stage of similar duration. Thus regular or irregular cycles, or constant estrus, may continue in some rats for even up to 2 years or longer; others may revert from the constant estrous or pseudopregnant state to irregular cycles; and some rats may go directly from cycling to the pseudopregnant state. I have not observed that any of the old anestrous rats return to the earlier patterns. The progression from irregular estrous cycles to the constant estrous state suggests that there is a gradual failure in the hypothalamo-pituitary mechanisms controlling LH release and ovulation. The explanation of why rats proceed from the constant estrous to the pseudopregnant state is not clear at this time. Aschheim (1961) reported that the corpora lutea of such pseudopregnant old rats are functional. The anestrous state has not previously been well-characterized, but it is now apparent that this state is usually found in the last period of life. These rats had atrophic ovaries and uteri, and all had pituitary tumors. A few old rats continued to exhibit irregular estrous cycles.

L-dopa was able to induce cycling in old constant estrous but not in the pseudopregnant or anestrous rats. The action of L-dopa was rapid, since vaginal cycling resumed within a few days after administration of this drug. This confirms previous observations that adrenergic drugs can induce cycling in old estrous rats (Clemens et al., 1969; Quadri et al., 1973), but shows that many of the cycles are not as regular as in normal mature cycling rats. There is a possibility that some other factor may be necessary in addition to L-dopa to normalize cycling in these rats. We have recently observed that very regular estrous cycles can be initiated in old constant estrous rats by the daily administration of small doses of progesterone (Huang, Marshall

and Meites, 1976 a).

Only a few cycles continued in some of the old constant estrous rats after termination of L-dopa treatment, probably reflection a carry-over effect of the drug. Rats that continued to show continuous estrus during L-dopa treatment had large cystic follicles filled with fluid, and their ovaries weighed about twice as much as the controls. High estrogen secretion was indicated in these 2 rays by increased weight of the uterus; this may have inhibited gonadotropin secretion and thus prevented stimulation by L-dopa.

The action of L-dopa in old constant estrous rats is believed to be exerted via the hypothalamus. L-dopa is the immediate precursor of dopamine, and injection of the latter into the third ventricle of rats has been reported to increase LRH release into the hypothalamo-pituitary portal circulation and to elevate serum LH and FSH values (Kamberi et al., 1970). Electrochemical stimulation of the hypothalamus of old constant estrous rats also resulted in prompt release of LH (Wuttke and Meites, 1973).

The failure of L-dopa to reinitiate estrous cycles in pseudo-pregnant old rats may be related to the presence of active corpora lutea in the ovaries. Electrochemical stimulation of the hypothalamus of old pseudopregnant rats was not found to evoke significant increases in LH release, in contrast to its effectiveness in old constant estrous rats (Wuttke and Meites, 1973). It is possible that progesterone secretion by the corpora lutea of these rats, combined with a deficiency of estrogen, inhibited the action of L-dopa on LH release.

The inability of L-dopa to induce estrous cycles in old anestrous rats seems to be due to the presence of large pituitary tumors. High levels of prolactin are secreted by the pituitary tumors (Huang and

Meites, 1976 b). Hypertrophied mammary glands and spontaneous mammary tumors are also present. A high incidence of pituitary tumors in old female and male rats, with the release of large amounts of prolactin, also has been reported by Moy et al. (1972).

TABLE 1. Effects of L-dopa on Vaginal Patterns in 3 Categories of Old Rats.

Group and no. of rats	Pre-Treatment State	Treatment	No. of Rats With Regular Cycles	No. of Rats With Irregular Cycles	CVC[1]	IP[2]	AS[3]	Post-Treatment State	Average Pituitary Weight(mg)	Average Ovarian Weight (mg)	Average Uterine Weight (mg)
1 (11)	CVC	Vehicle (Controls)		1	10			CVC	16.9 ± 0.98[4]	43.7 ± 2.18	650.0 ± 40.18
2 (12)	CVC	L-dopa	12					Cycles	17.0 ± 0.76	58.2 ± 2.30	648.2 ± 46.72
3 (4)	CVC	L-dopa		4				CVC	15.4 ± 0.55	36.3 ± 1.36	631.8 ± 60.21
4 (3)	CVC	L-dopa				3		IP	17.2 ± 0.60	86.7 ± 10.14	741.5 ± 60.20
5 (2)	CVC	L-dopa			2			CVC	19.0 ± 2.00	83.8 ± 3.75[5]	720.0 ± 10.00
6 (8)	IP	Vehicle (Controls)				8		IP	16.8 ± 2.00	107.3 ± 9.64	742.9 ± 25.40
7 (3)	IP	Vehicle (Controls)					3	AS	130.0 ± 65.24	20.6 ± 2.96	223.3 ± 21.86
8 (13)	IP	L-dopa				13		IP	20.5 ± 1.42	105.0 ± 8.62	767.5 ± 40.90
9 (3)	IP	L-dopa					3	AS	205.0 ± 70.29	19.75 ± 2.78	226.6 ± 24.03
10 (10)	AS	Vehicle (Controls)					10	AS	158.0 ± 33.00	19.7 ± 1.63	225.0 ± 21.92
11 (11)	AS	L-dopa					11	AS	199.0 ± 30.64	22.2 ± 1.25	220.0 ± 13.90

[1] CVC = Constant Vaginal Cornification
[2] IP = Irregular Pseudopregnancies
[3] AS = Anestrous State
[4] = Standard Error
[5] = Large Cystic Follicles

II. Induction of Estrous Cycles in Old Constant Estrous Rats by Progesterone, ACTH, Ether Stress or L-dopa

A. Objectives

The present study was undertaken to determine whether injections of progesterone, ACTH or subjection to ether stress could reinitiate estrous cycles in old constant estrous rats; these effects were compared with those of L-dopa, an agent previously shown to induce cycling in old constant estrous rats (Quadri et al., 1973). Serum LH was measured on the afternoon of the day of proestrus in rats induced to cycle by these treatments.

B. Materials and Methods

(1) Progesterone. 16-18 months old constant estrous rats were given injections of progesterone (0.5 mg / rat in 0.1 ml corn oil) s.c. twice daily at 1000 h and 1700 h. Controls were given s.c. injections of 0.1 ml / rat corn oil twice daily at 1000 h and 1700 h.

(2) ACTH. Rats were given s.c. injections of ACTH (porcine, grade II, Sigma Chemical Co., St. Louis, Mo.) twice daily at 1000 h and 1700 h in doses of 2.2 IU / rat in 0.1 ml of 0.85% NaCl. This dose was calculated from the amount of ACTH reported to be present in the plasma of rats during acute stress (Cook et al., 1973). Controls were given injections of the same volume of saline alone.

(3) Ether stress. The animals were placed in a glass chamber containing ether-dampened paper. When the animals became unconscious, they were removed from the ether chamber; anesthesia was continued for 2 mon with an ether-saturated nose cone. The rats were given this regimen twice daily at 1000 h and 1700 h. Control rats were treated similarly but were not given ether.

(4) L-dopa. L-dopa was dissolved in warm 0.5 n HCl and then

0.5 n NaOH was added to achieve a pH of 6.5 - 7.0 L-dopa, 30 mg per rat, was injected s.c. twice daily at 1000 h and 1700 h. Controls were given the vehicle only.

Assay of serum LH. Daily monitoring of estrous cycles continued throughout the treatment period of 30 days and the post-treatment, 30-40 days. When animals were induced to cycle, blood was collected from the orbital sinus under light ether anesthesia (about 30 sec exposure) at 2-h intervals from 1 to 9 p.m. on proestrous day of the 3rd or 4th cycle. Laparotomies were performed to observe the presence of new corpora lutea on diestrous day of the next cycle. Treatment were then terminated.

C. Results

(1) Progesterone

The number of old constant estrous rats treated with progesterone, and their responses during and after treatment are shown in Table 2. The 8 control estrous rats, given corn oil alone, remained in constant estrus during treatment and post-treatment periods. Of 26 constant estrous rats given progesterone, 23 rats showed regular 4- or 5-day cycles during the treatment period; 2 rats exhibited 2 regular cycles, each followed by a period of pseudopregnancy, and then irregular cycles and constant estrus during the post-treatment period. One rat remained in constant estrus during treatment and post-treatment periods. After progesterone administration was terminated, of the 23 rats induced to cycle normally, 5 returned to constant estrus within 48 h. Seven rats showed irregular cycles throughout the post-treatment period; 5 showed 3-4 irregular cycles followed by constant estrus, and 6, a period of pseudopregnancy followed by constant estrus.

Of 26 laparotomized rats, 21 rats had numerous corpora lutea in the ovaries, 4 had only a few, and 1 rat that remained in constant estrus had no corpora lutea. Representative vaginal patterns are shown before, during and after progesterone treatment in Figure 1.

(2) ACTH

Table 2 shows that all 6 controls exhibited no change in vaginal smear patterns during treatment and post-treatment periods. Ten of 12 rats given ACTH showed regular estrous cycles during the treatment period; the other 2 rats remained in constant estrus. Of the 10 rats that cycled regularly, 7 exhibited one irregular cycle early in the post-treatment period followed by constant estrus. Two rats showed irregular cycles during the post-treatment period, and one, a period of pseudopregnancy early in the post-treatment period followed by constant estrus. When the 12 ACTH-treated rats were laparotomized, corpora lutea were found in the 10 that had been induced to cycle regularly. Nine rats had large ovaries with numerous corpora lutea, and 1 rat had only a few corpora lutea. No corpora lutea were found in the remaining 2 rats. Representative vaginal patterns induced by ACTH are shown in Figure 2.

(3) Ether Stress

All 6 controls showed no change in vaginal patterns during and after the treatment period (Table 2). Of 22 rats treated with ether stress, only 8 rats showed regular 4- or 5-day cycles during the treatment period; 6 of these rats exhibited 1-3 irregular cycles early in the post-treatment period followed by constant estrus. The other 2 rats concinued to exhibit irregular cycles throughout the post-treatment period. Ten rats exhibited irregular cycles during treatment and 1-3 irregular cycles early in post-treatment followed by constant estrus.

Two rats showed a single period of pseudopregnancy followed by 2 irregular cycles during the treatment period; these rats reverted to constant estrus after treatment was terminated. The constant estrous state in 2 rats was not changed by ether stress. When the 22 treated rats were laparotomized, 4 rats had large ovaries with numerous corpora lutea, 6 rats had only a few corpora lutea in the ovaries (6 or less), and the remaining 12 rats had none. Corpora lutea were present only in rats showing regular cycles or pseudopregnancies during the treatment period. Representative vaginal patterns produced by ether stress are shown in Figure 3.

(4) L-dopa

The 6 controls showed no change in vaginal patterns during and after the treatment period (Table 2). Only 3 of 12 rats treated with L-dopa showed regular cycles, and these cycles became irregular after treatment was terminated. Five rats showed irregular cycles throughout the treatment and post-treatment periods. Two rats had a period of pseudopregnancy early in the treatment period followed by irregular cycles; 2 other rats exhibited no change in vaginal patterns. Of 12 laparotomized rats, 2 had large ovaries with numerous corpora lutea, 3 had only a few corpora lutea in the ovaries, and the remaining 7 rats had none. Corpora lutea were present only in rats showing estrous cycles or pseudopregnancies during the L-dopa treatment. Representative vaginal patterns from this experiment are shown in Figure 4.

Serum LH

Serum LH was measured in 20 old constant estrous rats induced to cycle regularly by progesterone; 14 of these rats exhibited LH surges exceeding 100 ng / ml on the afternoon of proestrus (Table 3). During the periods when blood was collected, from 1300 h to 1900 h, serum LH

values rose from basal levels of 7-28 ng / ml to a maximum of 2,186 ng / ml. A total of 3 rats showed LH peaks at 1300 h, 6 at 1500 h, 3 at 1700 h and 2 at 1900 h. Two rats had LH elevations of 84 and 87 ng / ml, respectively.

Serum LH levels in rats given ACTH injections are shown in Table 4. Six of 10 rats had serum LH surges of 1274-127 ng / ml. Serum LH peaks occurred in 2 rats at 1300 h, in 3 at 1500 h, in 1 at 1700 h and in another at 1900 h. Four rats apparently failed to show significant LH surges during times when blood was collected.

Serum LH levels on proestrous afternoon in rats treated twice daily with ether stress are shown in Table 5. Six of 20 rats had serum LH peaks of 110-760 ng / ml. One rat showed an LH peak at 1300 h, 4 at 1500 h and 1 at 1900 h, 14 rats apparently failed to show significant serum LH surges on the afternoon of proestrus.

Serum LH in rats given L-dopa injections are shown in Table 6. Only 2 of 10 rats had serum LH peaks greater than 100 ng / ml (207 and 473 ng / ml) and 1 rat had a peak of 74 ng / ml. Seven rats apparently failed to show serum LH surges on the afternoon of proestrus.

It is possible that LH surges also occurred in other rats induced to cycle by progesterone, ACTH, ether stress or L-dopa, but were missed because they occurred when blood samples were not collected. We have observed previously that placement of old constant estrous rats in an ether chamber for about 30 sec for blood collection did not result in elevations in serum LH.

D. Discussion

These results demonstrate that progesterone, ACTH, L-dopa or ether stress produced resumption of regular or irregular estrous cycles in old

constant estrous rats. Progesterone and ACTH were more effective than ether stress or L-dopa. A total of 23 of 26 rats given progesterone showed regular estrous cycles, and 10 of 12 ACTH-treated rats exhibited regular estrous cycles. Both of these groups had more fresh corpora lutea, as determined by laparotomy, and more LH peaks on the afternoon of proestrus, than ether-stressed or L-dopa-treated rats. Unlike normal cycling young rats, LH peaks did not always occur on the late afternoon of proestrous day but showed considerable individual variation. After termination of the different treatments, most of the rats ceased to cycle and reverted to constant estrus or irregular states. The ether-stressed and L-dopa-treated rats showed more irregular than regular cycles. Few of these rats showed LH peaks; some LH surges, however, may have been missed because blood samples were collected only on the afternoon of proestrus. It is not clear at present why ether stress and L-dopa were less effective than progesterone and ACTH in producing regular cycles in these rats.

Previously progesterone was reported to induce cycling in constant estrous rats (Everett, 1940), suggesting that progesterone may be deficient in these rats. Previous observations by our laboratory indicated that the ovaries of such rats do not contain corpora lutea (Clemens and Meites, 1971). In mature cycling rats, progesterone was shown to have a role in normal LH release and ovulation, and hastened these processes when administered (Everett, 1948). Progesterone induction of LH release apparently requires the mediation of hypothalamic catecholamines. Since administration of antiadrenergic drugs can block LH release by progesterone (Kalra et al., 1972). ACTH has been reported to increase progesterone release on the day of proestrus in order to faciliate release of ovulatory amounts of LH and to induce

mating behavior in female rats (Feder and Ruf, 1969; Feder et al., 1971; Lawton, 1970; Nequin and Schwartz, 1971; Barraclough et al., 1971). Injections of ACTH into old constant estrous rats could have acted in a similar manner. There also is evidence that ACTH also may act centrally to induce LH release in rabbits (Baldwin et al., 1974). Ether stress has been reported to be a potent stimulator of ACTH release (Cook et al., 1973), which in turn could promote progesterone release from the adrenals. There also is the possibility that ether acted centrally via the hypothalamic biogenic amines to elicit LH release. Ether stress has been reported to readily induce LH and prolactin release in the normal rat (Euker et al., 1975).

L-dopa induced mostly irregular cycling in the treated rats, in agreement with previous reports from our laboratory (Quadri et al., 1973). It is believed that L-dopa acts by increasing hypothalamic catecholamine activity, which results in release of LRH and LH. Hypothalamic norepinephrine is believed mainly responsible for LH release, since injecting norepinephrine into the 3rd ventricle of rabbits was reported to induce ovulation and to increase serum LH levels, whereas dopamine did not induce LH release (Sawyer et al., 1974). We had postulated earlier that hypothalamic catecholamine activity is low in old rats and may be partially responsible for the failure of ovulation in old constant estrous rats (Clemens et al., 1969; Clemens and Meites, 1971). Recent observations indicate that old male rats have significantly less hypothalamic norepinephrine and dopamine than young mature males (Simpkins et al., 1976). Finch (1973) also reported observations in old male mice suggestive of catecholamine deficiency. Thus a common hypothalamic mechanism may explain, at least in part, the ability of progesterone, ACTH, L-dopa and ether stress to

induce LH release and resumption of cycling in old constant estrous rats they increase hypothalamic catecholamine activity, thereby stimulating release of LRH into the portal circulation. However, other hypothalamic neurotransmitters, in addition to catecholamines, may be involved in mediating the actions of these agents.

Table 2. Effects of Progesterone, ACTH, Ether Stress and L-Dopa on Vaginal Patterns in Old Constant Estrous Rats

Treatment and No. of Rats	Treatment Response and No. of Rats	Corpora Lutea	Post-Treatment Response and No. of Rats
Controls (8) - (Corn oil)	CVC (8)	---	CVC (8)
.5 mg progesterone/rat, 2X daily (26)	RC (23)	+++ (19)	CVC (5)
		+ (4)	3-4IC, then CVC (5); 1 Pp, then CVC (6); IC (7)
	2 RC, then 1 Pp (2)	+++ (2)	2 IC, then CVC (2)
	CVC (1)	--- (1)	CVC (1)
Controls (6) - (Saline)	CVC (6)	--- (6)	CVC (6)
2.2 IU ACTH/rat, 2X daily (12)	RC (10)	+++ (9)	1 IC, then CVC (7);
		+ (1)	IC (2); 1 Pp, then CVC (1)
	CVC (2)	--- (2)	CVC (2)
Controls (6) - (No ether)	CVC (6)	--- (6)	CVC (6)
Ether stress, 2X daily (22)	RC (8)	+++ (2)	1-3 IC, then CVC (6);
		+ (6)	IC (2)
	1 Pp, then IC (2)	+++ (2)	CVC (2)
	IC (10)	--- (10)	1-3 IC, then CVC (10)
	CVC (2)	--- (2)	CVC (2)
Controls (6) - (Vehicle)	CVC (6)	--- (6)	CVC (6)
30 mg L-dopa/rat, 2X daily (12)	RC (3)	+ (3)	IC (3)
	1 Pp, then 2 IC (2)	+++ (2)	IC (2)
	IC (5)	--- (5)	IC (5)
	CVC (2)	--- (2)	CVC (2)

CVC = Constant vaginal cornification
RC = Regular cycle
IC = Irregular cycle
Pp - Pseudopregnancy

( ) = No. of rats
+++ = Many corpora lutea
+ = Few corpora lutea
--- = No corpora lutea

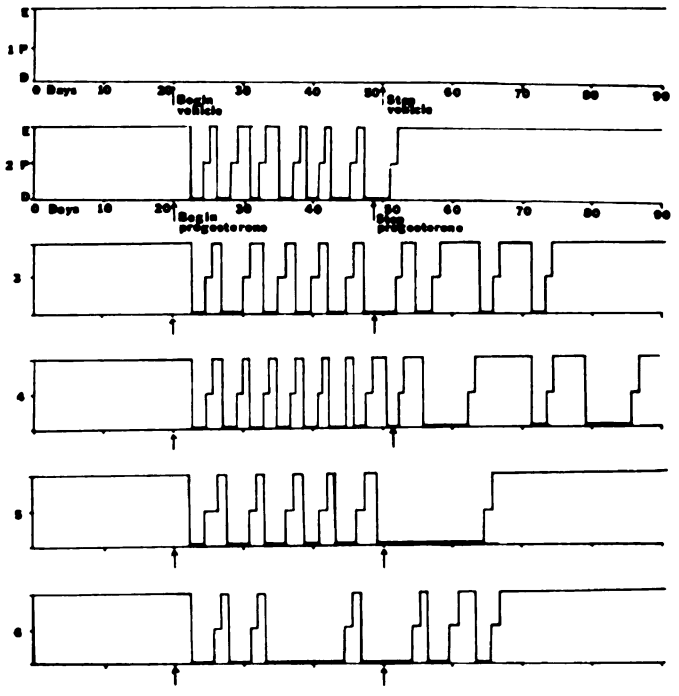

Figure 1. Vaginal smear patterns in 6 representative old constant estrous rats induced to cycle by progesterone. (1) control rat injected with vehicle alone, showing constant estrus. (2) rat given progesterone, showing regular cycles during treatment period and constant estrus after termination of treatment. (3) rat given progesterone, showing regular cycles during treatment and irregular cycles followed by constant estrus during post-treatment. (4) rat given progesterone, showing regular cycles during treatment and irregular cycles throughout post-treatment period. (5) rat given progesterone, showing regular cycles during treatment and 1 period of pseudopregnancy followed by constant estrus during post-treatment period. (6) rat given progesterone, showing 2 regular cycles and 1 period of pseudopregnancy during treatment, followed by irregular cycles and constant estrus during post-treatment. E = estrus, P = proestrus, D = diestrus.

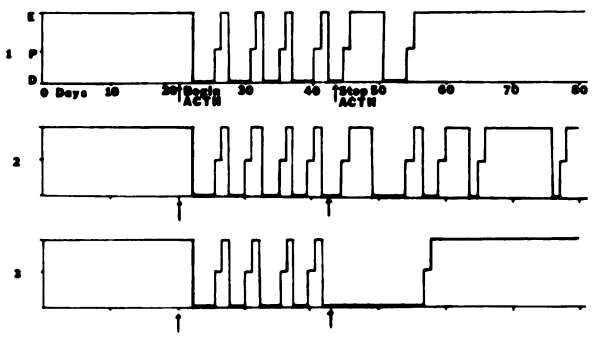

Figure 2. Vaginal smear patterns in 3 representative constant estrous rats treated with ACTH showing: (1) regular estrous cycles during treatment period, followed by 1 irregular estrous cycle and constant estrus during post-treatment; (2) regular estrous cycles during treatment period and irregular estrous cycles throughout post-treatment period; (3) regular cycles during treatment period and 1 period of pseudopregnancy early in post-treatment period followed by constant estrus.

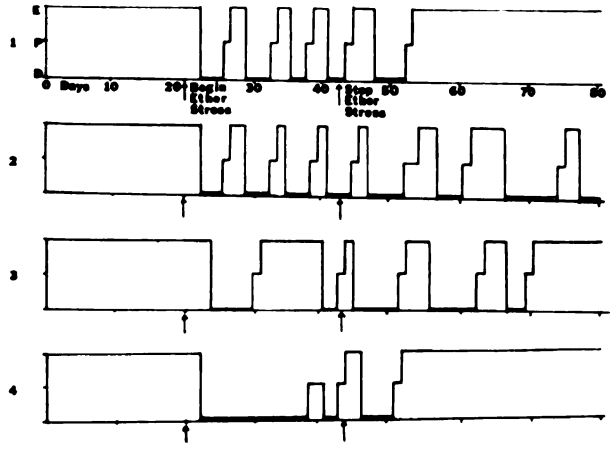

Figure 3. Vaginal smear patterns in 4 representative constant estrous rats treated with ether stress showing: (1) regular estrous cycles during treatment period, followed by 1 irregular estrous cycles early in post-treatment period and constant estrus thereafter; (2) regular estrous cycles during treatment period, followed by irregular estrous cycles throughout the post-treatment period; (3) irregular estrous cycles throughout treatment and post-treatment periods; (4) 1 period of pseudopregnancy during treatment period, followed by 1 irregular estrous cycle early in post-treatment period and constant estrus.

Figure 4. Vaginal smear patterns in 3 representative constant estrous rats treated with L-dopa showing: (1) regular estrous cycles during treatment period and irregular estrous cycles during post-treatment period; (2) irregular estrous cycles throughout treatment and post-treatment periods; (3) 1 period of pseudopregnancy during treatment period and irregular estrous cycles during post-treatment period.

Table 3. Serum LH (ng/ml) on Proestrous Afternoon in Old Constant Estrous Rats Induced to Cycle Normally by Progesterone

Rat No.	1300	1500	1700	1900	2100 h.
1	22	2186	316	217	140
2	1678	128	59	41	22
3	1326	26	26	50	41
4	68	1291	100	90	70
5	7	21	942	176	18
6	21	816	138	72	28
7	110	407	207	124	52
8	32	245	240	337	11
9	19	23	337	14	14
10	16	238	342	185	25
11	27	644	78	71	17
12	172	18	19	17	9
13	8	17	16	168	8
14	9	108	11	9	7
15	27	84	82	51	18
16	67	68	87	28	17
17	18	17	21	18	8
18	7	9	9	8	10
19	7	9	26	7	7
20	7	11	8	7	7

Table 4. Serum LH (ng/ml) on Proestrous Afternoon in Old Constant Estrous Rats Induced to Cycle Normally by ACTH

Rat No.	1300	1500	1700	1900	2100 h.
1	27	2147	498	80	56
2	18	691	1022	503	18
3	951	948	31	30	26
4	250	28	31	16	7
5	8	246	36	54	9
6	21	17	15	127	20
7	8	55	44	36	8
8	31	22	7	8	7
9	24	23	19	12	10
10	28	20	7	7	7

Table 5. Serum LH (ng/ml) on Proestrous Afternoon in Old Constant Estrous Rats Induced to Cycle by Ether Stress

Rat No.	1300	1500	1700	1900	2100 h.
		Regular Cycles			
1	17	760	602	293	116
2	176	361	123	96	72
3	26	268	230	119	25
4	274	200	108	110	85
5	25	28	15	120	72
6	26	110	18	24	7
7	48	23	31	14	11
8	29	21	21	15	14
		Irregular Cycles			
9	26	28	47	30	10
10	17	16	25	18	9
11	8	31	14	8	7
12	17	21	18	18	8
13	27	53	28	30	24
14	25	29	18	27	17
15	28	28	37	30	21
16	18	24	57	82	72
17	72	75	46	48	35
18	28	58	42	45	18
19	18	21	35	40	27
20	14	7	7	7	7

Table 6. Serum LH (ng/ml) on Proestrous Afternoon in Old Constant Estrous Rats Induced to Cycle by L-dopa

Rat No.	1300	1500	1700	1900	2100 h.
		Regular Cycles			
1	42	294	473	465	285
2	74	47	38	21	13
3	31	38	43	28	28
		Irregular Cycles			
4	207	22	24	17	16
5	16	25	22	24	24
6	34	18	15	13	8
7	29	15	20	13	10
8	18	26	49	14	14
9	30	55	32	25	9
10	9	8	8	9	17

III. Effects of Progesterone on Estrous Cycles of Ovariectomized Old Rats Bearing Ovaries from Young Rats, and Ovariectomized Young Rats Bearing Ovaries from Old Rats

A. Objectives

Since old constant estrous rats can be induced to ovulate and to resume regular estrous cycles by injections of progesterone, this suggests that deviation of estrous cycles could be due to dysfunction of the ovaries or of the hypothalamo-pituitary system. It was the purpose of the present study to observe whether the ovaries or the hypothalamo-pituitary system, or both, were responsible for cessation of regular estrous cycles in aging rats.

B. Materials and Methods

1. Ovariectomized Old Rats Transplanted with Ovaries from Immature Rats

(a) Eighteen to 20 month old constant estrous rats were ovariectomized, and at the same time were transplanted with two ovaries from 21 day old rats into the anterior chamber of the eye. Vaginal smears were monitored every day for 20 days after transplantation. At the time the eye was implanted with ovaries, the ovaries were observed for the presence of fresh corpora lutea. At the end of the experiment the grafted ovarian tissue was removed, weighed, and fixed in Bouin's fluid for histological examination. Four month old normal cycling rats were used for controls.

(b) Vaginal smears were carried out for 20 days after ovarian transplantation. Rats that showed constant vaginal cornification were treated with 1 mg progesterone per rat per day at 1000 h for 20 days. Controls were given corn oil. Vaginal smears were monitored during progesterone treatment and for 20 days after termination of progesterone

injections.

2. Ovariectomized Young Rats Transplanted with Ovaries from Old Rats

(a) Four month old normal cycling rats were ovariectomized and transplanted with two ovaries from old constant estrous (22-26 month old), old pseudo-pregnant (22-24 month old), or old anestrous rats (26-30 month old) into the kidney capsules (one ovary per kidney). Ovaries from 4 month old rats were used for controls. Vaginal smears were monitored for 20 days after transplantation. At the end of the experiment the animals were killed. The grafted ovaries and uteri were removed, weighed and fixed in Bouin's fluid for histological examination.

(b) Ovariectomized young rats bearing ovaries from old rats, which exhibited constant vaginal cornification, were injected with progesterone. Progesterone treatment and vaginal smearing were the same as in 1 (b). A laparotomy was done in each rat at the end of progesterone treatment to observe the presence of corpora lutea.

C. Results

Table 7 shows that when ovaries of old constant estrous rats were replaced by ovaries from young rats, they did not resume estrous cycles. All 10 old constant estrous rats continued to show constant vaginal cornification and no corpora lutea were present (Figure 5). All 6 young rats showed regular estrous cycles and formation of corpora lutea.

The effects of progesterone on the old rats given ovaries of young rats are shown in Table 8. During the treatment period, 8 of 13 rats exhibited regular 4 or 5 day cycles; 4 became pseudopregnant and 1 showed irregular cycles. Twelve of 13 rats had fresh corpora lutea

(Figure 6). After progesterone treatment was terminated, 5 returned to constant estrus, 2 showed pseudopregnancy, 1 exhibited irregular cycles, and 1 became anestrus. However, the old constant estrus controls did not change their vaginal patterns and no corpora lutea were observed throughout the experimental period.

Table 9 shows the results when ovariectomized young rats were transplanted with ovaries from old rats. Five of 6 controls continued to show regular cycles after ovarian transplantation. One rat showed irregular cycles. Ovarian and uterine weights did not change after transplantation. All 6 controls had corpora lutea in the ovaries.

Five of 14 young rats transplanted with ovaries from old constant estrous rats showed regular 4 or 5 day cycles; 1 showed irregular cycles and 8 showed constant estrus. Six of 14 rats which exhibited regular or irregular cycles after transplantation had corpora lutea in the grafted ovaries. At the end of the experiment, the grafted ovarian weight was significantly heavier than the original ovarian weight (57.1 ± 4.4 vs. 80.1 ± 4.5 mg).

Two of 10 rats transplanted with ovaries from old pseudopregnant rats had regular 4 or 5 day cycles, and 1 showed irregular cycles. Interestingly, 7 of 10 rats showed constant estrus and the large corpora lutea in the original ovaries disappeared at the end of the experiment. Only 3 of 10 rats had corpora lutea of normal size. The grafted ovarian weight was significantly less than the original ovarian weight (133.5 ± 8.3 vs. 79.4 ± 3.8 mg).

In the 8 young rats transplanted with ovaries from old anestrous rats, 2 showed regular 4 or 5 day cycles, 2 irregular cycles, 2 constant estrus and 2 prolonged diestrus. The atrophic ovaries (27.3 ± 2.6 mg) of anestrous rats grew to 76.8 ± 9.0 mg after they were grafted into

the young rats, and 3 rats exhibited corpora lutea.

The uterine weight and grafted ovarian weight of young rats transplanted with ovaries from these 3 groups of old rats were not different from those of young rats transplanted with young ovaries (controls). The histological picture of ovaries from old rats transplanted into young female rats are shown in Figures 7 to 12.

The effects of progesterone on vaginal patterns in young rats transplanted with ovaries from old rats are shown in Table 10. In the young rats transplanted with ovaries from old constant rats, 7 of 9 rats exhibited constant vaginal cornification but were induced to cycle regularly by daily injection of progesterone; 1 showed irregular cycles and 1 became pseudopregnant. Eight of 9 rats had corpora lutea in the grafted ovaries during the period of progesterone treatment. After progesterone treatment was terminated, 7 of 9 rats returned to constant vaginal cornification, 1 to irregular cycles and 1 remained pseudopregnant.

In the 6 young rats given ovaries from old pseudopregnant rats, all showed constant vaginal cornification and exhibited 4 or 5 day cycles and corpora lutea formation during progesterone administration. After progesterone administration was terminated, 2 rats continued to show 4 or 5 day cycles, 2 showed constant estrus, 1 showed irregular cycles, and 1 became pseudopregnant.

Two of 3 rats which showed constant vaginal cornification after receiving ovaries from old anestrous rats were induced to cycle regularly or to become pseudopregnant by progesterone. All 3 had corpora luteal formation during the period of progesterone administration, and returned to constant vaginal cornification when progesterone administration was stopped.

D. Discussion

Replacement of ovaries of old constant estrous rats with those from immature rats did not restore vaginal cyclicity. These observations confirm our previous report (Peng and Huang, 1972). However, old rats which bore ovaries from young rats could be stimulated to produce regular estrous cycles by daily administration of progesterone. This indicates that the effects of progesterone was mainly on the hypothalamo-pituitary system.

Most of the young rats which were given ovaries from old rats exhibited constant estrus or irregular cycles, only $\frac{1}{4}$ of the young rats showed regular cycles. These observations demonstrate that aged ovaries also can influence vaginal cyclicity. The number of oocytes in the ovaries becomes gradually reduced with aging, as indicated previously. The deviations in the estrous cycles of most young rats given transplants of ovaries from old rats was probably due to the reduced number of oocytes. Even under endogenous gonadotropin stimulation from the young rat, the aged ovaries still could not release enough estrogen to trigger a pre-ovulatory LH surge and induce ovulation. The young rats could be induced to ovulate and to exhibit regular estrous cycles by administration of progesterone. Progesterone may potentiate the effects of estrogen and reduce the estrogen threshold for producing pre-ovulatory gonadotropin release.

Table 7. Behavior of Immature Rat Ovaries Transplanted into Ovariectomized Old Rats

Group, Age, and No. of Rats	Estrous Cycles Before Graft	Estrous Cycles After Graft	Ovarian Wt. of Immature Rat before Graft and No. of Rats with CL (mg)	Ovarian Wt. of Immature Rat After Graft and No. of Rats with CL (mg)	Recipient Ovaries Wt. and No. of Rats with CL (mg)
Control Young Rats 4 mo. (6)	RC (6)	RC (6)	18.7 ± 0.4 None	34.4 ± 0.7 (6)	76.4 ± 1.8 (6)
Old Rats 18-20 mo. (10)	CVC (10)	CVC (10)	17.6 ± 0.7 None	23.3 ± 2.2* None	55.1 ± 4.4* None

CL = Corpora lutea.
RC = Regular.
CVC = Constant vaginal cornification.
( ) = No. of rats.
* = Significantly different from young controls.

Table 8. Effects of Progesterone on Vaginal Patterns in Ovariectomized Old Constant Estrous Rats Given Ovaries from 21 Day Old Rats

Treatment and No.	Pretreatment State	Treatment Response and No. of Rats	No. of Rats with CL	Post-treatment Response and No. of Rats
Control (6)	CVC	CVC (6)	None	CVC (5) IC (1)
1 mg Progesterone /rat/day (13)	CVC	RC (8) PP (4) IC (1)	(12)	CVC (7) PP (2) IC (3) AS (1)

CL = Corpora lutea.
CVC = Constant vaginal cornification.
RC = Regular cycle.
IC = Irregular cycle.
PP = Pseudopregnancy.
AS = Anestrus.
( ) = No. of rats.

Table 9. Behavior of Old Rat Ovaries Transplanted into Ovariectomized Young Mature Rats

Group, Age of Ovary, and No. of Rats	Estrous Cycles Before Graft	Estrous Cycles After Graft	Ovarian Weight Before Graft and No. of Rats with CL (mg)	Ovarian Weight After Graft and No. of Rats with CL (mg)	Uterine Weight After Graft (mg)
Control (6) Young Mature Rats, 4 mo.	RC (6)	RC (5) IC (1)	75.4 ± 1.6 (6)	84.0 ± 3.8 (6)	440.8 ± 17.7
Old Constant Estrous Rats 20-26 mo. (14)	RC (14)	RC (5) IC (1) CVC (8)	57.1 ± 4.4* None	80.1 ± 4.5 (6)**	438.7 ± 19.6
Old Pseudo-pregnant Rats 20-24 mo. (10)	RC (10)	RC (2) IC (1) CVC (7)	133.5 ± 8.3 (10)*	79.4 ± 3.8 (3)**	435.9 ± 19.8
Anestrous Rats 24-28 mo. (8)	RC (8)	RC (2) IC (2) CVC (2) PD (2)	27.3 ± 2.6* None	76.8 ± 9.0 (3)**	419.5 ± 18.8

CL = Corpora lutea.
CVC = Constant vaginal cornification.
RC = Regular cycle.
IC = Irregular cycle.
PD = Prolonged diestrus.
( ) = No. of rats.
* = Significantly different from young controls.
** = Significantly different from ovarian weight before graft.

Table 10. Effects of Progesterone on Vaginal Patterns in Ovariectomized Young Mature Rats Given Ovaries from Old Rats

Group, Age of Ovary and No. of Rats	Pretreatment State	Treatment Response and No. of Rats	No. of Rats with CL	Post-Treatment Response and No. of Rats
Old Constant Estrous Rats 20-26 mo. (9)	CVC	RC (7) IC (1) PP (1)	(8)	CVC (7) IC (1) PP (1)
Old Pseudo-pregnant Rats 20-24 mo. (6)	CVC	RC (6)	(6)	RC (2) CVC (2) IC (1) AS (1)
Old Anestrous Rats 24-28 mo. (3)	CVC	RC (2) PP (1)	(3)	CVC (3)

CL = Corpora lutea.
CVC = Constant vaginal cornification.
IC = Irregular cycle.
AS = Anestrus.
PP = Pseudopregnant.

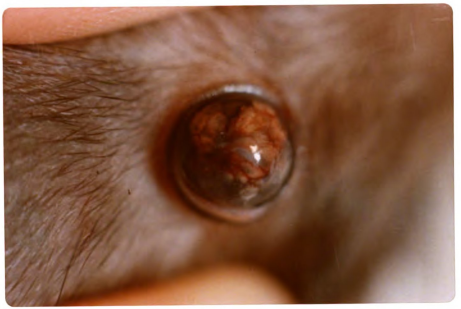

Figure 5. Two ovaries from a 21-day-old immature rat, transplanted into the anterior chamber of the eye of an ovariectomized old constant estrus rat.

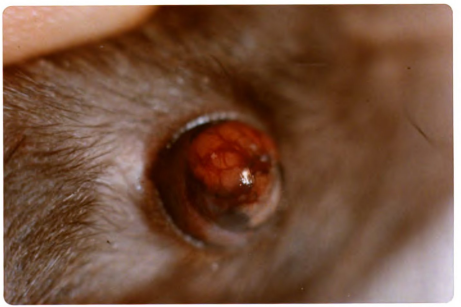

Figure 6. Two ovaries from a 21-day-old immature rat, transplanted into the anterior chamber of the eye of an ovariectomized old constant estrous rat, and induced to ovulate. Shows formation of corpora lutea by daily injection of progesterone.

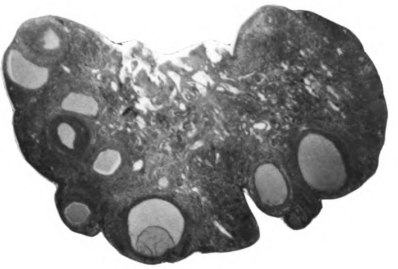

Figure 7. Ovary from control constant estrous rat with well-developed follicles but no corpora lutea. x 6.8.

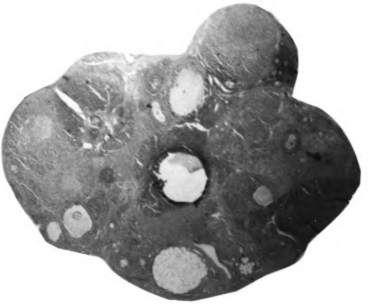

Figure 8. Ovary from old constant estrous rat transplanted into the kidney capsule of an ovariectomized young mature rat. Shows corpora lutea. x 6.8.

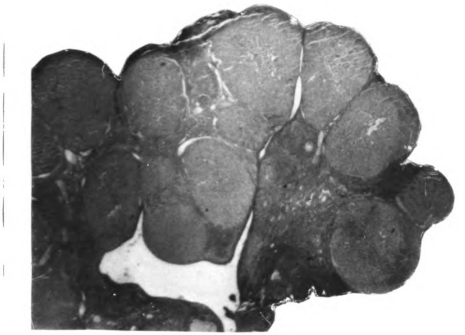

Figure 9. Ovary from pseudopregnant rat showing numerous large corpora lutea. x 6.8.

Figure 10. Ovary from pseudopregnant rat transplanted into kidney capsule of ovariectomized young mature rat. x 6.8.

Figure 11. Atrophic ovary from anestrous rat with no follicular or luteal elements. x 6.8.

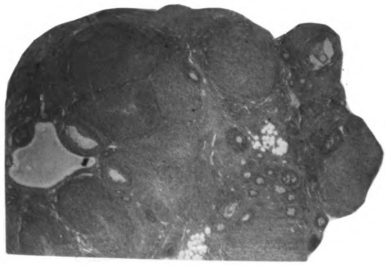

Figure 12. Ovary from old anestrous rat transplanted into kidney capsule of ovariectomized young mature rat. Shows large follicle and corpora lutea. x 6.8.

IV. Effects of Partial Ovariectomy in Pre-pubertal Rats on Subsequent Estrous Cycles

A. Objectives

When ovaries of young cycling rats were replaced with ovaries from 22-30 month old female rats, the vaginal patterns became irregular or showed constant estrus in most animals. These results do not completely agree with previous reports by Aschheim (1964-65) and by Peng and Huang (1972) who observed mainly normal cycles. The purpose of the present study was to observe how amount of ovarian tissue present influences vaginal cycles with advance of age.

B. Materials and Methods

1. Effects of Partial Ovariectomy on Estrous Cycles

Immature rats, 21-25 days old, were laparotomized and part of the ovaries were removed. In the first group 1 ovary was removed, in the second group 1½, in the third group 1 3/4, and in the fourth group 1 7/8 ovaries, respectively. Intact immature rats of the same age were used for controls. About 1 month after surgery, vaginal smears were monitored in these 5 groups of rats for 6 months.

2. Effects of Progesterone on Estrous Cycles in Partially Ovariectomized Rats

When 4 month old, the estrous cycles of partially ovariectomized rats began to become irregular or showed constant estrus. Rats which showed constant estrus were injected with 1 mg progesterone per rat per day at 1000 h. Vaginal smears were monitored for 15 days after progesterone administration was terminated.

3. Replacement of Ovaries from Partially Ovariectomized Rats with Ovaries from Immature Rats

Partially ovariectomized rats were completely ovariectomized when

they showed constant estrus. At the same time, the rats were transplanted with 2 ovaries from 21 day old immature rats into the kidney capsules. At the end of the experiment, the animals were killed. The transplanted ovaries were removed, weighed, and fixed in Bouin's fluid for histological examination. Controls were of the same age as in the intact rats.

4. LH Feedback in Partially Ovariectomized Rats

When the rats with 1 7/8 ovarian tissue removed were 2 months old, estrous cycles were regular. During this period, 1 ml blood was sequentially taken from each rat at 1000 h on the days of diestrous, at 1500, 1700, 1900 h on proestrous day, and at 1000 h on estrous days. Four months later, the rats were completely ovariectomized after they had progressed to constant estrus. Two weeks after the operation, each rat was given a single sc injection of estradiol benzoate (Nutritional Biochemical Co., Cleveland, OH) 10 ug/100 g body weight at 1000 h. Seventy-two hours later, they received an injection of progesterone (Searle Chemicals Inc., Chicago, Ill.), 0.5 mg/100 g body weight. Controls were given the same amount of corn oil. A volume of 1 ml blood was collected at 1200, 1600, and 1800 h on the day of progesterone administration. Blood samples also were taken at 1200 h at 7, 14 and 28 days after injection of progesterone. Controls were of the same age as the intact rats.

C. Results

1. Effects of Partial Ovariectomy on Estrous Cycle (Table 11)

The partially ovariectomized rats had 4 or 5 day estrous cycles when they were 2 or 3 months old. However, half of the rats with 1 7/8 ovaries removed progressed to irregular cycles or constant

estrus when they were 4 months old. No regular estrous cycles were found when the rats were 5 months old. And the same ages, intact and hemiovariectomized rats still showed regular cycles. Five of 12 rats with 1 3/4 ovaries removed at 4 months showed abnormal estrous cycles, 9 of 12 at 5 months, 11 of 12 at 6 months, and all at 7 months. Four of 12 rats with $1\frac{1}{2}$ ovaries removed exhibited abnormal estrous cycles when they were 5 months old, 8 of 12 at 6 months, and 10 of 12 at 7 months. Only 5 of 12 hemi-ovariectomized rats showed regular cycles when they were 7 months old. At the same ages, only 1 of 12 intact rats showed irregular cycles.

These results suggest that when ovarian tissue is removed, and the amount of functional tissue is reduced, the estrous cycles of rats progress to irregular cycles or to constant estrus at an earlier age.

2. Effects of Progesterone on Vaginal Patterns of Partially Ovariectomized Rats (Table 12)

Nine of 10 partially ovariectomized rats which exhibited constant estrus showed 4 or 5 day cycles as a result of injection of progesterone. After progesterone administration was terminated 8 of 10 rats returned to constant estrus and 2 animals showed irregular cycles.

3. Appearance of Ovaries of Partially Ovariectomized Rats Given 2 Ovaries from 21 Day Old Rats (Table 13)

Nine of 10 old rats which had constant estrus were restored to 4 or 5 day cycles by given them 2 ovaries from young rats. One showed pseudopregnancy. At the end of the experiment, all 10 rats had corpora lutea in the transplanted ovaries and ovarian weight was significantly heavier than the original weight of the recipient ovaries (77.6 $\pm$ 7.0 vs. 18.6 $\pm$ 0.6 mg; 77.6 $\pm$ 7.0 vs. 36.0 $\pm$ 6.4 mg). The histological features of ovaries of partially ovariectomized rats are shown in

Figures 13 and 14.

4. Serum LH Values of Partially Ovariectomized Rats Showing Regular Cycles (Figure 15)

There were no statistical differences in basal and peak values of LH as compared to the same age of intact cycling rats. The release of LH in response to ovarian steroid administration in partially ovariectomized rats after they had progressed to constant estrus are shown in Figures 16 and 17. The surge of LH in the partially ovariectomized rats treated with estradiol benzoate or estradiol benzoate followed by progesterone were the same as those of intact controls (Figures 16 and 17). After termination of ovarian steroid administration, the rise of LH in the partially ovariectomized was as high as in intact controls (Figure 18).

D. Discussion

These results demonstrate that partial ovariectomy of prepubertal rats still resulted in regular estrous cycles when the animals were 2 to 3 months old. Thereafter, rats with more ovarian tissue removed exhibited earlier irregular cycles or constant estrus. Since partial ovariectomy reduced the total number of oocytes in the ovaries with advance of age, the ovaries of partially ovariectomized rats could not function normally as long as those of intact rats. Thus, the partially ovariectomized rats exhibited abnormal vaginal patterns earlier than intact rats. Normal vaginal patterns could be restored in partially ovariectomized rats by replacement with two ovaries from immature rats. This indicates that the total number of oocytes in the ovaries may play an important role in control of estrous cycles. Rats showed irregular cycles or constant estrus when the total number of oocytes in the ovaries were less than a certain number (threshold number). Thus, in

partially ovariectomized rats, the principal mechanism involved in loss of normal cycles lies in the ovaries, whereas in rats with intact ovaries, the principal factor responsible for loss of cycling with aging appears to lie in the hypothalamus. The negative and positive feedback response to ovarian steroids for release of LH in partially ovariectomized rats were not different from those of intact rats of the same age. These findings are not in agreement with the view of Aschheim (1976) who reported that hemi-ovariectomy hastened changes in hypothalamo-pituitary function, and thus led to the deviation of estrous cycles, as in female rats during aging. Abnormalities or cessation of sexual cycles in female rats apparently can occur as a result of disfunction or impairment in any part of the reproductive system. Therefore, it is necessary to observe the function of each component of the reproductive control system in aging rats to determine the causes of reproductive senescence.

Table 11. Effects of Partial Ovariectomy on the Vaginal Patterns of Rats

Group and No. of Rats	Age of Rats					
	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	7 mo.
Intact Controls (12)	RC (12)	RC (12)	RC (12)	RC (12)	RC (12)	RC (11) IC (1)
Hemi-Ovx. (12)	RC (12)	RC (12)	RC (12)	RC (12)	RC (12)	RC (5) IC (6) CVC (1)
1 and ½ Ovx. (12)	RC (12)	RC (12)	RC (12)	RC (8) IC (3) CVC (1)	RC (4) IC (5) CVC (3)	RC (2) IC (5) CVC (5)
1 and 3/4 Ovx. (12)	RC (12)	RC (12)	RC (7) IC (3) CVC (2)	RC (3) IC (4) CVC (5)	RC (1) IC (3) CVC (8)	RC (0) IC (4) CVC (8)
1 and 7/8 Ovx. (12)	RC (12)	RC (10) IC (2)	RC (6) IC (4) CVC (2)	RC (0) IC (6) CVC (6)	RC (0) IC (4) CVC (8)	RC (0) IC (5) CVC (7)

RC = Regular cycle.
CVC = Constant vaginal cornification.
IC = Irregular cycle.
( ) = No. of rat.

Table 12. Effects of Progesterone on the Vaginal Patterns of Partially Ovariectomized Rats

Group, Age, and No. of Rats	Pretreatment State	Response of Treatment	Post-treatment State
1 + 1/2 Ovx. (3) 6-7 mo.	CVC	RC (3)	CVC (2) IC (1)
1 + 3/4 Ovx. (5) 5-6 mo.	CVC	RC (4) IC (1)	CVC (4) IC (1)
1 + 7/8 Ovx. (2) 5-6 mo.	CVC	RC (2)	CVC (2)

CVC = Constant vaginal cornification.
RC = Regular cycle.
IC = Irregular cycle.
( ) = No. of rats.

Table 13. Behavior of Immature Rat Ovaries Transplanted into Partial Ovariectomized Rats

Group	No. of Rats	Age	Estrous Cycles Before Graft	Estrous Cycles After Graft (4-5 Days)	Ovarian Wt. of Immature Rat Before Graft and No. of Rats with CL (mg)	Ovarian Wt. of Immature Rat After Graft and No. of Rats with CL (mg)	Recipient Ovaries: Wt. and No. of Rats with CL (mg)
1 and 3/4 Ovx.	5	5-6 mo.	CE (5)	RC (4) PP (1)	18.6 + 0.6 ($\bar{0}$)	77.6 + 7.0* ($\bar{5}$)	36.0 + 6.4 ($\bar{0}$)
1 and 7/8 Ovx.	5	5-6 mo.	CE (5)	RC (5)	18.5 + 1.0 ($\bar{0}$)	83.6 + 7.6* ($\bar{5}$)	23.2 + 3.0 ($\bar{0}$)

CL = Corpora Lutea.
CE = Constant estrus.
PP = Pseudopregnancy.
( ) = No. of Rats.
* = Significantly different from original and recipient ovaries.

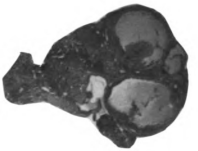

Figure 13. Ovary from partially ovariectomized rat with well developed follicles but no corpora lutea. x 6.8.

Figure 14. Ovary from 21-day-old rat transplanted into the kidney capsule of partially ovariectomized rat. Shows numerous corpora lutea. x 6.8.

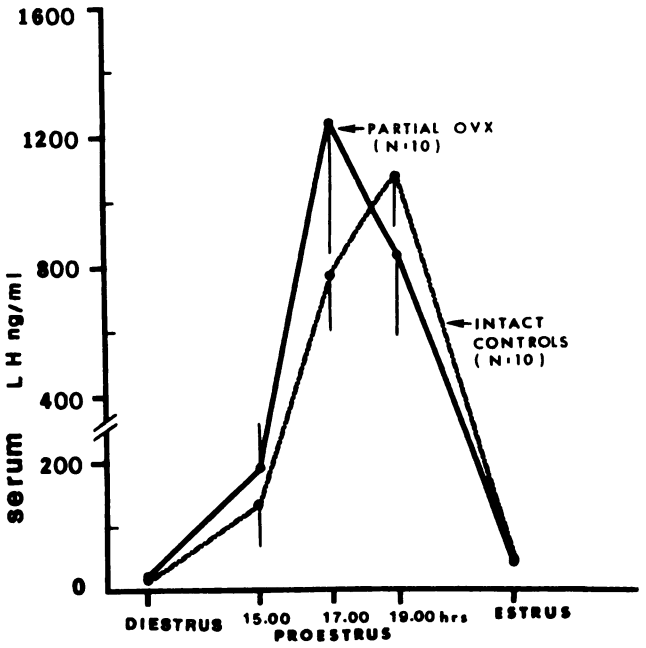

Figure 15. Serum L H in partially ovariectomized rats at 2 months of age.

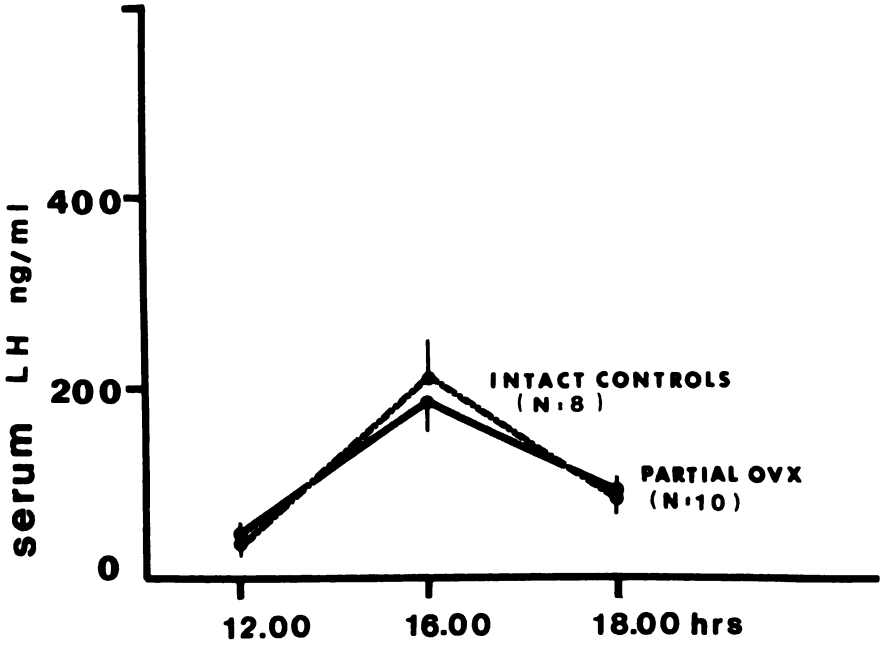

Figure 16. LH surge in ovariectomized rats 3 days following injection of 10 ug/100 g bw of estradiol benzoate. Before ovariectomy, one group of rats was partially ovariectomized and showed constant estrus, the intact control group cycled normally.

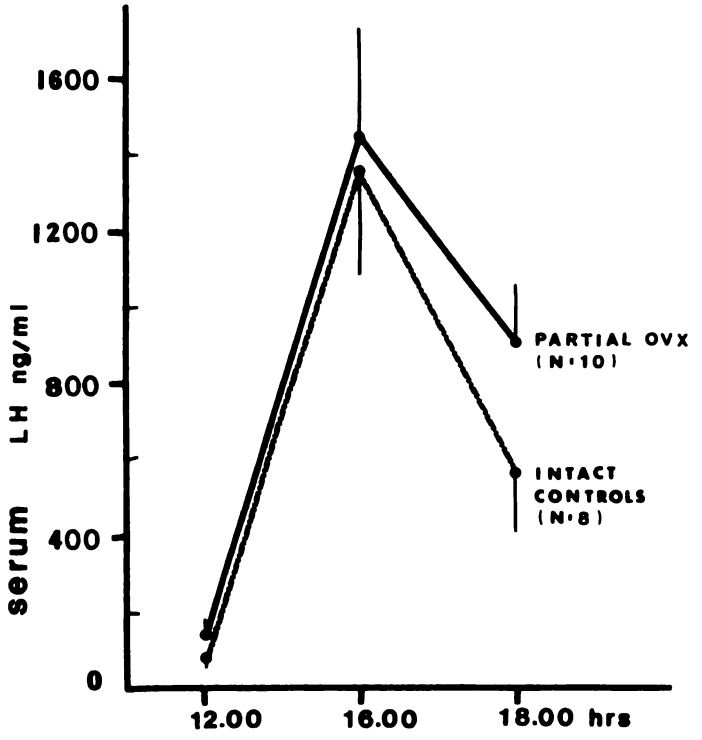

Figure 17. LH surge in ovariectomized rats 3 days after injection of 10 ug/100 g bw of estradiol benzoate followed by an injection of progesterone (0.5 mg/100 g bw). Before ovariectomy one group of rats was partially ovariectomized and showed constant estrus. The intact control group cycled normally.

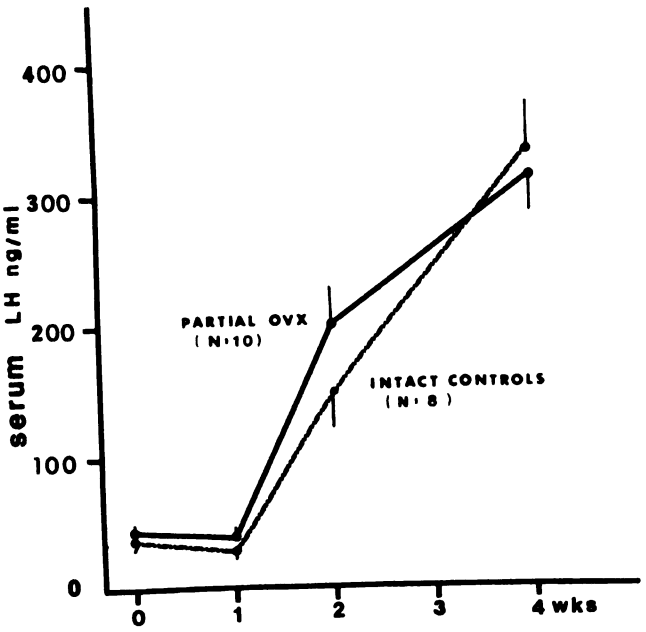

Figure 18. LH levels in ovariectomized rats after withdrawal of ovarian steroid replacement. Before ovariectomy, one group of rats was partially ovariectomized and showed constant estrous, the intact control groups cycled normally.

V. Capacity of Old Versus Young Female Rats to Secrete LH, FSH and Prolactin

A. Objectives.

It was the purpose of the present investigation to measure serum LH, FSH and prolactin in old constant estrous, pseudopregnant, anestrous and young cycling rats; to determine the effects of ovariectomy and estrogen administration on serum levels of these 3 hormones; and to attempt to relate the serum hormone levels to the reproductive patterns exhibited by these rats.

B. Materials and Methods

Twenty two to 24 month old constant estrous, pseudopregnant rats and 26-30 month old anestrous rats were used for this study. Controls were 4 to 5 month old regular cycling female rats.

Two ml of blood were collected between 1200 and 1300 h before bilateral ovariectomy and thereafter at the same time once every week for 7 weeks. Estradiol benzoate (EB) (Nutritional Biochemical Co., Cleveland, OH) was dissolved in corn oil and injected s.c. at a dose of 0.5 ug/100 g bodyweight between 1000 and 1100 h daily beginning on day 1 of the eighth week and continuing for 8 days. Two ml of blood were collected 2-3 h after EB injection on days 2. 5. 8, and at 1 and 2 weeks after steroid treatment was terminated.

C. Results

Serum LH

Figure 19 shows that in intact young rats serum LH was significantly higher on the day of estrus than on diestrous day 2 (34.8 $\pm$ 4.0 ng/ml

vs. 20.7 ± 3.2 ng / ml). By 7 weeks after ovariectomy, serum LH increased to 736.1 ± 14.4 ng / ml. After daily injection of estrogen for 8 days, serum LH fell to 84.2 ± 4.2 ng / ml. Intact old constant estrous rats had significantly higher serum LH (52.4 ± 6.3 ng/ml) than young rats on the day of estrus, and intact old pseudopregnant rats had about the same serum LH (20.8 ± 2.7 ng / ml) as young rats in diestrus. After ovariectomy serum LH increased significantly in both old constant estrous and pseudopregnant rats to 169.3 ± 18.4 ng / ml and 291.3 ± 32.4 ng / ml, respectively, but these increases were significantly less than in the young rats. After estrogen injections the fall in serum LH in both groups of old rats was significantly less than in the young rats, declining to 40.5 ± 7.6 ng / ml in the old constant estrous rats and to 49.2 ± 2.9 ng / ml in the old pseudopregnant rats. Two weeks after termination of EB treatment, serum LH increased from 84.2 ± 4.2 to 225.1 ± 14.5 ng / ml in the young controls, but only from 40.5 ± 7.6 ng / ml to 66.1 ± 9.0 ng / ml in the old constant estrous rats and from 49.2 ± 2.9 ng / ml to 69.5 ng / ml in the old pseudopregnant rats. in the old anestrous rats; serum LH was not detectable (less than 3 ng / ml) under any condition of treatment, and remained undetectable after ovariectomy or estrogen treatment.

Serum FSH

Figure. 20. shows that in intact young rats serum FSH was significantly higher on the day of estrus than during diestrus (124.0 ± 11.5 ng / ml vs. 26.5 ± 3.9 ng / ml). By seven weeks after ovariectomy, serum FSH increased to 485.9 ± 18.8 ng / ml in the young rats, and 8 days after daily injection of EB serum fell to 140.3 ± 12.3 ng / ml. Intact old constant estrous and pseudopregnant rats showed about the same serum

FSH values (121.5 ± 8.1 ng / ml and 85.7 ± 25.7 ng / ml, respectively) as in intact young estrous rats, but both old groups exhibited a signnificantly smaller FSH rise after ovariectomy and a signficantly smaller FSH decline after EB treatment than young rats. Seven weeks after ovariectomy, serum FSH in the old constant estrous rats rose from 121.5 ± 8.1 ng / ml to 250.8 ± 41.5 ng / ml and 8 days after EB treatment fell to 137.0 ± 9.8 ng / ml. In the old pseudopregnant rats, serum FSH rose from 85.7 ± 25.7 ng / ml 300.5 ± 30.4 ng / ml after ovariectomy, and declined to 116.8 ± 13.3 ng / ml after estrogen treatment. After estrogen treatment was terminated, serum FSH rose more quickly and to higher levels in the young rats than in both groups of old rats, increasing from 140 ± 12.3 ng/ml to 776.0 ± 39.4 ng/ml in the young rats and from 137.0 ± 9.8 ng / ml to 234.8 ± 34.0 ng / ml in the old constant estrous rats and from 116.8 ± 13.3 ng / ml to 224.3 ± 32.0 ng / ml in the old pseudopregnant rats. The old anestrous rats showed only a small rise in serum FSH after ovariectomy, from 47.6 ± 9.9 ng / ml to 70.0 ± 2.9 ng/ml, and no change occurred as a result of estrogen treatment or after termination of estrogen treatment.

Serum Prolactin

Fig. 21. shows that serum prolactin trends in the old rats generally were opposite to those of the gonadotropins. Serum prolactin was significantly higher in the constant estrous and anestrous old rats than in the young rats, but the old pseudopregnant rats had values as high as in young rats on the day of estrus. Serum prolactin concentration in the intact young rats was significantly higher during estrus than on diestrus (175.5 ± 27.8 ng/ml vs. 108.7 ± 18.8 ng/ml). By 7 weeks after ovariectomy, serum prolactin of young rats fell to 66.5 ± 9.4 ng / ml,

and after 8 days of estrogen treatment it rose to 338.5 ± 16.8 ng / ml. The intact old constant estrous rats had significantly higher serum prolactin (395.0 ± 34.1 ng / ml) than the intact young rats, and this decreased to 127.6 ± 28.8 ng / ml by 7 weeks after ovariectomy, and increased to 487.7 ± 56.1 ng / ml after 8 days of estrogen treatment. The intact old pseudopregnant rats had about the same serum prolactin values (188.4 ± 42.7 ng / ml) as young estrous rats, showed only a slight but non-significant decline to 165.0 ± 25.0 ng / ml by 7 weeks after ovariectomy, and exhibited a significant rise to 665.2 ± 55.4 ng / ml after 8 days of estrogen treatment. The intact old anestrous rats showed much greater serum prolactin levels (807.8 ± 135.5 ng / ml) than any of the other groups, and no fall in serum prolactin after ovariectomy. Estrogen administration increased serum prolactin to 1169.0 ± 51.7 ng / ml. One week after termination of estrogen treatment, serum prolactin values in the young and old anestrous rats had returned to ovariectomy levels, but in the old constant estrous and pseudopregnant rats serum prolactin remained elevated.

D. Discussion

The present study indicates the presence of different patterns of secretion of LH, FSH and prolactin in old non-cyclic female as compared to young cycling female rats. It is clear that the capacity to increase FSH and LH release in response to ovariectomy is significantly reduced in all categories of old female rats, whereas the ability to secrete prolactin is enhanced. These results are in agreement with the recent report on the ability of old female rats to secrete LH and prolactin as compared to young female rats (Shaar *et al.*, 1975), but extend our ob-

servations to show the relationship of FSH, LH and prolactin to each category of old rats. Ovariectomy of old constant estrous and pseudopregnant rats resulted in a much smaller rise in serum FSH and LH than in young rats, and the old anestrous rats showed only a small rise in serum FSH and no rise in serum LH. Estrogen administration after ovariectomy also produced a relatively smaller decrease in serum FSH and LH in the old constant estrous and pseudopregnant rats than in the young rats, and had no effect on FSH or LH in the old anestrous rats. Cessation of estrogen treatment also elicited only a relatively minor rise in serum FSH and LH in the old constant estrous and pseudopregnant rats as compared to the young rats, and no rise at all in the old anestrous rats.

Serum prolactin was significantly higher in the old as compared to the young rats, and in the old pseudopregnant rats was as high as in young rats on the day of estrus. This agrees with previous reports of high pituitary and serum prolactin levels in old as compared to young rats (Clemens and Meites, 1971, Shaar et al., 1975). The extraordinary high levels of serum of prolactin in the old anestrous rats have not been reported previously. These rats all had pituitary tumors, as observed by us previously in old anestrous rats (Huang and Meites, 1975), and apparently are similar to prolactin secreting pituitary tumors observed in other strains of old rats (Ito et al., 1972). The higher pituitary and serum levels of prolactin observed in the present and earlier studies by us in old rats is believed to account at least in part for the increased incidence of spontaneous mammary tumors in old female rats (Meites et al., 1972).

The failure of old female rats to cycle is believed to be associated with an altered capacity of the hypothalamopituitary system to

secrete gonadotropins in response to physiological stimuli. Thus the constant estrous syndrome in old rats may be due, at least in part to a deficiency in the LH release mechanism as indicated by the significantly reduced capacity to release LH after ovariectomy when compared to young female rats. It has been reported that old constant estrous rats do not respond to cervical stimulation with induction of pseudopregnancy (Clemens et al., 1969) as do cycling rats on the day of estrus. The ovaries of old constant estrous rats also do not contain corpora lutea (Clemens and Meites, 1971; Huang and Meites, 1975) and probably are deficient in progesterone secretion. We recently found that administration of small doses of progesterone to such rats induces regular cycling (Huang et al., 1976a).

The old pseudopregnant rats appear to have relatively greater capacity to increase release of LH and FSH after ovariectomy than old constant estrous rats, which may explain the occasional ovulations observed in these rats. The sustained high serum prolactin levels in these old rats could account for the maintenance of the corpora lutea during each pseudopregnancy period. The anestrous state observed in the oldest female rats apparently results from the inability of the pituitary tumors to secrete sufficient gonadotropins. The ovaries of these anestrous rats were atrophic, contained only few small follicles, and apparently secreted little or no estrogen as indicated by the presence of an infantile uterus, in agreement with our earlier report (Huang and Meites, 1975). The increase in serum prolactin as a result of estrogen administration to these anestrous rats probably is due to the direct stimulatory action of estrogen on pituitary prolactin secretion (Meites et al., 1972).

We previously have expressed the opinion that major charges occur in the hypothalamo-pituitary system of aging female rats (Clemens et al., 1973 Huang et al., 1975), and have provided some evidence to substantiate this view. Thus we reported that old constant estrous rats can be induced to ovulated by eletrochemical stimulation of the preoptic area or by injections of progesterone or epinephrine (Clemens et al., 1969; Wuttke and Meites, 1973). Regular cycles were induced in old constant estrous rats by daily injection of progesterone or ACTH (Huang et al., 1976a) and mainly irregular cycles by daily injection of epinephrine (Clemens et al., 1969), L-dopa, iproniazid or ether stress (Quadri et al., 1973; Huang et al., 1976a). We have suggested that old female rats may be deficient in hypothalamic catecholamines (Clemens et al., 1969; Clemens and Meites, 1971; Quadri et al., 1973), and preliminary evidence indicates that old rats have less norepinephrine and dopamine in the hypothalamus than young mature rats (Simpkins et al., 1976). Finch (1973) also reported that old male mice have lower hypothalamic norepinephrine and dopamine uptake than young mature mice. Hypothalamic catecholamines, particularly norepinephrine, have been strongly implicated in the release of gonadotropins (Sawyer, 1975), whereas dopamine has been shown to inhibit prolactin release (Meites et al., 1972). It also is possible that the hypothalamus of old rats exhibits increased serotonergic activity, as shown recently (Simpkins, et al., 1976). Sero- has been demonstrated to inhibit gonadotropin (Kamberi et al., 1971) and to promote prolactin release (Meites et al., 1972).

Although aging female rats usually cease to cycle, their ovaries remain capable of a considerable degree of function. We have observed that transplantation of ovaries from old constant estrous, pseudopreg-

nant or even anestrous rats to young ovariectomized females results in stimulation of these ovaries and in frequent resumption of cycling (Huang and Meites, Unpublished). It appears that the ovaries of old rats remain capable of nearly normal function throughout their lifespan, and the primary cause of cessation of cycling lies in the hypothlamo-pituitary axis.

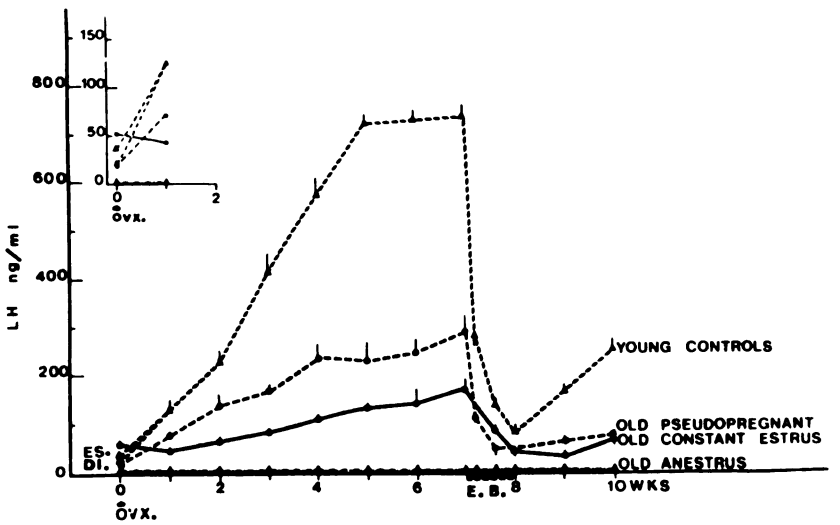

Figure 19. Serum LH in intact rats, during 7 weeks after ovariectomy, during estradiol benzoate (EB) treatment for 8 days, and during 2 weeks post-EB treatment. Groups consisted of old constant estrous (N = 8), old pseudo-pregnant (N = 6), old anestrous (N = 6) and young control rats (N = 12). In young intact rats serum LH levels were measured during estrus and diestrus (N = 6). ES = Estrus, DI = Diestrus, OVX = Ovariectomized, WKS = Weeks.

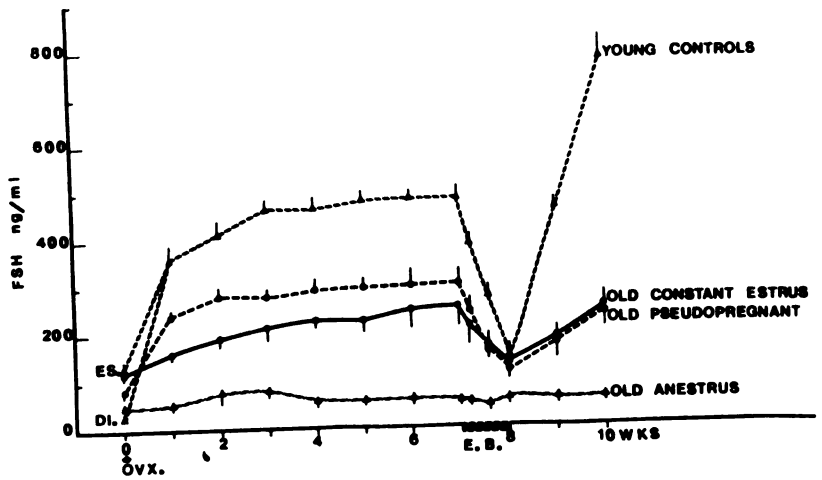

Figure 20. Serum FSH in blood samples collected from same rats as in Figure 19.

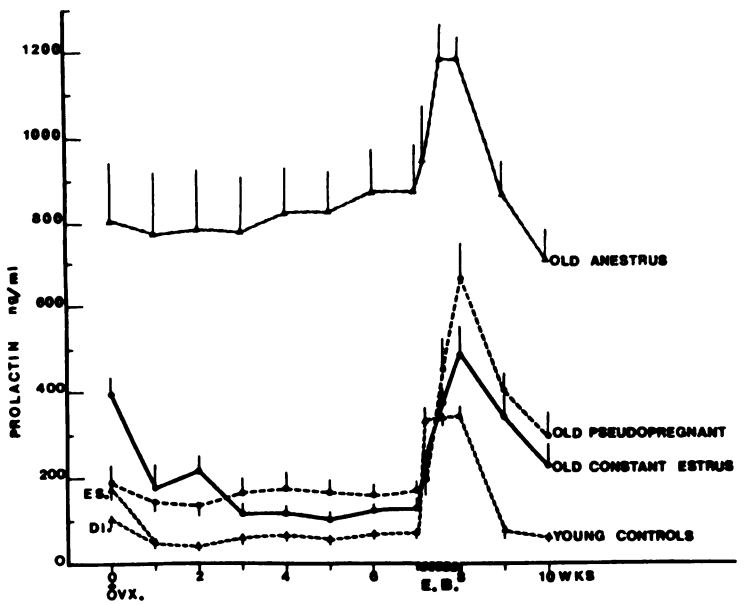

Figure 21. Serum prolactin in blood samples collected from same rats as in Figure 19.

VI. Positive Feedback of Ovarian Steroids on LH and FSH Release in Old and Young Female Rats.

A. Objectives

Old constant rats can be induced to ovulate and cycle regularly by daily injections of progesterone or ACTH (Huang et al., 1976a) but not by injection of estrogen (Huang and Meites, unpublished observation). Since estrogen, either alone or in combination with progesterone, can exert a positive feedback in many species and is now accepted as an essential part of the process leading to ovulation in spontaneously ovulating mammals (Schwartz 1969; Caligaris, et al., 1971 a,b; Brown-Grant and Naftolin, 1972), it was of interest to compare the ability of ovarian steroids to exert a positive feedback on LH and FSH release in old constant estrous and young rats after ovariectomy.

B. Materials and Methods

Sixteen to 18 month old constant estrous rats were used in this experiment and 4 month old rats of the same strain with 4 days estrous cycles were used as controls.

1. Serum LH and FSH in Intact Old Constant Estrous and Young Rats.

Serum LH and FSH from 8 intact old constant estrous rats were first measured in sequential blood samples (1.2 ml each) collected at 1000, 1700, 2400 h on the first day, at 1000 h on the second day and the third day by orbital sinus puncture under light ether anethesia. Blood was similarly collected from 8, 4-day cycling young rats at the same time periods on proestrus, estrus and diestrus.

2. Effect of Estradiol Benzoate on Serum LH in Old Constant Estrous

and Young Rats 2 weeks after Ovariectomy.

Both old constant estrous and young cycling rats were bilaterally ovariectomized. Two weeks after ovariectomy, each rat was given a single s.c. injection of 10 ug estradiol benzoate (Nutritional Biochemical Cl., Cleveland, Ohio) in 0.1 ml corn oil per 100 g body weight at 0900 h. Blood samples were collected at 0900, 1300, 1700, 2400 h on day 3 after estradiol benzoate administration and in the same rats at 1300 h on day 4. A volume of 0.1 ml corn oil per 100 g body weight was given to each rat at 1000 h on day 3 after estradiol benzoate injection.

3. Effect of Progesterone on Serum LH and FSH in Estrogen-Primed Ovariectomized Old and Young Rats.

Old and young ovariectomized rats were first treated with estradiol benzoate (10 ug per 100 g body weight) and this was followed 3 days later by a single s.c. injection of 0.5 mg progesterone (Searle Chemicals, Inc., Chicago, Ill) in 0.1 ml corn oil per 100 g body weight at 1000 h. Blood samples were collected at 0900, 1300, 1700, 2400 h on day 3 after estradiol benzoate administration and in the same rats at 1300 h on day 4.

C. Results

1. Serum LH and FSH in Intact Old Constant Estrous and Young Rats.

Serum LH and FSH values on proestrus, estrus and diestrus in young intact rats and during a similar time course in old constant estrous rats are shown in Fig. 22. In the young rats LH rose from 43 ± 4 ng / ml at 1000 on proestrus to a peak of 590 ± 159 ng / ml at 1700 h on proestrus,

then declined to basal levels (64 ± 8 ng / ml) at midnight on proestrus.. None of the 8 old constant estrous rats exhibited surges of LH In young rats, FSH increased gradually from 43 ± 5 ng / ml at 1000 h on estrus (134 ± 25 and 139 ± 20 ng / ml respectively); thereafter FSH fell to basal levels (39 ± 6 ng / ml)at 1000 h on diestrus. The old constant estrous rats did not show any FSH surges. However, they had higher basal levels than young rats (83 ± 5 vs. 43 ± 5 ng/ml). No corpora lutea were found in the ovaries of the old rats, but they had mature follicles.

2. Effect of Estradiol Benzoate on Serum LH and FSH in Old Constant Estrous and Young Rats 2 Weeks after Ovariectomy

Fig. 23. shows the serum LH and FSH values in young and old rats given a single s.c. injection of estradiol benzoate 2 weeks after ovariectomy. All 8 young rats exhibited surges of LH at 1700 h on day 3 after estrogen administration. No surges of LH were observed in any of the 8 old rats. In the young rats LH rose from basal levels (105 ± 12 ng / ml) at 0900 h to peak values (288 ± 46 ng / ml) at 1500 h, and then fell to 140 ± 26 ng / ml at 2400 h. Since no surges of LH occurred in the old rats, LH values did not show great fluctuation. Interestingly, both young and old rats exhibited surges of FSH on day 3 after estrogen injection. FSH increased from 457 ± 47 ng / ml at 0900 h to peak values (605 ± 40 ng / ml) at 1700 h and (545 ± 44 ng / ml) at 2400 h, and then declined to 346 ± 33 ng / ml at 1300 h on day 4 in the young rats. In the old rats, FSH rose from 312 ± 19 ng / ml at 0900 to peak values (433 ± 20 ng / ml at 1700 h and (415 ± 17 ng / ml) at 2400 h, and thereafter dropped to 262 ± 17 ng / ml at 1300 h on day 4. As compared with young rats, both the basal levels and peak values of FSH in the old rats were

were significantly lower than in the young rats.

3. Effect of Progesterone on Serum LH and FSH in Estrogen-Primed Ovariectomized Old and Young Rats.

The effects of estradiol benzoate followed by progesterone treatment on serum LH and FSH in ovariectomized young and old rats are shown in Fig. 24. Both young and old rats consistently exhibited surges of LH and FSH which appeared at 1700 h on day 3 after estrogen priming. Serum LH in the young rats rose from 107 ± 11 ng/ml at 0900 h to a peak of 945 ± 104 ng / ml at 1700 h and fell to 85 ± 9 ng / ml at 2400 h. However, serum LH in the old rats showed much smaller rises than in the young rats, increasing from 58 ± 7 ng / ml at 0900 h to a peak of 320 ± 57 ng / ml at 1500 h. Thereafter there was a decline to 84 ± 25 ng / ml at 2400 h. The basal serum LH values in the young rats was 2 fold greater than in the old rats, but the peak of LH in the young rats was about 3 times greater than in the old rats. Serum LH increased about 9-fold in the young rats, but only about 5-fold in the old rats. Serum FSH in the young rats increased from 414 ± 42 ng / ml at 0900 to a peak of 822 ± 74 ng / ml at 1700 h and fell to 372 ± 25 ng / ml at 1300 h on day 4. Serum FSH in the old rats rose from 309 ± 32 ng / ml at 0900 to a peak of 577 ± 51 ng/ml at 1700 h and declined to the basal levels (225 ± 23 ng / ml) at 1300 h on day 4. Both of the basal levels and peak levels in old the rats were significantly lower than in the young rats. However, serum FSH increased about 2 fold in both young and old rats.

D. Discussion

The present results support our previous hypothesis that the failure of old constant estrous rats to cycle may be due mainly to an altered capacity of the hypothalamo-pituitary system to release gonadotropins in

response to normal physiological stimuli (Huang et al., 1976b). No surges of LH and FSH, and no ovulations were observed in intact old constant estrous rats. However, many mature follicles were found in their ovaries. This may be due to the high levels of FSH in these rats. Old constant estrous rats were induced to ovulate by injection of LH (Aschheim, 1965) or synthetic gonadotropin realeasing hormone (GnRH) (Huang and Meites, unpublished), or by electrical stimulation of the preoptic hypothalamic area (Clemens, et al., 1969). This suggests that the constant estrous state in old rats may be due in part to a faulty mechanism in the hypothalamo-pituitary system.

The preovulatory surge of gonadotropins that occurs on the afternoon and evening of proestrus in rats has been shown to be preceded by an elevation of estrogen (Schwartz, 1964; Callantine et al., 1966; Shirley, et al., 1968; Labhsetwar, 1970). Prevention of the estrogen rise or inhibition of estrogen action have been shown to inhibit the proestrous LH surge and prevent ovulation. A regimen of estrogen to ovariectomized young rats resulted in daily surges of for 3 to 4 days (Caligaris et al., 1971a; Legan et al., 1975). However, the ovariectomized old constand estrous rats in the present study showed no surges of LH and a smaller rise in FSH in response to estrogen administration. This indicates that the sensitivity of the hypothalamopituitary system to the positive feedback from estrogen is significantly reduced during aging. Since old constant estrous rats were still capable of producing FSH surges in response to estrogen injection, this suggests that the rate of the decline in control of FSH release is slower than that of LH in old rats. This also can explain why old constant estrous rats have mature follicles but do not show ovulation. The response of the hypothalamo-pituitary system to estrogen may be directly related to the ability of ovarian

steroids to bind to the anterior hypothalamus. Estrogen-uptake was reported to be significantly reduced in the anterior hypothalamus of old constant estrous rats as compared with that of young rats (Peng and Peng, 1973). This suggests that the progressive decline and ultimate cessation of estrous cycles in aging rats may be related to a gradual reduction in ovarian steroid receptors in the hypothalamus.

The positive feedback action of estrogen was potentiated by a single injection of a small dose of progesterone in both old and young ovariectomized estrogen-primed rates. Both old and young rats showed surges of LH and FSH, and the peak values were much higher than in rats treated with estrogen alone. This can explain why old constant estrous rats can be induced to ovulate and cycle regularly by daily injection of a small dose of progesterone, but not by estrogen.

Clemens et al., (1969)suggested that the failure to ovulate and show estrous cycles in old female rats may be due to a deficiency in hypothalamic catecholamines. Administration of drugs that increase catecholamine were found to induce cycling in old constant estrous rats (Quadri et al., 1973). A small dose of progesterone potentiated the action of estrogen. It may be that progesterone increase estrogen receptors in the anterior hypothalamus which in turn may alter catecholamine turnover. Progesterone alone was ineffective in stimulating gonadotropin release in ovariectomized rats, but a response was observed when it was preceded by an injection of estrogen. This action of progesterone on the gonadotropin surge required the medication of hypothalamic catecholamines (Kalra et al., 1972). Thus the reduced capacity of old constant estrous rats to show a positive gonadotropin response to estrogen and progesterone may be due largely to a reduced uptake by the hypo-

thalamus of estrogen, to a deficiency in hypothalamic catecholamines, and perhaps also to an increase in hypothalamic serotonin.

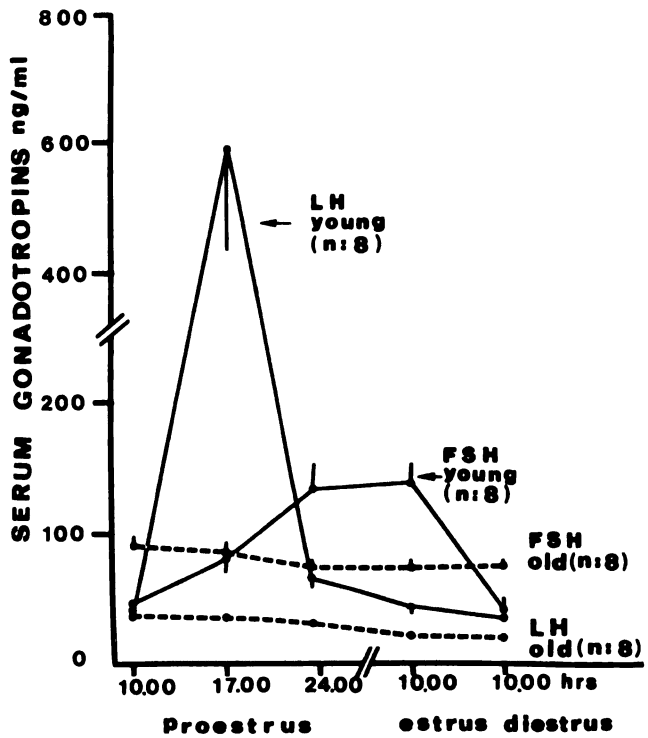

Figure 22. Serum LH and FSH in intact old constant estrous and young cycling rats.

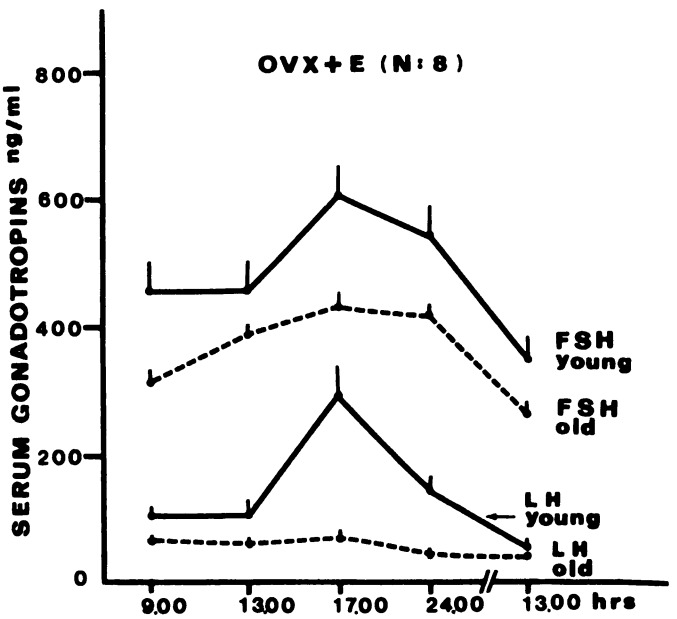

Figure 23. Effects of a single dose of estadiol benzoate (EB) on serum LH and FSH in ovariectomized old and young rats. Ovariectomized rats were injected with 10 ug EB / 100 g body weight, and serum LH and FSH were measured on days 3 and 4 after EB administration.

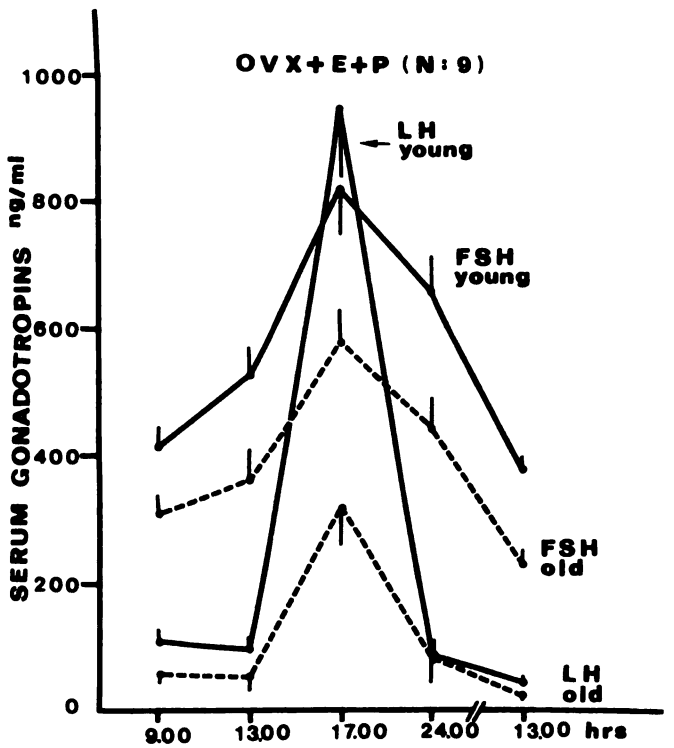

Figure 24. Effects of injection of 0.5 mg / 100 g body weight progesterone into ovariectomized, estrogen-primed old and young rats on serum LH and FSH. Ovariectomized rats were treated with a single dose of estradiol benzoate (10 ug / 100 g body weight) followed 3 days later with a single dose of progesterone, serum LH and FSH were measured on days 3 and 4 after injecting the estrogen priming dose.

VII. Induction of Ovulation by GnRH, and Pituitary Response to GnRH in Old Constant Estrous Rats.

A. Objectives.

Injection of LH has been reported to evoke ovulation in old constant estrous rats (Aschheim, 1965). Hypophysectomized young rats can be made to undergo estrous cycles and fertility by transplanting pituitaries from old rat (Peng and Huang, 1972). Positive feedback action of ovarian steroids on gonadotropin release was much less in old than in young rats. (Lu et al., 1977; Huang and Meites, unpublished). The purpose of the present study was to test the capacity of the pituitary of old constant estrous rats to release gonadotropins and cause ovulation under stimulation of gonadotropin releasing hormone (GnRH).

B. Materials and Methods

1. Induction of LH Release, ovulation and Formation of Corpora Lutea in Old Constant Estrous Rats.

Sixteen to 18 month old constant estrous rats and 4 month old rats with 4 day estrous cycle were used in this study.

(a) Old constant estrous rats were injected subcutaneously 3 times at 30 minute intervals with 150 ng of synthetic GnRH, A 41070, lot # 3549-215 B+C, Abbott laboratories, North Chicago, Ill.) and controls were injected with saline only. At 15 hours after the last GnRH injection, the animals were laparotomized and the oviducts were removed for counting the number of ova.

(b) Similar constant estrous rats were given 9 consecutive s.c. injections of 50 ng GnRH / 100 g body weight at 20 minute intervals

in the first hour, and at 30 minute intervals for the following 3 hours. The time of injection was from 1100 to 1500 h. Controls were treated with saline only. A volume of 1.2 ml of blood was collected for measuring LH at 10 minutes after the third injection, at the end of the first hour, and after the second injection at every hour for 3 hours. Pre-treatment blood was taken 30 minutes before GnRh injections. Two days after the last injection, the ovaries were removed to determine whether fresh corpor lutea were present, and the ovaries were weighed, fixed in Bouin's fluid, and sectioned and stained with eosin and hematoxyline for histological examination.

2. Release of Pituitary LH and FSH in Response to GnRH in Old Constant Estrous and Young Cycling Rats.

Old constant estrous rats and young proestrous and estrous rats were treated with 9 consecutive s.c. injections of GnRH. The dose, and the time courses of injections and blood sampling were the same as in I (b).

3. Pituitary Release of LH and FSH in Response to GnRH in Ovariectomized Ovarian Steroid-Primed Old and Young Rats.

Both old constant estrous and young cycling rats were bilaterally ovariectomized. Two weeks after ovariectomy, each rat was given a single s.c. injection of 10 ug estradiol benzoate (Nutritional Biochemical Co, Ohio) in 0.1 ml corn oil/ 100 g body weight at 1000 h. and this was followed 3 days later by a single s.c. injection of 0.5 mg progesterone (Searle Chemicals. Inc., Chicago Ill) in 0.1 ml corn oil / 100 g body weight at 1000 h. The controls were injected only with the same amount of corn oil. GnRH treatments and blood sampling were the same as in I

(b).

4. Pituitary Release of LH and FSH in Response to GnRH in Vitro in Old Constant Estrous and Young Cycling Rats.

Anterior pituitaries from old constant estrous rats and normal cycling female rats on proestrous and estrous days were used for in vito incubations. The rats were decapitated by guillotine at about 1300 h, and the anterior pituitaries were quickly removed, hemisected, and weighed. Each anterior pituitary was placed in a 5-ml culture tube containing 2 ml of medium-199 at a pH of 7.4. Medium-199 for the incubation was prepared by dissolving 1.10 g of medium-199 in powder form (Difco Labs., Detroit, Mich) and 180 mg of $NaHCO_3$ (J.T. Baker Chemical Co., Phillisburg, NJ) in 100 ml of deionized distilled water. Incubations were carried out in a Dubnoff metabolic incubator (Precision Schientific Co., Chicago, Ill), 60 cycles / min, under constant gassing with 95% O_2 - 5% CO_2 at 37 $\pm$ 0.5° C. After 1 hour pre-incubation, the medium was removed and replaced with 2.0 ml of frech medium-199 containing 60 ng of GnRH or medium-199 alone. One anterior pituitary half from each rat was incubated in medium-199 alone, while the other half was incubated with GnRH in medium-199. The incubated medium was removed from each tube and replaced with fresh medium at hourly intervals for 4 hours. The incubated medium was immediately frozen and stored for hormone assays.

C. Results.

1. Induction of LH Release, Ovulation and Formation of Corpora Lutea in Old Constant Estrous Rats.

Table 14 shows that 10 of 11 rats treated with GnRH ovulated

and produced 8.1 ± 0.8 ova per rat. None of the saline-injected controls ovulated. Serum LH was greatly increased in the rats given GnRH but there was no rise in serum LH in the control rats (Table 15). It also can been seen (Table 14 lower panel) that all 9 GnRH treated rats had ovaries that weighted significantly more than in the controls, due to the presence of corpora lutea (78.0 ± 36 vs. 46.6 ± 3.2 mg). None of the 12 saline-treated old constant estrous rats (controls) showed fresh corpora lutea. By histological examination, large and some cystic follicles were seen in the ovaries of the controls. Many fresh corpora lutea appeared in the ovaries of the GnRH treated rats. No ova were seen trapped in these corpora lutea. This indicated that the formation of these corpora lutea was not due to anovulatory luteinization.

2. Pituitary Release of LH and FSH in Response to Consecutive Injections of GnRH in old Constant Estrous and Young Cycling Rats.

Pituitary release of LH and FSH in response to GnRh are shown Fig. 25 and Fig. 26 (FSH). There was no difference in the pretreatment values of serum LH among the old constant estrous, young estrous and young rats on proestrous morning (1000 to 1100 h), (65 ± 8, 56 ± 4 and 54 ± 7 ng / ml, respectively). Very interestingly, in old constant estrous rats, pituitary release of LH in response to GnRH stimulation was similar to young rats on proestrous stage. However it was more responsive than in young rats during the estrous stage. During the first 2 hours of consecutive injection of GnRH, serum LH in the old constant estrous rats rose from 65 ± 8 ng / ml to a peak of 3934 ± 345 ng / ml and 2 hours later fell to 2782 ± 218 ng / ml. Serum LH increased from

54 ± 7 to a peak of 4200 ± 156 ng / ml and declined to 2517 ± 267 ng / ml 2 hours later in the young proestrous rats, whereas in the young estrous rats serum LH rose from 56 ± 4 ng / ml to a plateau of 1104 ± 120 ng/ml at the second hour during GnRH injections. There was no change in serum LH during continuing injections of GnRH for two more hours. Serum LH in the old constant estrous and young proestrous rats were significantly higher than in the young estrous rats at the corresponding intervals during GnRH treatment.

Serum FSH in the pretreatment old constant estrous rats was significantly higher than in the young rats on proestrous morning (132 ± 10 vs. 30 ± 3 ng / ml), but not different from young estrous rats (148 ± 18 ng / ml). Serum FSH increased progressively in all three groups during 4 hours of consecutive GnRH injections (Fig. 26). In the old constant estrous rats serum FSH rose from 132 ± 10 ng / ml to 798 ± 46 ng/ml, from 30 ± 3 ng / ml to 399 ± 18 ng / ml in the young proestrous rats and from 148 ± 18 ng / ml to 484 ± 51 ng / ml in the young estrous rats respectively at the end of GnRH injections. Thus, the pituitary release of FSH in response to GnRH in old constant estrous rats was significantly higher than in both groups of young rats. However, there was no difference between these two groups of young rats.

3. Pituitary Release of LH and FSH in Response to Consecutive Injections of GnRH in Ovairectomized Ovarian Steroid-Primed Old and Young Rats.

Figs, 27 and 28 show pituitary release of LH and FSH in response to GnRH stimulation in ovariectomized steroid primed rats. There was no significant difference in release of LH and FSH between old and young rats treated with estrogen alone or with estrogen followed by pro-

gesterone at corresponding intervals during GnRH injections. In the old rats treated with estrogen alone, serum LH increased from 122 ± 15 ng / 100 to 3072 ± 324 ng / ml, and FSH from 649 ± 22 ng / ml to 1291 ± 70 ng / ml at the end of the first hour of GnRH injections. In the young controls serum LH increased from 118 ± 19 ng/ml to 5132 ± 205 ng / ml and FSH from 878 ± 53 ng / ml to 2182 ± 361 ng / ml. Thus, at the end of the first hour of GnRH injections the old rats released significantly less LH and FSH than young rats. However, continued injections of GnRH the old rats released as much LH and FSH as in young rats at each corresponding hourly intervals. In the old rats serum LH rose to 6818 ± 558 ng / ml and FSH to 2545 ± 174 ng / ml at the end of the fourth hour of GnRH injections. In the young controls, serum LH increased to 7018 ± 482 ng / ml and FSH to 3052 ± 227 ng / ml.

Pituitary release of LH and FSH in response to GnRH in estrogen - progesterone treated rats were similar to that of rats treated with estrogen alone. At the end of the first hour of GnRH injection serum LH increased from 120 ± 16 ng / ml to 2814 ± 516 ng / ml and FSH from 649 ± 22 ng / ml to 1291 ± 70 ng / ml in the old rats. Serum LH rose from 157 ± 22 ng / ml to 4503 ± 516 ng / ml and FSH from 827 ± 66 ng / ml to 1829 ± 151 ng / ml in the young controls. However, at the end of the fourth hour of GnRH injections serum LH rose to 6676 ± 334 ng / ml and FSH to 2715 ± 133 ng / ml in the old rats. In the young controls serum LH increased to 7138 ± 607 ng / ml and FSH to 3189 ± 160 ng / ml. Thus, the pituitary of estrogenprogesterone treated old rats showed significantly less response than young controls only at the first hour of GnRH stimulation, but no statistical difference during the remainder of time.

4. Pituitary Release of LH and FSH in Response to GnRH in Vitro in Old Constant Estrous and Young Cycling Rats.

Pituitary release of LH in response to GnRH stimulation in vitro is shown in Figure 29 and FSH in Figure 30. There was no statistical difference in the hourly and total amount of LH and FSH released by the medium-199 incubated anterior pituitaries from old constant estrous, young estrous and young proestrous rats. During the first hour o f GnRH incubation, the anterior pituitaries from old constant estrous rats released 8350 ± 791 and 8835 ± 650 LH ng / half pituitary respectively. Thus, the amount of LH release during the first hour incubation in old constant estrous rats was significantly less than in young rats. However, beginning at the second hour and continuing to 4 hours, the amount of LH released by the pituitaries from old constant estrous rats were similar to those of young proestrous rats, but were significantly greater than those of young estrous rats. The total amounts of LH released by the anterior pituitaries incubated with GnRH for four hours in old constant estrous rats and young proestrous rats were significantly greater than in young estrous rats (35.0 ± 2.2, 39.3 ± 1.8 and 28.7 ± 1.8 ug / half pituitary, respectively).

The anterior pituitaries from old constant estrous rats released 558 ± 74 FSH ng / half pituitary after 1 hour incubation, whereas the anterior pituitary from young proestrous and young estrous rats released 362 ± 51 and 843 ± 64 FSH ng / half pituitary, respectively. Thus, during the first hour of incuvation the amount of FSH released by the pituitaries of old rats was significantly greater than that of young proestrous rats, but significantly less than that of young estrous rats. However, beginning at the second hour of incubation and continuing to 4 hours, the amounts of FSH released by the anterior pituitaries from

these three groups of rats showed no statistical difference.

5. Body and Pituitary Weights

Table 16 shows there was no significant difference in body weight between young and old constant estrous rats. However, the pituitaries of the old constant estrous rats were significantly heavier than those of the young rats.

D. Discussion.

These results demonstrate that GnRH administration to old constant estrous rats not only can promote LH and FSH release, but also can elicit ovulation and formation of corpora lutea. These data also demonstrate that multiple injections of GnRH in intact old constant estrous rats can induce as much LH release as in young mature female rats during the proestrous stage and release more FSH than young rats during estrus or proestrus. These results do not agree with those of Watkins et al (1975). They reported that old rats released less LH in response to a single injection of GnRH than young rats. This difference may be due to the different methods of GnRH injections. In vitro, the anterior pituitaries of old constant estrous rats released about the same amounts of LH and FSH as young rats during the proestrous stage. This further indicates that the capacity of the pituitary to secrete and release gonadotropins in the old constant estrous rats does not decline, although such rats exhibit cessation of estrous cycles.

The responsiveness of the pituitary to GnRH appears to be mainly influenced by the ovarian steroids. To prevent fluctuation of ovarian steroids during the estrous cycle, rats were ovariectomized and given exogenous ovarian steroids. Under a constant ovarian steroid dose, the pituitaries of old constant estrous rats released as much LH and FSH as

those of young rats during multiple injections of GnRH. This again demonstrates that the functional capacity of the pituitary of old constant estrous rats is maintained as well as in young rats. This also can indicate that the reduced rise of LH and FSH in the old rats after ovariectomy, with no LH surges or smaller LH surges in the old rats treated with ovarian steroids, is due mainly to a fault in the hypothalamus and not in the pituitary. We have reported that the ovaries of old constant estrous rats contained as many LH and FSH receptor sites as the ovaries of young mature rats (Steger et al., 1976a). The present results provide further evidence that the cessation of estrous cycles in the rat during aging is due primarily to dysfunction of the central nervous system (hypothalamus).

Table 14. Effects of GnRH on Luteinization, Number of Ova and Ovarian Weight in Old CE Rats

Treatment and No. of Rats	No. of Rats with Fresh Corpora Lutea	No. of Ova	(mg) Av. Ovarian Wt.
Controls (10)	(0/10)	0	
GnRH (11)	(10/11)	8.1 ± 0.8	
Controls (12)	(0/12)		46.6 ± 3.2
GnRH (9)	(9/9)		78.0 ± 3.6*

CE = Constant estrus.
± = Means of standard error.
* = Significantly different from controls ($P < .01$).

Table 15. Effects of GnRH on Serum LH in Old CE Rats

Treatment and No. of Rats	Serum LH (ng/ml) Time (hours) -0.5	1	2	3	4
Controls (12)	54 ± 3	47 ± 3	40 ± 3	38 ± 3	36 ± 3
GnRH (9)	65 ± 8	2433 ± 337[a]	3934 ± 345[a,b]	2782 ± 176[a]	2187 ± 174[a,b]

CE = Constant Estrus.

[a] = Significantly different from controls at corresponding time intervals.

[b] = Significantly different from pretreatment period and from 2 hour period.

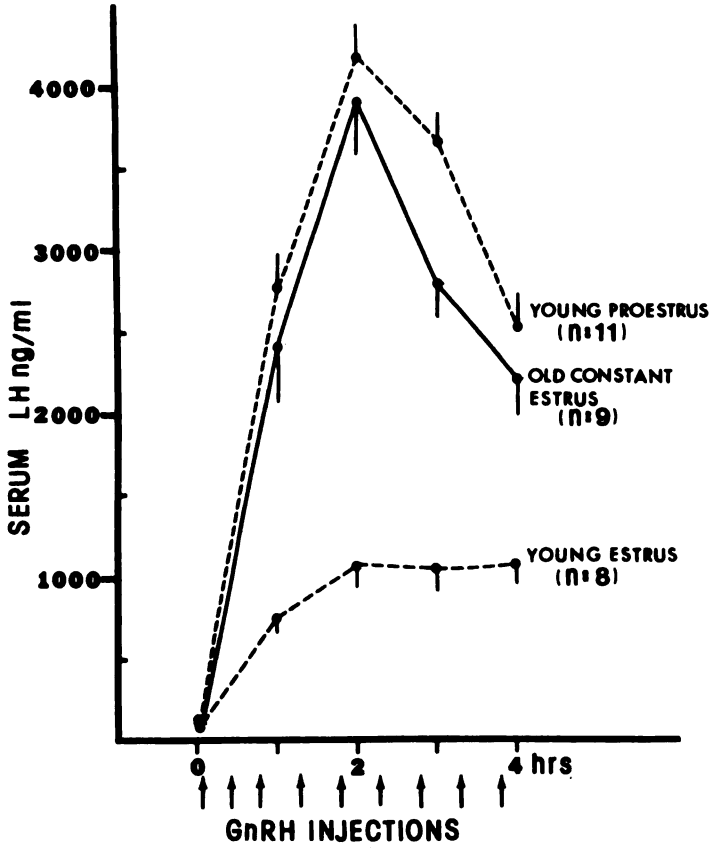

Figure 25. Effects of 9 consecutive injections (Arrows) of GnRH on serum LH in old constant estrous, young proestrous and estrous rats.

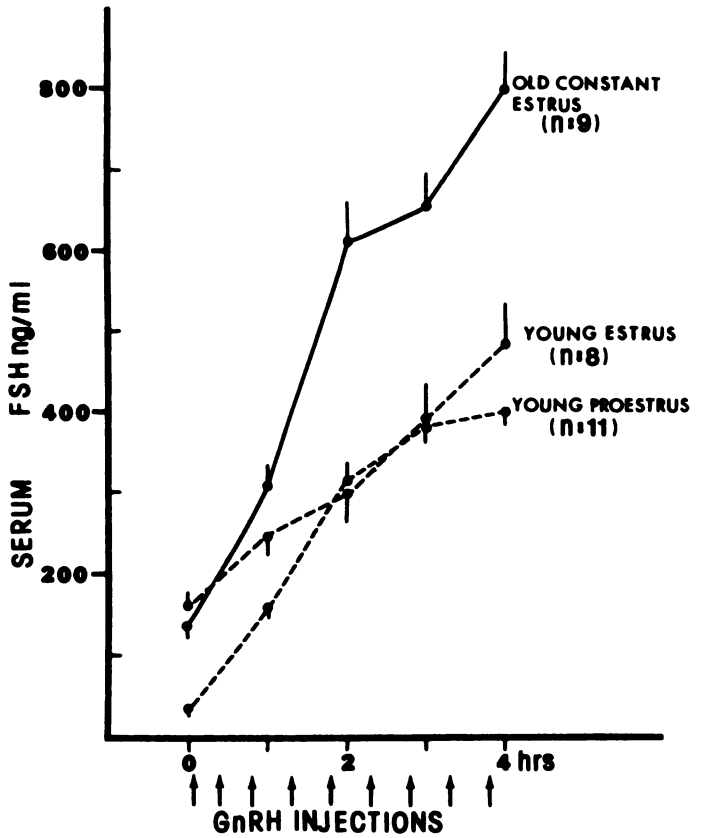

Figure 26. Effects of 9 consecutive injections of GnRH (Arrows) on serum FSH in old constant estrous, young proestrous and estrous rats.

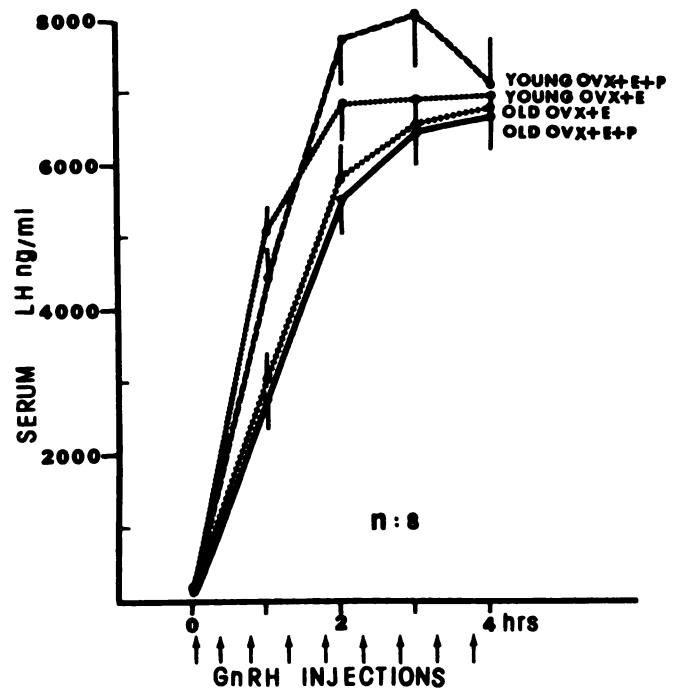

Figure 27. Effects of 9 consecutive injections of (Arrows) on serum LH in estrogen or estrogen-progesterone treated ovariectomized old and young rats.

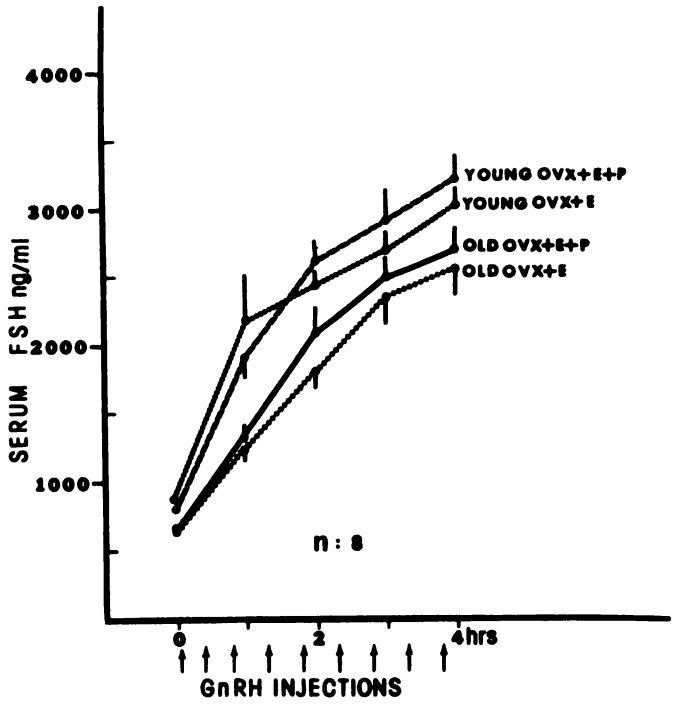

Figure 28. Effects of 9 consecutive injections (Arrows) on serum FSH in estrogen or estrogen-progesterone treated ovariectomized old and young rats.

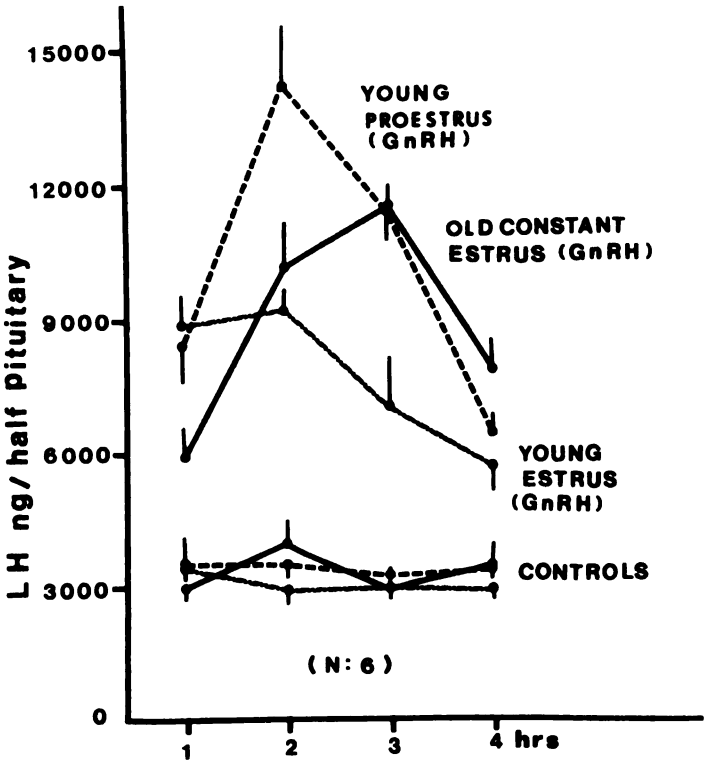

Figure 29. Total LH in incubation medium after 1,2,3 and 4 hours of GnRH incubation with hemi-pituitaries from old constant estrous, young proestrous and estrous rats. Controls were incubated with medium alone.

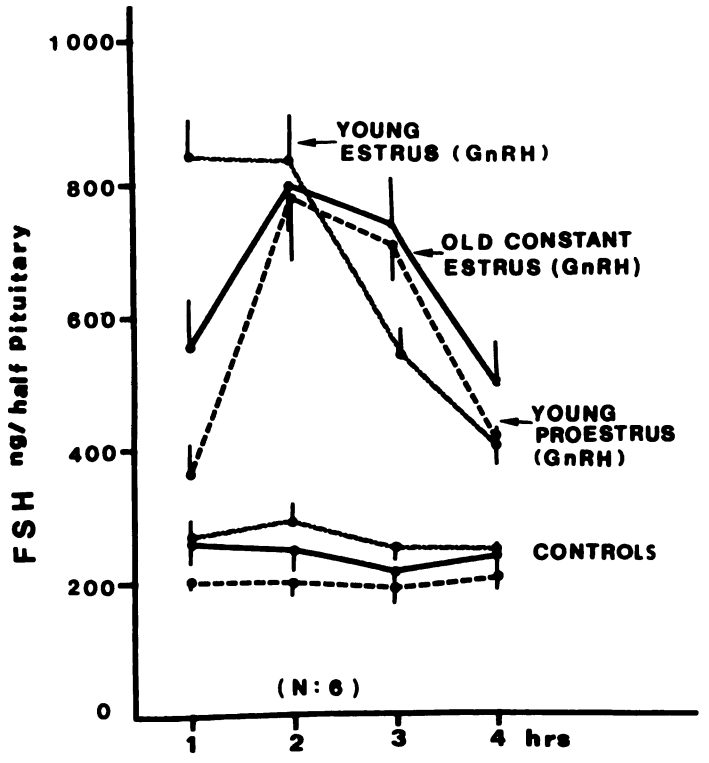

Figure 30. Total FSH in incubation medium after 1,2,3 and 4 hours of GnRH incubation with hemi-pituitaries from old constant estrous, young proestrous and estrous rats. Controls were incubated with medium alone.

Table 16. Body and Pituitary Weights of Young and Old Constant Estrous Rats

Group and No. of Rats	Av. Body Wt., g	Av. Pituitary Wt., mg	Av. Pituitary Wt./ 100 g Body Wt. (mg/100g)
Old Constant Estrous (6)	333 ± 13	18.8 ± 0.9*	5.9 ± 0.2*
Young Proestrous (6)	330 ± 4	13.4 ± 0.7	4.1 ± 0.2
Young Estrous (6)	310 ± 9	14.2 ± 0.2	4.6 ± 0.1

* = $P < 0.05$; Old vs. Young.
± = Standard error of mean.

VIII. Hypothalamic Norepinephrine and Dopamine Turnover and Relation to LH, FSH and Prolactin Release in Intact and Ovariectomized Old Rats.

A. Objectives.

Old female rats show less capacity to secrete LH and FSH and more capacity to secrete prolactin than young cycling female rats. After ovariectomy or estrogen treatments, old female rats release less LH and FSH, but more prolactin than young females (Shaar et al., 1975; Huang et al., 1976b). These findings are believed to largely explain why old female rats exhibit deviations of the estrous cycle.

The influence of catecholamines on control of anterior pituitary function is now well established. We have suggested that changes in anterior pituitary function in old rats may be due to the depressed hypothalamic catecholamine activity (Clemens et al., 1969; Shaar et al., 1975; Huang et al., 1976b). The present study was undertaken to determine whether there are any differences in hypothalamic dopamine and norepinephrine turnover between young and old female rats.

B. Materials and Methods

Eighteen to 20 month old constant estrous rats and 4 to 5 month old rats during the proestrous or estrous stage were used in this study. In experiment 1, animals were injected 1.p. with 250 mg alpha-methyl-para-tyrosine (α- MPT) / kg body weight or its vehicle, 0.9% NaCl at 1330-1400 h. Forty five minutes later, the rats were killed by decapitation and trunk blood was collected for measuring hormones. Brains were quickly removed from the cranium and placed on ice. The anterior

hypothalamus was dissected by making cuts at the hypothalamic sulci, rostral to the optic chiasma, and caudal to the tuber cinereum. The posterior hypothalamus was dissected by making cuts at the hypothalamic sulci, rostral to tuber cinereum, and caudal to the mammillary bodies. The cube produced by cutting a 2-3 mm deep piece of tissue was frozen immediately for dopamine (DA) and norepinephrine (NE) assays.

In experiment 2, rats were bilaterally ovariectomized. Ten days later the animals were sacrified for measuring catecholamines. The treatments and brain removals were same as in experiment 1.

C. Results.

Norepinephrine concentration in the anterior hypothalamus was significantly lower in old than in young rats on proestrous or estrous day (1210 ± 62 vs. 1543 ± 123, 1509 ± 98 ng per gram wet weight, respectively Figure 31). Forty-five minutes after α-MPT injection, NE decreased by 28 ± 3% in old rats, 41 ± 0.3% in young proestrous rats and 42 ± 4% in young estrous rats ($P<0.05$). Figure 31.). Ten days after ovariectomy, NE concentrations were not altered in either old or young rats (1227 ± 143, 1619 ± 59 ng per gram wet weight, respectively Figure 31). However, 45 minutes after α-MPT injection, NE decreased by 53 ± 3% in young rats and 34 ± 3% in old rats (Figure 31). NE turnover was increased significantly after ovariectomy in the anterior hypothalamus of young rats (53 ± 3% vs. 41 ± 3%, 42 ± 4% respectively, $P < 0.05$), but not in old rats (28 ± 3% vs. 34 ± 3%).

The anterior hypothalamic DA concentration and turnover were not significantly different between intact old and young rats (438 ± 42 vs. 370 ± 36; 437 ± 48 ng per gram wet weight; 48 ± 6% vs. 46 ± 6%; 51 ± 3% respectively) (Figure 32). Ten days after ovariectomy, DA concentra-

tion in the anterior hypothalamus was increased similarly in both old and young rats (540 ± 47, 522 ± 37 ng per gram wet weight, respectively. Figure 32). However, DA turnover was increased significantly in young rats (69 ± 4% vs. 46 ± 6%, 51 ± 3%, respectively, $P < 0.05$) but not in old rats (48 ± 1% vs. 48 ± 6%) (Figure 32).

The posterior hypothalamic NE and DA concentrations and turnover were not different between old and young rats, and were not altered by ovariectomy (Figures 33 and 34).

The hormone levels in saline and α-MPT treated young and old rats used in catecholamine study are shown in Figure 35 (LH). Figure 36 (FSH), and Figure 37 (Prolactin). Serum LH, FSH and prolactin were the same in saline treated intact old and young rats. Serum LH and FSH in intact rats were not altered by injection of α-MPT. However, prolactin was greatly increased in both intact old and young rats after α-MPT injection. The prolactin rise in old rats was significantly higher than in young controls (1016 ± 63 vs. 597 ± 38 and 772 ± 74 ng / ml $P < 0.05$).

By 10 days after ovariectomy, serum LH and FSH in old rats was about half of that in young rats (216 ± 30 vs. 455 ± 62; 536 ± 73 vs. 836 ± 59 ng / ml respectively), whereas prolactin was about 2-fold higher than in young rats (109 ± 10 vs. 61 ± 10 ng / ml). After injection of α-MPT, serum LH in old rats fell from 216 ± 30 to 108 ± 18 ng / ml and from 455 ± 62 to 284 ± 52 ng / ml in young rats. However, serum prolactin in old rats rose from 109 ± 10 to 356 ± 66 ng / ml and from 61 ± 10 to 238 ± 33 ng / ml in young rats. Serum FSH in both age groups was not changed by α-MPT.

D. Discussion

These results support our previous hypothesis that the decrease in catecholamine activity in the hypothalamus is associated with a reduction in LH and FSH release and loss of cycling, and with an increase in prolactin release (Clemens et al., 1969; Quadri et al., 1973; Huang and Meites 1975; Huang et al., 1976b). Involvement of catecholamines in the control of gonadotropin release has been well established, although controversy remains as to whether they inhibit or stimulate release of LH and FSH (Sawyer, 1975). Many investigators now agree that NE stimulates LH release and induces ovulation. An increase of NE turnover at proestrus in the adult cycling female rats has been reported by Fuxe et al., in 1976. The effects of DA on release of LH and FSH are not completely understood, and contraditory results have been reported by different workers. However, the inhibitory effects of DA on release of prolactin have been well established (Meites et al., 1977).

Concentration and turnover of NE in the anterior hypothalamus were significantly lower in old constant estrous rats than in young cycling rats, but not in the posterior hypothalamus. An decrease in hypothalamic concentration and turnover of NE in the hypothalamus of old male rats has been reported by Simpkins et al., (1976). In old male mice, Finch (1973) reported a decreased uptake of dopamine by the hypothalamus. The constant estrous state in old rats can be attributed in large part to the failure of the preovulatory LH surge mechanism, suggesting a functional change in the anterior hypothalamus. Reinitiation of regular or irregular cycles was induced in old constant estrous rats by injections of epinephrine, a catecholamine, or by electric stimulation of the anterior hypothalamus (Clemens et al., 1969). or by injections of L-dopa or iproniazid (Quadri et al., 1973; Huang et al.,

1975), drugs that increase brain catecholamines. This study directly proves that the cessation of estrous cycle is mainly due to a deficeency of NE in the anterior hypothalamus, and not due to DA.

Castration of either male or female rats results in increased catecholamine turnover in the hypothalamus whereas administration of gonadal steroids decreases hypothalamic turnover of catecholamines (Fuxe et al., 1976). In the present study, the effects of ovariectomy in young cycling rats are in agreement with previous reports. In old constant estrous rats, DA concentration in the anterior hypothalamus was significantly increased by ovariectomy. However, NE concentration and turnover of NE and DA in the anterior hypothalamus were only slightly increased, and were not statistically different. The post-castration rise of LH and FSH and decline of prolactin were much less in old than in young rats. This may account in large part for the decreased responsiveness and reduced functional capacity of the center in the hypothalamus that regulates feedback control in old rats.

It is possible that other neurotransmitters, such as serotonin, acetylcholine and γ-aminobutyric acid, also change with advance of age and alter the release of anterior pituitary hormones.

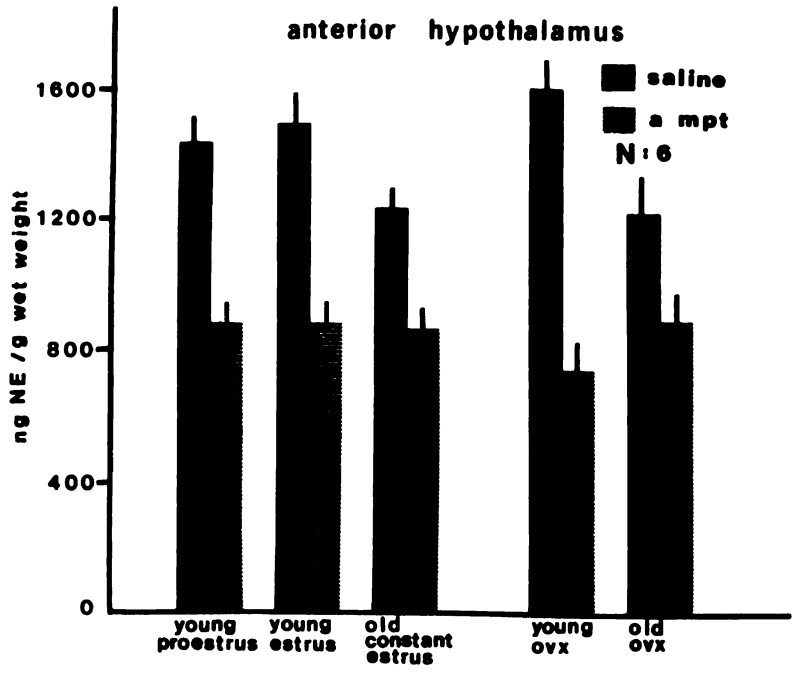

Figure 31. Norepinephrine steady state concentration and concentration after α -MPT treatment in the anterior hypothalamus of intact or ovariectomized old and young rats. Vertical lines indicate standard error of mean.

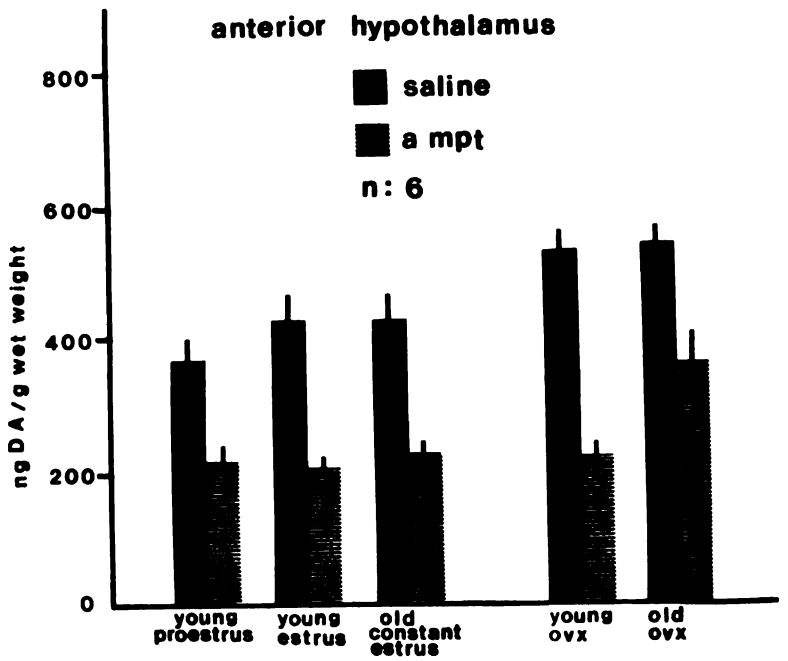

Figure 32. Dopamine steady concentration and concentration after α-MPT treatment in the anterior hypothalamus of intact and of ovariectomized old and young rats. Vertical lines indicate standard error of mean.

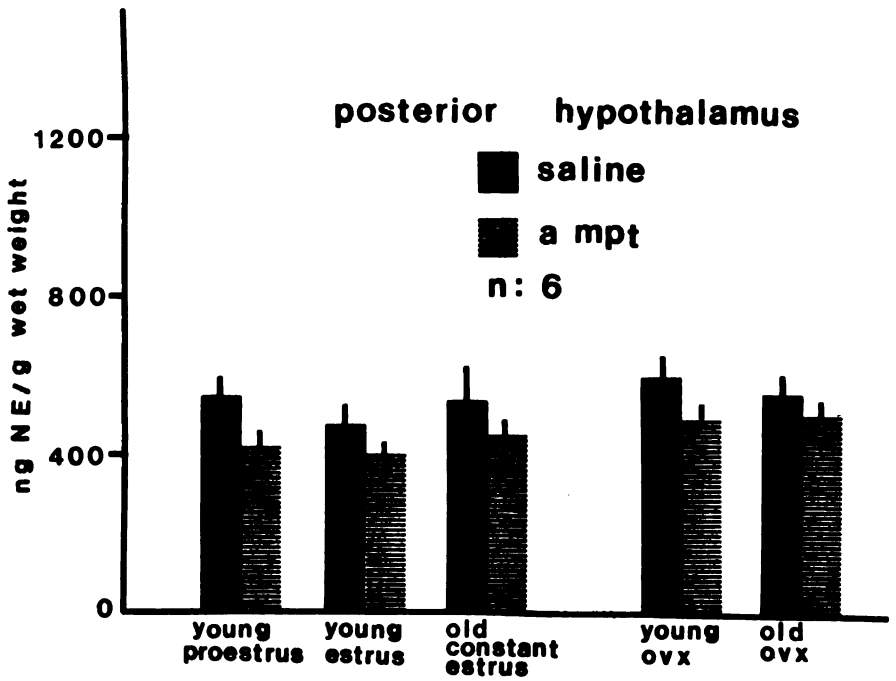

Figure 33. Norepinephrine steady state concentration after α-MPT treatment in the posterior hypothalamus of intact or ovariectomized old and young rats. Vertical lines indicate standard error of mean.

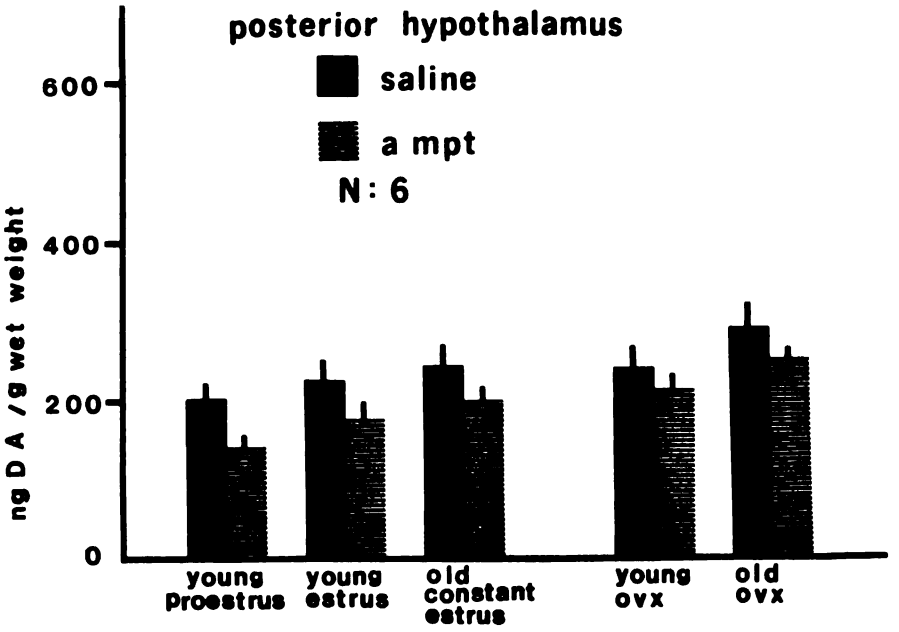

Figure 34. Dopamine steady state concentration and concentration after α-MPT treatment in the posterior hypothalamus of intact or of ovariectomized old and young rats. Vertical lines indicate standard error of mean.

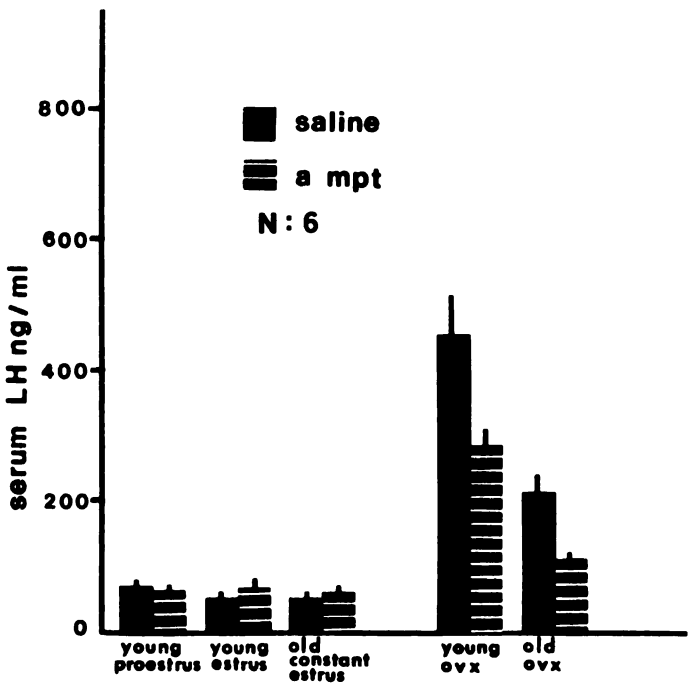

Figure 35. Serum LH concentration and concentration after α-MPT treatment in intact or ovariectomized old and young rats. Vertical lines indicate standard error of mean.

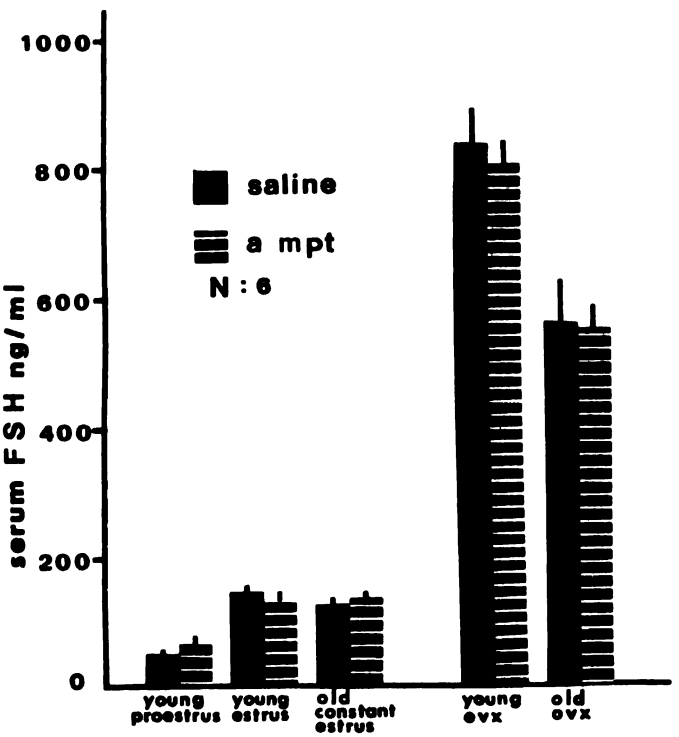

Figure 36. Serum FSH in blood samples collected from same rats as in Figure 35.

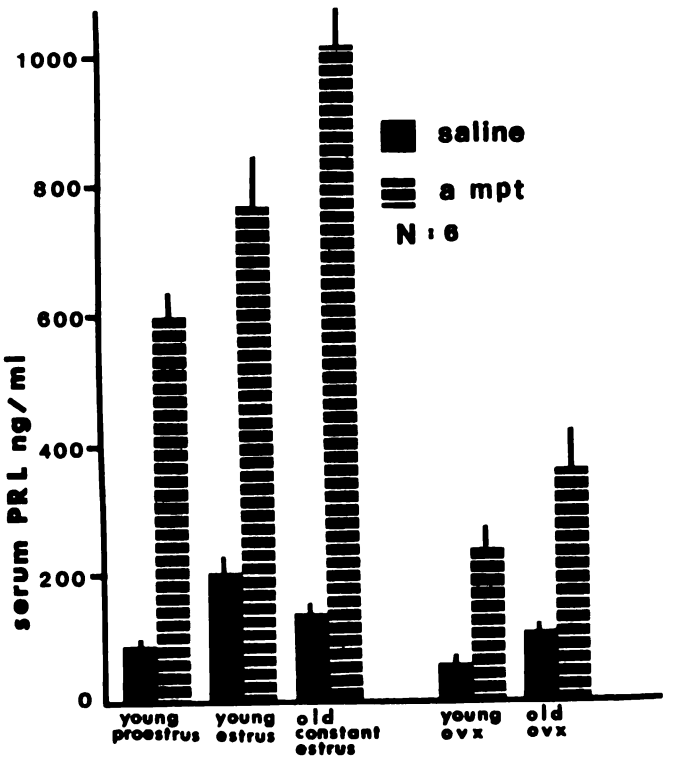

Figure 37. Serum prolactin in blood samples collected from same rats as in Figure 35.

IX. Hypothalamic Norepinephrine and Dopamine Turnover in Ovariectomized Old Rats Treated with Gonadal Steroids.

A. Objectives

Old constant estrous rats do not exhibit preovulatory LH and FSH surges. They can be induced to ovulate and cycle regularly by daily injections of progesterone (Huang et al., 1976a). Positive feedback of ovarian steroids on LH and FSH release in old constant estrous rats is much less than in young rats (see experiment VII). It has been reported that progesterone induction of the LH surge requires the mediation of hypothalamic catecholamines (Kalra et al., 1972). The purpose of this study was to determine whether there was a difference in hypothalamic catecholamine activity between young and old rats in response to ovarian steroid treatments.

B. Materials and Methods

Twenty to 22 month old constant estrous rata and 4 to 5 month young cycling rats both were bilaterally ovariectomized. Two weeks after ovariectomy, each rat was given a single s.c. injection of 10 ug estradiol benzoate in 0.1 ml corn oil per 100 g body weight at 0900 h.Seventy two hours later, one group of animal was injected with α-MPT or saline, and 45 minutes later was killed for measuring hypothalamic catecholamine activity. The other group of animal was given 0.5 mg progesterone per 100 g body weight by a single s.c. injection. At 1300 h (4 hours after progesterone injection), the rats were given α-MPT and 45 minutes later were sacrified for catecholamine assay. The α-MPT treatments, brain tissue collection, and methods of catecholamine measurements were the same as in experiment VIII.

C. Results

Figure 38. shows that the concentration and turnover of NE in the anterior hypothalamus were significantly lower in old than in young rats. At 0900 h. the anterior hypothalamic NE concentration in old rats was 1819 ± 165 ng per gram wet weight. Forty five minutes after α-MPT injection, NE decreased by 17 ± 4.5%, whereas in young rats NE concentration was 2486 ± 62 ng per gram wet weight and decreased 27 ± 2.3% after α-MPT treatment. It was about the same at 1300 h. NE concentration in old rats was 1729 ± 136 ng per gram wet weight and reduced to 19 ± 4.5% after α- MPT treatment, However, NE concentration was 2479 ± 98 ng per gram wet weight in young rats and decreased to 27 ± 1.5% after α-MPT injection.

The anterior hypothalamic DA concentration and turnover are shown in Figure 39. There were no significant difference in DA concentration between old and young rats at 0900 h and 1300 h (940 ± 27 vs. 840 ± 58; 987 ± 51 vs. 892 ± 150 ng per gram wet weight, respectively). Forty five minutes after α-MPT injection, the % of DA decrease at 0900 h in old and young rats were same (43 ± 3.4% vs 44 ± 2.2). Interestingly, at 1300 h, the % of DA decrease was significantly less in young than in old rats (22 ± 4.7% vs 44 ± 1.9). It also was significantly lower as compared with young rats at 0900 h. This indicates that DA turnover of young rats was decreased by 4 hours after progesterone injection.

The concentration and turnover of NE and DA in the posterior hypothalamus were slightly higher in young than in old rats, but there were no statistical difference (Figures 40 and 41). The hormone levels in old and young rats used in the present study are shown in Figures 42 (LH) 43 (FSH) and 44 (Prolactin). As in our previous experiments,

serum LH and FSH were significantly higher in young than in old rats at 0900 h (172 ± 28 vs. 110 ± 22 ng / ml; 498 ± 30 vs 372 ± 33 ng / ml respectively). However, serum prolactin was significantly lower in young than in old rats (98 ± 18 vs. 199 ± 24 ng/ml). Forty five minutes after α-MPT, serum LH and FSH were not altered in both age of groups (237 ± 33 vs. 129 ± 34; 510 ± 21 vs. 365 ± 52 ng / ml respectively). However, serum prolactin rose from 98 ± 18 to 586 ± 43 ng / ml in young rats and rose from 199 ± 24 to 1028 ± 116 ng / ml in old rats after α-MPT. At 1300 h (4 hours after progesterone), serum LH and FSH increased significantly more in young than in old rats (369 ± 110 vs. 233 ± 30; 660 ± 57 vs. 336 ± 22 ng / ml, respectively). Serum prolactin was increased about the same in both of young and old rats 283 ± 63 vs. 350 ± 36 ng / ml). The rise of serum LH after progesterone injection was suppressed in both age of groups by α-MPT (from 369 ± 110 to 115 ± 14 ng / ml in young rats; from 233 ± 30 to 51 ± 11 ng / ml in old rats). Serum FSH was not changed by α-MPT in both young and old groups (660 ± 57 vs. 684 ± 42 ng / ml in young rats; 336 ± 22 vs. 332 ± 30 ng / ml in old rats, respectively). However, serum prolactin was greatly increased by α-MPT (from 283 ± 63 to 728 ± 56 ng / ml in young rats; 350 ± 36 to 927 ± 74 ng / ml in old rats).

D. Discussion

The present results provide additionally evidence that reduced hypothalamic catecholamine activity may be involved in the decline in reproductive functions and in the increase in prolactin actions observed in aging rats. It is well established that the anterior hypothalamus of the rat is the main site controlling the positive feedback of gonado-

tropin release, ovulation and estrous cycles. The stimulatory effects of ovarian steroids on gonadotropin release is mediated by hypothalamic neuro-transmitters (Kalra, et al., 1972). The rise of LH and FSH in estrogen-progesterone treated rats can be blocked by catecholamine depressants such as α-MPT. The positive feedback release of LH and FSH in response to ovarian steroids in old rats is much less than in young rats. This may account for the lower NE concentration and turnover in the anterior hypothalamus of old constant estrous rat. It also is possible that catecholamine activities in the anterior hypothalamus of old rats is altered less in response to ovarian steroid treatment. The old rats did not exhibit a decrease in DA turnover during the critical period preceding gonadotropin surges as in young rats.

A number of investigators have postulated that the DA neurons of the tuberoinfundibular region inhibit the discharge of luteinizing hormone releasing hormone (FRH) and prolacting inhibiting factor (PIF). Hence they would act to decrease LH and FSH but to increase prolactin release. Consistent with this hypothesis are reports of enhanced tuberinfundibular DA turnover during metestrus-diestrus as compared to proestrus-estrus. Decreased DA turnover is associated with the critical period on the day preceding ovulation (Hökfelt and Fuxe et al., 1972). Contrariwise, there also is evidence in favor of a stimulatory dopaminergic mechanism for the release of LRH, FRH and PIF with subsequent facilitation of LH, FSH secretion and inhibition of prolactin secretion (McCann et al., 1972).

Present data support the hypothesis that DA inhibits LH, FSH and prolactin release. It is well known that LH , FSH and prolactin all show simultaneous pre-ovulatory surges. This suggests that the pre-ovulatory rise of LH, FSH and prolactin are not controlled by one particu-

lar neuro-transmitter such as dopamine. It may be influenced by several other neuro-transmitter, as well, such as acetylcholine, serotonin, GABA, etc. Some are inhibitory and some are stimulatory to release of these hormones (Meites et al., 1977). The levels of LH, FSH and prolactin in the serum can be influenced by the ratio of stimulatory and inhibitory neuro-transmitter activities during any particular physiological state.

Serotonin also may be involved in the decline of reproductive function in old female rats. We have reported an increase in hypothalamic serotonin activity in old male rats (Simpkins et al., 1976). Serotonin has been shown to decrease LH release when infused intraventricularly in rats (Schneider and McCann, 1970; Kamberi et al., 1970). Cessation of estrous cycles and higher prolactin levels in old female rats may be due in part to the increase of hypothalamic serotonin activity during aging. The relationship between alterations in central amine activities and changes in serum hormone levels in old rats requires further clarification. Additional studies are needed to elucidate the multifactorial regulation of anterior pituitary hormone secretion.

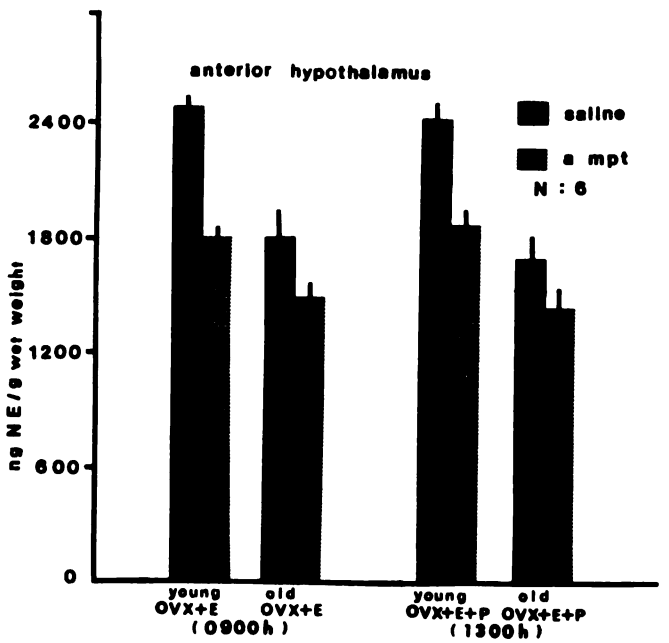

Figure 38. Norepinephrine concentration and concentration after α-MPT treatment in the anterior hypothalamus of estrogen or of estrogen-progesterone treated ovariectomized old and young rats. Vertical lines indicate standard error of mean.

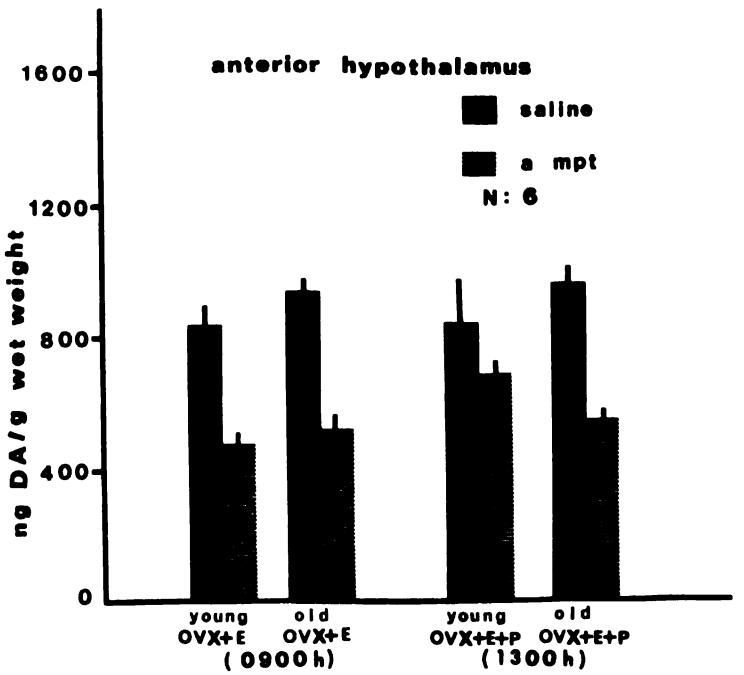

Figure 39. Dopamine concentration and concentration after α- MPT treatment in the anterior hypothalamus of estrogen or of estrogen-progesterone treated ovariectomized old and young rats. Vertical lines indicate standard error of mean.

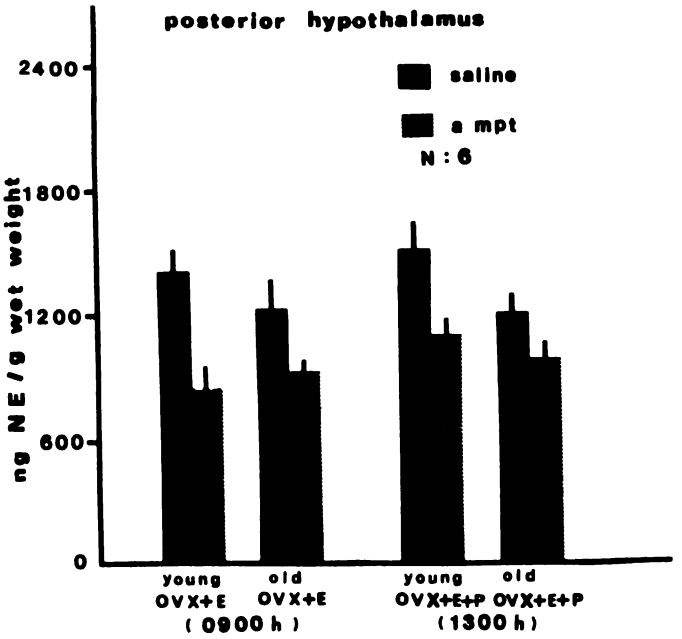

Figure 40. Norepinephrine concentration and concentration after α-MPT treatment in the posterior hypothalamus of estrogen or of estrogen-progesterone treated ovariectomized old and young rats. Vertical lines indicate standard error of mean.

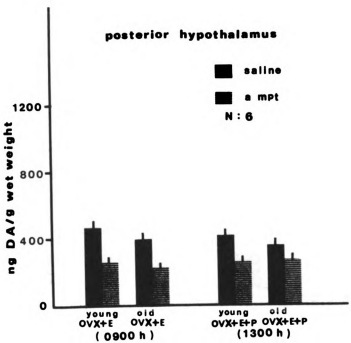

Figure 41. Dopamine concentrations and concentrations after α-MPT treatment in the posterior hypothalamus of estrogen or of estrogen-progesterone treated ovariectomized old and young rats. Vertical lines indicate standard error of mean.

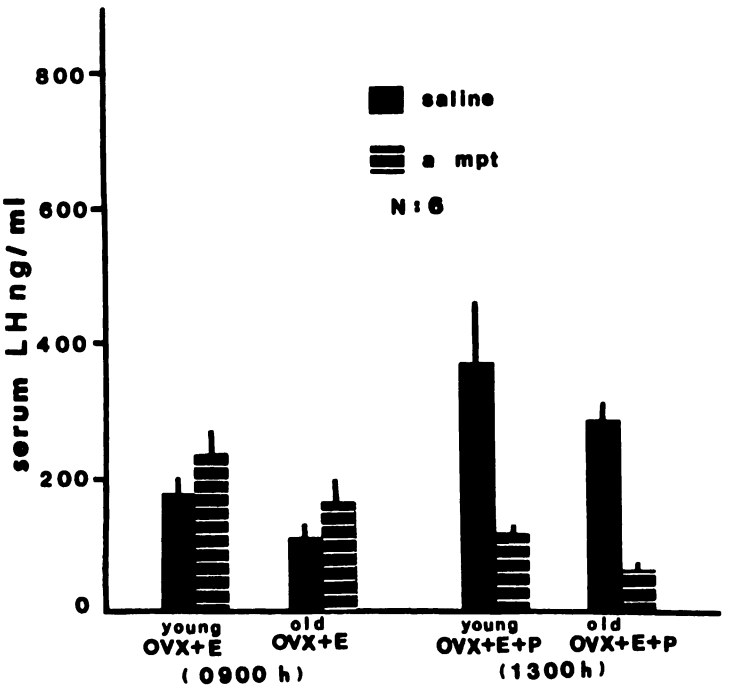

Figure 42. Serum LH concentration and concentration after α-MPT treatment in estrogen or estrogen-progesterone treated ovariectomized old and young rats.

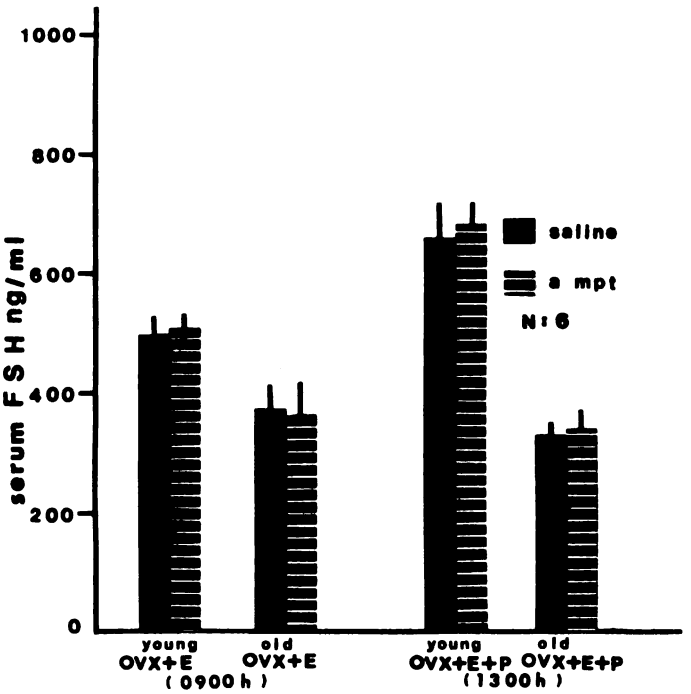

Figure 43. Serum FSH in blood samples collected from same rats as in Figure 42.

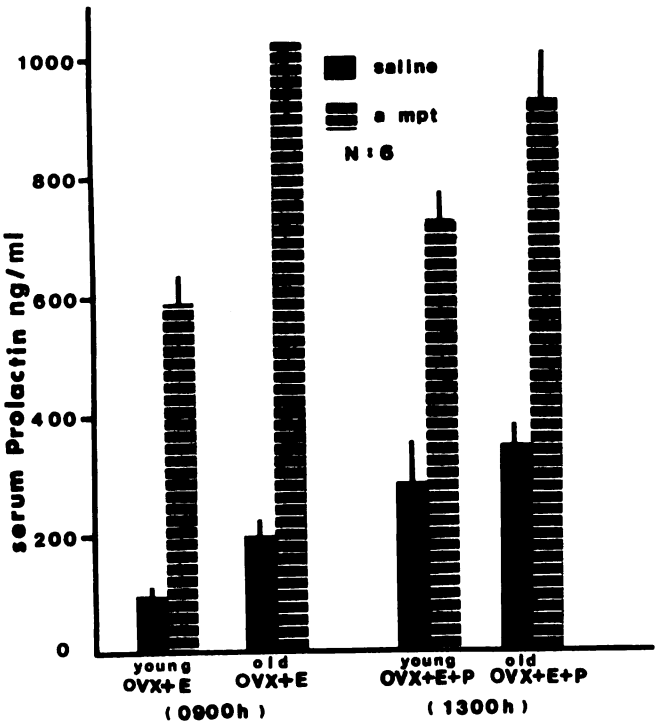

Figure 44 Serum prolactin in blood samples collected from same rats as in Figure 42.

X. Effects of Mating Old Female Rats.

A. Objectives.

The ovaires of old constant estrous rats contain mainly normal but some cystic follicles and no corpora lutea. Upon administration of progesterone, ACTH, L-dopa, epinephrine, iproniazed or ether stress, these animals were induced to ovulate and cycle (Huang et al., 1976a, Quadri et al., 1973). The ovaries of old constant estrous rats and pseudopregnant rats recently were found to retain their capacity to specifically bind^{125} I-labeled LH and FSH in a manner qualitatively similar to that of ovaries from young cycling rats, but ovaries with cystic follicles showed minimal gonadotropin binding capacity (Steger et al., 1976a). It appears possible, therefore, that old constant estrous rats are still capable to being fertilized and carry through a successful pregnancy provided the uterine tract can implant the fertilized ova, form a placenta and carry the fetuses to term. In the present study old constant estrous rats were treated with progesterone to induce regular cycles and mated with fertile male rats.

B. Materials and Methods.

Sixteen to 18 month old constant estrous rats were used in this study. One group of 12 rats served as controls and were injected s.c. daily with corn oil. Two groups of 10 or 12 rats each were injected daily with 1 mg progesterone in corn oil, and after they showed 2 consecutive regular estrous cycles, fertile male 5 to 6 months of age were placed in the breeding cages of both the progesterone treated and control rats. Rats that showed vaginal plugs or sperm in vaginal smears were considered as possibly pregnant. Progesterone at a dose of 2 mg daily was

continued in one group for 21 more days. Vaginal smears were monitored and the males were permitted to remain in the cages throughout the experiment. At about 10-12 days after mating, the abdomens of rats were palpated for the presence of fetuses, and those found to be pregnant were observed continuously 20 more days. The rats which did not show the presence of fetuses were laparotimized, and ovaries, oviducts and uterus were examined. Rats that had fetuses but did not undergo parturition by 21-23 days were laparotomized at 30-32 days after mating.

C. Results.

It can be seen in Table 16 that 7 of 12 control constant estrous rats became pregnant. However, the fetuses of 4 pregnant rats were dead *in utero* by mid-gestation; only 1 rat continued into late pregnancy but failed to undergo parturition and the fetuses were macerated when examined at the end of 30 days. Two rats examined at mid-gestation showed infection of the oviduct. In the group given 1 mg progesterone daily until mating, 6 of 10 rats became pseudopregnant and 4 rats became pregnant but the fetuses were dead by mid-pregnancy or later, infection was observed in the oviducts of 2 of these rats. In the rats given 1 mg progesterone daily initially and 2 mg daily for 21 days after mating, 6 rats became pregnant and 6 rats became pseudopregnant, but none of the fetuses survived. Three of these rats showed oviductal infection.

D. Discussion.

It is apparent that mating of old constant estrous rats, whether treated with progesterone to induce regular estrous cycles or

not, resulted in pseudopregnancies in most of the rats and pregnancies in the remainder, but none of the fetuses survived or underwent parturition. Thus mating of old constant estrous rats with fertile male rats is more effective than stimulation of the uterine cervix with a glass rod for producing pseudopregnancies (Clemens, et al., 1969), but not as effective as mating of young mature female rats for producing normal pregnancies. The reasons for the pregnancy failure in the old rats are not entirely clear, but may be due to aging or other faults in the ova, decreased uterine sensitivity to ovarian hormones, deficient hormone secretion by the ovaries and placenta, and infection of the oviduct.

A decline in the number of ova ovulated and viability of ova with aging has been observed in a variety of laboratory animals including the mouse, rat and hamster; delayed fertilization also may occur in aging animals, as well as a decline in number of implantation sites. Post-implantation reproductive failures have been observed to increase with maternal aging in mice and hamsters (Talbert, 1968). In aging female rats treated with ovarian steroids, traumatization of the uterine Horns produced little or no decidual response in contrast to many positive responses in similarly treated young female rats (Chatterjee and Mukherjee, 1974). We observed that the uterine endometrium of aging rats showed a loss of microvilli and diminuation of cell contact when compared with young mature female rats (Steger et al., 1976 b). These and perhaps other deficiencies contribute to the pregnancy and parturition failures reported here in aging female rats.

Table 17. Effects of Mating Old CE Rats with Fertile Males

Group and Treatment	No. of Rats	No. of Rats Pseudo-pregnant	No. of Rats With Dead Fetuses in utero	No. of Rats With Prolonged Pregnancy	No. of Rats With Infection in Oviducts
Controls (corn oil)	12	7	4	1	2/12
1 mg progesterone daily to produce regular cycles, until mating	10	6	3	1	2/10
1 mg progesterone daily to produce regular cycles, 2 mg daily after mating	12	6	4	2	3/12

General Discussion

The causes of aging are largely unknown, but they are believed to be multiple. It is likely that the primary control of aging is built into the DNA of the gene, whose expression is programmed as a function of time. The rats of aging and maximal duration of life appear to be determined by the interaction between the genetic program in the cell and environmental factors such as radiation, temperature, nutrition, stress, etc. The interactions between the genetic program and environment may be mediated through the hypothalamic-peripheral endocrine system.

The reproductive functions of animals are regulated by the interaction of the hypothalamic, pituitary, gonadal and reproductive tract systems. The concept that emerges from our and related studies by others is that the female rat ceases to cycle and to reproduce usually by or even before the first half of its potential life span of about 3 years. The hypothalamus, pituitary and ovaries, however, remain capable of normal function under appropriate stimulation with the exception of the pituitaries of anestrous rats. Whether the uterus is capable of normal function still remains to be determined. With aging, deficiencies begin to appear in each of these organs, but the primary problem appears to occur in the hypothalamus. The hypothalamus becomes less responsive to stimuli that normally produce release of LH and FSH, and becomes more responsive to stimuli that normally produce release of prolactin. This is believed to be associated with a decrease in hypothalamic catecholamines and an increase in serotonin activity, which apparently reduce release of LHRH and PIF into the portal circulation and result in decreased release of LH and FSH and increased release of prolactin. There is as yet no explanation for these changes in hypothalamic biogenic

amine activity with aging in the rat. It is possible that turnover of other brain neurotransmitters also are altered in the aging female rat.

Despite these changes in the hypothalamus-pituitary-ovarian system that occur with aging, it is apparent that cycling can be reinitiated in old constant estrous and pseudopregnant rats, and pseudopregnancy or pregnancy can occur at least in some old constant estrous rats when mated with fertile male rats. In the old anestrous rats, the presence of pituitary tumors appears to rule out the possibility of reestablishing cycles, since the changes in the pituitary appear to be irreversible. Development of pituitary tumors may be considered to be part of the normal aging pattern in female and also male rats. Nonetheless, the atrophic ovaries of these old rats can become functional upon appropriate stimulation by gonadotropins, as indicated by transplantion to young ovariectomized rats. Thus the ovaries of female rats apparently remain capable of function during the entire life-span of the rat.

Still to be measured in aging rats are the levels of growth hormone, ACTH, steroid hormones and thyroid hormones, and to determine how they influence reproductive functions. Little is yet known about age changes in the ability of target organs to respond to hormonal stimulation. The degree of tissue responsiveness may be directly related to the amount of hormone bound to specific receptors, and is worthy of study. There are some indications that receptors for some hormones may be decreased with aging.

That cyclic-AMP (c-AMP) and postaglandins play an important role in several steps of the reproductive process has been indicated in a number of studies. Experimental evidence suggests that c-AMP may be a second messenger for the action of LHRH on pituitary LH release (Ratner,

1970). Extension of such studies to other hormones involved in aging of the reproductive system might be revealing.

It is of interest to speculate at least briefly on the comparative changes in neuroendocrine function that occur at about the time of puberty and at the time of reproductive senescence in rats. At the onset of puberty in female rats, there is a rise in LH and FSH release preceded by an increase in turnover of hypothalamic norepinephrine (Advis, Simpkins, and Meites, unpublished). In aging female rats that have ceased to cycle, just the opposite appears to occur since the capacity to release gonadotropin is reduced and hypothalamic turnover of catecholamines is decreased. Can the period of reproductive senescence in rats therefore be considered as a reversion to the prepubertal state? The ovaries as well as the hypothalamo-pituitary system of pre-pubertal rats can be stimulated to hasten the onset of puberty and initiation of estrous cycles, as can the hypothalamo-pituitary-ovarian system of aging non-cycling rats to induce resumption of cycling. Thus there are some parallels between the prepubertal state and the period of reproductive senescence in aging rats.

The possible relationship of the observations in aging female rats to the problem of reproductive aging in human subjects is of obvious interest. Many gaps remain in our knowledge of hypothalamo-pituitary-gonadel function in women before and after the menopause. Women cease to undergo menstrual cycles and to conceive well before termination of their normal life span, and in this respect are similar to female rats that usually stop cycling even before the middle of their potential life span. For several years prior to the menopause, the cycles of women tend to become irregular and there is an increase in number of anovulatory cycles; there is inadequate development and early involution of the

corpus luteum; estrogen and progesterone secretion appear to decline and the ovaries show a reduced capacity to respond to gonadotropins (Timiras, 1972). Some of these changes appear to be similar to those observed in aging female rats, whose estrous cycle first become irregular, followed by constant estrus with ovaries showing well developed and sometimes cystic follicles but no ovulation. However, the ovaries of aging rats appear to remain responsive to the action of gonadotropins unlike the ovaries of premenopausal women. There is evidence that secretion of gonadotropins increase even as early as 10 years prior to the menopause in women, whereas gonadotropin secretion in amounts sufficient to produce ovulation apparently decrease in aging rats showing irregular estrous cycles, constant estrous or repeated periods of pseudopregnancy. The capacity for producing a preovulatory surge of LH in aging female rats is reduced, and this also may occur in pre-menopausal women.

In the post-menopausal period in women, ovulation rarely occurs. The relatively few ova and follicles present gradually disappear, and the ovaries tend to become fibrotic and unresponsive to gonadotropic stimulation. In reaction to the marked decline in ovarian hormones, the hypothalamo-pituitary system responds with a marked elevation in secretion of both FSH and LH which may continue for at least 15 years after the menopause. A sharp fall in gonadotropins has been observed in women about 80 years of age (Albert, et al., 1956). It is evident that there are some important differences between the post-menopausal period in women and the related events in aging female rats. The greatest difference is that the ovaries of women show exhaustion of ova and follicles and non-responsiveness to gonadotropic hormone stimulation, whereas the ovaries of aging female rats retain some ova and

follicles, and can respond to gonadotropins until the end of their life span. Even in the old anestrous rats, whose atropic ovaries and reproductive tract appear to most closely parallel the post-menopausal state in women, the capacity to respond to gonadotropins remains. Thus the primary cause for termination of menstrual cycles in women appears to lie in the ovaries, whereas in female rats the primary cause for cessation of normal estrous cycles lies in the hypothalamus.

There is some evidence in aging human subject (male and female) of a decrease in brain catecholamine and an increase in monoamine oxidase (Robinson, et al., 1972), but whether this is related to reproductive senescence is unknown. Little is as yet known as to whether there are changes in responsiveness of the hypothalamo-pituitary system in aging women and men to stimuli that release gonadotropins and other hormones that influence the reproductive system. The rat has served as a worthy model to obtain basic information and insight into the mechanism controlling reproductive senescence, and some of the approaches used on the rat should be applicable to studies on aging human subjects.

LIST OF REFERENCES

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Albert, A., R. V. Randal, R. A. Smith and Johnsen. 1956. Hormones and the Aging Process, Academic Press, Inc., New York, N. Y.

Aschheim, P. 1961. La Pseudogestation a repetition chez les rattes se niles. C. R. Acad. Sci. 253:1988-1990.

Aschheim, P. 1964-65. Resultats fournis par la greffe heterchrone des ovaires dans letude de la regulation hypothalamo-hypophyso-ovarienne de la ratt senile. Gerontologia 10:65-75.

Aschheim, P. 1965. La reactivation de l'ovaire des rattes en oestrus permanent au moyen d'hormones gonadotropes de la mise a l'obscurite. C. R. Acad. Sci. 260:5627-5630.

Aschheim, P. 1968. Contenu hypophysaire en hormone luteinisante (LH) et reaction histophysiologique a la LH circulante du tissu interstitiel ovarien chez divers types de rattes seniles. C. R. Acad. Sci. 267:1397-1400.

Aschheim, P. 1976. Aging in the hypothalamic-hypophyseal ovarian axis in the rat. In: Hypothalamus, Pituitary and Aging, edited by A. V. Everitt and J. A. Burgess, pp. 376-418. Charles C. Thomas, Springfield, Ill.

Adamopoulos, D. A., J. A. Loraine and G. A. Dove. 1971. Endocrinological studies in women approaching the menopause. J. Obstet. Gynaecol. Br. Common 78:62-79.

Adams, J. H., P. M. Daniel and M. M. L. Prichard. 1965. Observations on the portal circulation of the pituitary gland. Neuroendocrinology 1:193-213.

Ajika, K., L. Krulich, C. P. Fawcett and S. M. McCann. 1972. Effects of estrogen on plasma and pituitary gonadotropins and prolactin and on hypothalamic releasing and inhibiting factors. Neuroendocrinology 9:304-315.

Allen, E. 1922. The oestrous cycle in the moust. Am. J. Anat. 30:297-371.

Anden, N. E., A. Rubenson, K. Fuxe and T. Hökfelt. 1967. Evidence for dopamine receptor stimulation by apomorphine. J. Pharmacol. 19:627-629.

Anderson, E. 1966. Adrenocorticotropin-releasing hormone in peripheral blood: increase during stress. Science 152:379-380.

Antunes-Rodrigues, J. and S. M. McCann. 1967. The effect of suprachiasmatic lesions on the regulation of LH secretion in the female rat. Endocrinology 81:666-670.

Asdell, S. A. 1969. Historical introduction. In: Reproduction in Domestic Animals, edited by H. H. Cole and P. T. Cupps, pp. 1-16. Academic Press, New York, N. Y.

Bailey, P. and F. Bremer. 1921. Experimental diabetes insipidus. Arch. Intern. Med. 28:73.

Baldwin, D. M., C. K. Haun and C. H. Sawyer. 1974. Effects of intraventricular infusions of $ACTH^{1-24}$ and $ACTH^{4-10}$ on LH release, ovulation and behavior in the rabbit. Brain Res. 80:291-301.

Bargmann, W. and E. Scharrer. 1951. The site of origin of the hormones of the posterior pituitary. Am. Scientist 39:255-259.

Barraclough, C. A. 1967. Modifications in reproductive function after exposure to hormones during the prenatal and postnatal period. In: Neuroendocrinology, Vol. II, edited by W. F. Ganong and L. Martini, pp. 61-69, Academic Press, New York, N. Y.

Barraclough, C. A., R. Collu, R. Massa and L. Martini. 1971. Temporal interrelationships between plasma LH ovarian secretion rates and peripheral plasma progestin concentration in the rat; effects of nembutal and exogenous gonadotropins. Endocrinology 88:1437-1447.

Barraclough, C. A. 1973. Sex steroid regulation of reproductive neuroendocrine process. In: Handbook of Physiology, Vol. II, edited by R. O. Greep, Sec. 7, pp. 29-56. American Physiological Society, Washington, D. C.

Barrnett, R. J. and R. P. Greep. 1951. The direction of flow in the blood vessel of infundibular stalk. Science 113:185.

Bassett, D. L. 1943. The changes in the vascular pattern of the ovary of the albino rat during the estrous cycle. Am. J. Anat. 73:251-291.

Bjersing, L. 1967. On the morphology and endocrine function of granulosa cells in ovarian follicles and corpora lutea. Acta. Endocrinol. Suppl. 125:1-23.

Bjorkstein, E. 1968. The cross linkage theory of aging. J. Am. Geriatr. Soc. 16:408-418.

Blackwell, R. E. and M. S. Amoss, Jr. 1971. A sex difference in the rate of rise of plasma LH rats following gonadectomy. Proc. Soc. Exp. Biol. Med. 136:11.

Blake, C. A. 1976. Effects of intravenous infusion of catecholamines on rat plasma luteinizing hormone and prolactin concentrations. Endocrinology 98:99-104.

Blumenthal, H. T. 1955. Aging processes in the endocrine glands of various strains of normal mice: relationship of hypophyseal activity to aging changes in other endocrine glands. J. Gerontol. 10:253-267.

Bogdanove, E. M. 1963. Direct gonado-pituitary feedback: an analysis of effects of intracranial estrogenic depots on gonadotropin secretion. Endocrinology 73:696-712.

Bogdanove, E. M. 1964. The role of the brain in the regulation of pituitary gonadotropin secretion. Vitamins and Hormones 22:205-260.

Bowers, C. Y., H. G. Friesen and K. Folkers. 1973. Further evidence that TRH is also a physiological regulator of PRL secretion in man. Biochem. and Biophys. Res. Comm. 51:512-521.

Bowers, C. Y., H. G. Friesen, P. Huang, H. J. Guyda and K. Folkers. 1971. Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl-prolinamide. Biochem. Biophsy. Res. Comm. 45:1033-1041.

Bowers, C. Y., A. Weil, J. K. Chang, H. Sievertsson, Enzmann and K. Folkers. 1970. Activity-structure relationships of the thyrotropin releasing hormone. Biochem. Biophsy. Res. Comm. 40:683.

Brazeau, P., J. Rivier, W. Vale and R. Guillemin. 1974. Inhibition of growth hormone secretion in the rat by synthetic somatostatin. Endocrinology 94:184-187.

Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, R. Rivier and R. Guillemin. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77-79.

Brizze, K. R., P. A. Cancilla, N. Sherwood and P. S. Timiras. 1969. The amount and distribution of pigments in neurons and glia of the cerebra cortex. J. Gerontol. 24:127-135.

Brody, H. 1955. Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. J. Comp. Neuro. 102:511-556.

Brown-Grant, K. 1967. The effects of a single injection of progesterone on the oestrous cycle, thyroid gland activity and uterus-plasma concentration ratio for radioiodide in the rat. J. Physiol. 1960:101-121.

Brown-Grant, K., F. Naftolin. 1972. Facilitation of luteinizing hormone secretion in the female rat by progesterone. J. Endocrinol. 53:37-46.

Browstein, M. J., M. Palkovits, J. J. Saavedra and J. S. Kizer. 1976. Distribution of hypothalamic hormones and neurotransmitters with diencephalon. In: Frontiers in Neuroendocrinology, Vol. IV, edited by W. F. Ganong, pp. 1-23, Raven Press, New York, N. Y.

Bruni, J., H. H. Huang, S. Marshall and J. Meites. (in press). Effects of single and multiple injections of synthetic GnRG on serum LH, FSH and testosterone in young and old male rats. Biology of Reproduction.

Burgus, R., T. F. Dunn, D. Desiderio, D. N. Ward, W. Vale and R. Guillemin. 1970. Characterization of ovine hypothalamic hypophysiotropic TSH-releasing factor. Nature (London) 226:321-325.

Byrnes, W. W. and R. K. Meyer. 1951. Effect of physiological amounts of estrogen on the secretion of follicle stimulating and luteinizing hormones. Endocrinology 49:449-60.

Caligaris, L., J. J. Astrada and S. Taleisnik. 1971 a. Release of LH induced by estrogen injection into ovariectomized rats. Endocrinology 88:810-815.

Caligaris, L., J. J. Astrada and S. Taleisnik. 1971 b. Biophasic effect of progesterone on the release of gonadotropin in rats. Endocrinology 89:331-337.

Callantine, M. R., R. R. Humphrey, S. L. Lee, B. L. Windsor, M. Schottin and O. P. O'Brien. 1966 a. Release of luteinizing hormone induced by estrogen injection into ovariectomized rats. Endocrinology 79:153-157.

Callantine, M. R. R., R. R. Humphrey and B. L. Nesset. 1966 b. LH release by 17- -estradiol in the rat. Endocrinology 79:455-456.

Ceresa, F. and L. Lacroix. 1951. La funzione gonadotropa della preipofisi nei maschi in eta senile. Acta. Gerontol. 1:3-13.

Chatterjee, A. and A. P. Mukherjee. 1974. The aging uterus and its refractoriness to appropriate sex steroids in the development of deciduoma. Endokrinologie 63:166-168.

Chen, C. L., E. J. Bixler, A. I. Weber and J. Meites. 1968. Hypothalamic stimulation of prolactin release, from the pituitary of turkey hen and poults. Gen. Comp. Endocrinol. 11:489-494.

Chen, C. L., H. Minaguch and J. Meites. 1967. Effects of transplanted pituitary tumors on host pituitary-prolactin secretion. Proc. Soc. Exp. Biol. Med. 126:317-320.

Chen, H. J., G. P. Mueller and J. Meites. 1974. Effect of L-dopa and somatostatin on suckling induced release of prolactin and growth. Endocrine Res. Comm. 1:283-291.

Chirigos, J. A., P. Greengard and S. Udenfriend. 1960. Uptake of tyrosine by rat brain in vivo. J. Biol. Chem. 235:2075-2079.

Clemens, J. A., Y. Amenomori, T. Jenkins and J. Meites. 1969. Effects of hypothalamic stimulation, hormones and drugs on ovarian function in old female rats. Proc. Soc. Exp. Biol. Med. 132:561-563.

Clemens, J. A. and J. Meites. 1968. Inhibition by hypothalamic prolactin implants of prolactin secretion, mammary growth and luteal function. Endocrinology 82:878-881.

Clemens, J. A. and J. Meites. 1971. Neuroendocrine status of old constant estrous rats. Neuroendocrinology 7:249-256.

Convey, E. M., V. G. Smith, K. Mongkonpunya, J. Zolman and H. D. Hafs. 1975. Clinical applications of hypothalamic hormones in animals. In: Hypothalamic Hormones, edited by E. S. E. Hafez and J. R. Reel, pp. 101-128. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.

Cook, D. M., J. W. Kendall, M. A. Greer and R. M. Kramer. 1973. The effect of acute or chronic ether stress on plasma ACTH concentration in rats. Endocrinology 93:1019-1024.

Cooper, E. R. A. 1925. The Histology of the More Important Human Organs at Various Ages, Oxford University Press, London.

Coyle, J. T. and D. Henry. 1973. Catecholamines in fetal and newborn rat brain. J. Neurochem. 21:61-67.

Cramer, O. M. and C. A. Barraclough. 1971. Effect of electrical stimulation of the preoptic area on plasma LH concentrations in proestrous rats. Endocrinology 88:1175-1183.

Croes-Buth, S. F., J. A. Paesi and S. E. DeJong. 1959. Stimulation of ovarian follicles in hypophysectomized rats by low dosage of estradiol benzoate. Acta. Endocrinol. 32:399-410.

Curtis, H. J. and L. Gebhard. 1958. Comparison of life shortening effects of toxic and radiation stresses. Radiat. Res. 9:104.

Curtis, H. J., J. Tilley, C. Crowley and M. Fuller. 1966. The role of genetic factor in the aging process. J. Gerontol. 21:365-368.

Dahlström, A., and K. Fuxe. 1964. Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta. Physiol. (Scand.) 62 (suppl. 232):1-55.

Daniel, P. M. 1966. The anatomy of the hypothalamus and pituitary gland. In: Neuroendocrinology, edited by L. Martini and W. F. Ganong, pp. 15-80. Academic Press, New York, N. Y.

Daniel, P. M. and M. M. L. Prichard. 1956. Anterior pituitary necrosis. Infraction of the pars distalis produced experimentally in the rat. Quart. J. Exp. Physiol. 41:215-229.

Daniel, P. M. and M. M. L. Prichard. 1957. Anterior pituitary necrosis in the sheep produced by section of the pituitary stalk. Quart. J. Exp. Physiol. 42:248-254.

Davidson, J. M. 1969. Feedback control of gonadotropin secretion. In: Frontiers in Neuroendocrinology, edited by W. F. Ganong and L. Martini, pp. 343-388, Oxford University Press, New York, N. Y.

Davidson, J. M., R. F. Welch, E. R. Smith and R. Dominguez. 1970. Feedback mechanisms in relation to ovulation. Fed. Proc. 29:1900-1906.

Deane, H. W. 1952. Histochemical observations on the ovary and oviduct of the albino rat during the estrous cycle. Am. J. Anat. 91:363-414.

DeGroot, J. 1959. The Rat Forebrain in Stereotaxic Coordinates. N. V. Noord-Hollandsche Maatschappiu, Amsterdam.

Dempsey, E. W. and Bassett. 1943. Observations on the fluorence, birefringence and histochemistry of rat ovary during the reproductive cycle. Endocrinology 33:384-401.

Descline, L. and Flament-Durand. 1963. Presence de neurosecretat dans le lobe posterieur d'hypophyses greffes l'hypothalamus chez le rat hypophysectomise. Ann. Endocrinol. (Paris) 24:131-135.

Dey, F. L. 1941. Changes in ovaries and uteri in guinea pigs with hypothalamic lesions. Am. J. Anat. 69:61-87.

Dey, F. L. 1943. Evidence of hypothalamic control of hypophysial gonadotropic functions in the female guinea pig. Endocrinology 33:75-82.

Dibbet, J. A., J. F. Bruni, G. P. Mueller, H. J. Chen and J. Meites. 1974. In vivo and in vitro stimulation of prolactin (PRL) secretion by synthetic TRH in rats. The 55th meeting of the Endocrine Society, Abst. 182:A-139.

Dickerman, S., J. Clark, E. Dickerman and J. Meites. 1972. Effects of haloperidol on serum and pituitary prolactin and on hypothalamic PIF in rats. Neuroendocrinology 9:332-340.

Dilman, V. M. 1971. Age-associated elevation of hypothalamic threshold to feedback control, and its role in development, aging and disease. Lancet 11:1211.

Dilman, V. M. 1976. The hypothalamic control of aging and age-associated pathology. The elevation of mechanism of aging. In: Hypothalami, Pituitary and Aging, edited by A. V. Everitt and J. A. Burgess, pp. 634-667. Charles G. Thomas, Springfield, Ill.

Docke, F. and G. Dörner. 1966. Facilitative action of progesterone on the induction of ovulation by estrogen. J. Endocrinol. 36:209-210.

Donoso, A. O., W. Bishop, C. P. Fawcett, L. Krulich and S. M. McCann. 1971. Effect of drugs that modify brain monoamine concentrations on plasma gonadotropin and prolactin levels in the rats. Endocrinology 89:774-784.

Dörner, G. and F. Döcke. 1967. The influence of intrahypothalamic and intrahypophysial implantation of estrogen or progesterone on gonadotrophin release. Endocrinologia Exp. 2:65-71.

Douglas, W. W. and A. M. Poisner. 1964. The role of calcium in the release of vasopressin from the neurohypophysis. J. Physiology (London) 1972:1-18.

Duncan, L. E., D. H. Solomon, E. K. Rosenberg, M. P. Nichols and N. W. Shock. 1952. The metabolic and hematologic effects of chronic administration of ACTH to young and old men. J. Gerontol. 7:351-374.

Ellis, R. S. 1920. Norms for some structure changes in the human cerebellum from birth to old age. J. Comp. Neuro. 32:1-33.

Euker, J. S., J. Meites and G. D. Riegle. 1975. Effects of acute stress on serum LH and prolactin in intact, castrated and dexamethasone-treated male rats. Endocrinology 96:85-92.

Everett, J. W. 1940. The restoration of ovulatory cycles and corpus lutea formation in persistent estrous rats. Endocrinology 27: 681-686.

Everett, J. W. 1947. Hormonal factor responsible for deposition of cholesterol in the corpus luteum of the rat. Endocrinology 41:364-377.

Everett, J. W. 1948. Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat. Endocrinology 43:389-405.

Everett, J. W., C. H. Sawyer and J. E. Markee. 1949. A neurogenic timing factor in control of ovulatory discharge of luteinizing hormone in the cyclic rats. Endocrinology 44:219-234.

Everett, J. W. and C. H. Sawyer. 1949. A neural timing factor in the mechanism by which progesterone advance ovulation in the cyclic rat. Endocrinology 45:581-595.

Everett, J. W. 1961. Sex and Internal Secretion, Vol. II, 2nd ed., edited by William C. Young, pp. 497-555. Williams and Wilkins, Baltimore, Maryland.

Everett, J. W. 1964. Central neural control of reproductive functions of the adenohypophysis. Physiol. Rev. 44:373-431.

Everitt, A. V. 1976. The nature and measurement of aging. In: Hypothalamus, Pituitary and Aging, edited by A. V. Everitt and J. A. Burgess, Chapter 2. Charles C. Thomas, Springfield, Ill.

Falck, B., N. A. Hillarp, G. Thieme and A. Thorp. 1962. Fluorence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. 10:348-354.

Feder, H. H., K. Brown-Grant and C. S. Corker. 1971. Preovulatory progesterone, the adrenal cortex and the "critical period" for luteinizing hormone release in rats. J. Endocrinol. 50:29-39.

Feder, H. H., J. A. Resko and R. W. Goy. 1968. Progesterone levels in the adrenal plasma of pre-ovulatory and ovariectomized rats. J. Endocrinol. 41:563-564.

Feder, H. H. and K. B. Ruf. 1969. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. Endocrinology 84:171-174.

Finch, C. E. 1973. Catecholamine metabolism in the brains of male mice. Brain Res. 52:261-276.

Flament-Duran, J. 1964. Morphologie et fontion de transplants hypophysaires dan l'hypothalamus chez le rat. Compt. Rend. Acad. Sci. 259:4376-4378.

Flament-Durand, J. 1965. Observations on pituitary transplants into the hypothalamus of the rat. Endocrinology 77:446-454.

Flerko, B. and V. Bardos. Absence of compensatory ovarian hypertrophy in rats with anterior hypothalamic lesions. Acta. Endocrinol. 36:180-184.

Flerko, B. 1969. Control of gonadotropin secretion in the female. In: Neuroendocrinology, edited by L. Martini and W. F. Ganong, pp. 613-53. Academic Press, New York, N. Y.

Flerko, B. 1974. Hypothalamic mediation of neuroendocrine regulation of hypophysial gonadotropic functions. In: MTP Internal Review of Science. Reproductive Physiology, Physiology Series One, Vol. 8, edited by A. C. Guyton and D. Horrobin, pp. 1-31. University Park Press, Baltimore, Maryland.

Fonda, M. L., D. W. Acree and S. B. Auerbach. 1973. The relationship of gamma-amino butyrate levels and its metabolism in brains of mice. Arch. Bioch. Biophys. 159:622-628.

Frolkis, V. V., V. V. Bezrukov, Yu K. Duplenko and E. D. Genis. 1972. Exp. Gerontol. 7:169-184.

Frolkis, V. V. 1976. The hypothalamic mechanisms of aging. In: Hypothalamus, Pituitary and Aging, edited by A. V. Everitt and J. A. Burgess, pp. 614-633, Charles C. Thomas, Springfield, Ill.

Fujimoto, W., J. Ensinck and R. Williams. 1974. Somatostatin inhibits insulin and glucagon release by monolayer cultures of rat endocrine pancreas. Life Science 15:1999-2004.

Fuxe, K. 1963. Cellular localization of monoamines in the median eminence and infundibular stem of some mammals. Acta. Physiol. Scand. 58:383-384.

Fuxe, K. 1965. Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine nerve terminals in the central nervous system. Acta. Physiol. (Scand.) 64 (Suppl. 247):37-85.

Fuxe, K. and T. Hökfelt. 1966. Further evidence for the existence of tuberoinfundibular dopamine neurons. Acta. Physiol. (Scand.) 66:245-246.

Fuxe, K. and T. Hökfelt. 1969. Catecholamines in the hypothalamus and pituitary gland. In: Frontiers in Neuroendocrinology, edited by L. Martini and W. T. Ganong, pp. 47-98, Oxford University Press, New York, N. Y.

Fuxe, K. and T. Hökfelt. 1970. Participation of central monoamine neurons in the regulation of anterior pituitary function with special regard to the neuroendocrine role of tubero-infundibular dopamine neurons. In: Aspects of Neuroendocrinology, edited by W. Bargmann and [illegible]. Scharrer, pp. 275-314. Springer-Verlag, Berlin, Germany.

Fuxe, K., T. Hökfelt, L. Agnati, A. Löfstom, B. J. Everitt, O. Johansson, G. Jonsson, W. Wuttke, and Goldstein. 1976. Neuroendocrine Regulation of Fertility, edited by T. C. Anand Kumar, pp. 124-140, S. Karger, Basel, Switzerland.

Gallo, R. V., J. Rabbi and G. P. Moberg. 1975. Effect of methysergide, a blocker of serotonin receptors on plasma prolactin levels in lactating and ovariectomized rats. Endocrinology 97:1096-1105.

Ganong, W. F. 1963. The central nervous system and the synthesis and release of adrenocorticoid hormone. In: Advances in Neuroendocrinology, edited by Nalbandov, pp. 92, University of Illinois Press, Evanston.

Gay, V. L. and A. R. Midgley, Jr. 1969. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology 84:1359-1365.

Glowinski, J. and J. Axelrod. 1966. Effects of drugs on the uptake, release and metabolism of H^3-norepinephrine in the rat brain. J. Pharmacol. Exp. Ther. 149:43-49.

Glowinski, J., I. Kopin and J. Axelrod. 1965. Metabolism of H^3-norepinephrine in the rat brain. J. Neurochemo. 12:25-30.

Gourdji, D. and A. Tixier-Vidal. 1966. Mise en evidence d'un control hypothalamique stimulant de la prolactin hypophysaire chez le canard. Compt. Rend. Acad. Sci. 263:162-165.

Green, J. D. 1947. Vessels and nerves of amphibian hypotheses. A study of the living circulation of the hypophysial vessels and nerves. Anat. Record. 99:21-53.

Green, J. D. and G. W. Harris. 1949. Observation of the hypophysioportal vessels of the living rat. J. Physiol. 108:359-369.

Greep, R. O., H. B. VanDyke and B. F. Chow. 1942. Gonadotropins of the swine pituitary. I. Various biological effects of purified thylakentrin (FSH) and pure metakentrin (ICSH). Endocrinology 30:635-649.

Guillemin, R., W. R. Hern, W. R. Cheek and D. E. Housholder. 1957. Control of corticotropin release; further studies with in vitro methods. Endocrinology 60:488-506.

Halasz, B. 1969. The endocrine effects of isolation of hypothalamus from the rest of the brain. In: Frontiers in Neuroendocrinology, edited by W. F. Ganong and L. Martini, pp. 307-342, Oxford University Press, New York, N. Y.

Halasz, B. and R. A. Gorski. 1967. Gonadotrophic hormone secretion in female after partial or total interruption of neural afferents to the medial basal hypothalamus. Endocrinology 80:608-622.

Halasz, B., L. Pupp and S. Uharik. 1962. Hypophysiotropic area in the hypothalamus. J. Endocrinol. 25:147-154.

Halasz, B., L. Pupp, S. Uhlarik and L. Tima. 1965. Further studies on the hormone secretion of the anterior pituitary transplanted into the hypophysitropic area of the rat hypophysiotropic area of the rat hypothalamus. Endocrinology 77:343-355.

Halasz, B. and J. Szentagothai. 1962. The tropic dependence of the anterior pituitary on the hypothalamus. In: Hypothalamic Control of the Anterior Pituitary, pp. 266-276, Publ. House Hung Acad. Sci., Budapest, Hungary.

Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11:298-300.

Harman, D. 1961. Prolongation of the normal lifespan and inhibition of spontaneous cancer by antioxidants. J. Gerontol. 16:247-254.

Harman, D. 1968. Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF mice. J. Gerontol. 23:476-482.

Harris, G. W. 1937. The induction of ovulation in the rabbit by electrical stimulation of the hypothalamo-hypophyseal mechanism. Proc. Roy. Soc. (London), B 122:374-394.

Harris, G. W. 1948. The control of the pituitary gland. Physiol. Rev. 28:139-179.

Harris, G. W. 1949. Neural control of the pituitary gland. Physiol. Rev. 29:

Harris, G. W. 1950. Oestrus rhythm, pseudopregnancy and pituitary stalk in the rat. J. Physiol. (London) 111:347-360.

Harris, G. W. 1955. Neural Control of the Pituitary Gland. Edward Arnold, London, England.

Harris, G. W. 1961. The pituitary stalk and ovulation. In: Control of Ovulation, edited by C. A. Vilee, pp. 56-74. Pergamon Press, London, England.

Harris, G. W. 1972. Humours and Hormones. J. Endocrinol. 53: ix-xiii.

Harris, G. W. and D. Jacobsohn. 1952. Functional grafts of the anterior pituitary gland. Proc. Roy. Soc. (London) B 139: 263-279.

Hayflick, L. 1965. The limited in vitro lifetime of human diploid cell strains. Exp. Cell Res. 37:614-636.

Hayflick, L. 1975. Current theories of biological aging. Fed. Proc. 34:9-13.

Hayflick, L. and P. S. Moorhead. 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25:585-621.

Hess, G. D. and G. D. Riegle. 1970. Adrenocortical responsiveness to stress and ACTH in aging rats. J. Gerontol. 25:354-358.

Hess, G. D. and G. D. Riegle. 1972. Effects of chronic ACTH stimulation on adrenocortical function in young and aged rats. Am. J. Physiol. 222:1458-1461.

Hill-Samli, M. H. and R. W. MacLeod. 1974. Interaction of thyrotropin releasing hormone and dopamine on the secretion of prolactin and ^{14}C-Glucose oxidation by rat anterior pituitary incubated in vitro. Fed. Proc. 195:

Himwich, W. A. 1973. Neurochemical patterns in the developing and aging brain. In: Development and Aging in the Nervous System, edited by M. Rockstein, pp. 151-170.

Hohlweg, W. 1934. Verander ungen des hypophsen-vonderlappens und des ovariums nach behandlimgen mit grossen doser von follikelhormon. Klin. Wochinsch 11:92-95.

Hohleg, W. and K. Junkmann. 1932. Die hormonal-nervose regulierung der funktion des hypophysen vorderlappens. Klin. Wochinsch 11:321-323.

Hökfelt, T. and K. Fuxe. 1976. Brain-Endocrine Interaction: Median Eminence: Structure and Function, edited by K. M. Knigge, D. E. Scott and A. Weindle, pp. 181-223, S. Karger, Basel, Switzerland.

Hollander, J. and C. H. Barrows. 1968. Enzymatic studies in senescent rodent brains. J. Gerontol. 23:174-179.

Holliday, R. 1969. Errors in protein synthesis and clonal senescence in fungi. Nature 221:1224-1228.

Holzbauer, M. and H. M. Newport. 1967. The effect of stress on the concentration of 3 B-hydroxylpregn-3, 20-on3 (pregnenolone) and pregn-4-ene-3, 20-dione (progesterone) in the adrenal gland of the rats. J. Physiol. (London) 193:131-40.

Houssay, B. A., A. Biosotti and R. Samartino. 1935. Modifications fonctionelles de l'hypophyse apres les lesions infundibuloteriennes chez le crapaud. C. R. Hebd. Seanc. Soc. Biol. (Paris) 120: 725-727.

Huang, H. H. and Meites J. 1975. Reproductive capacity of aging female rats. Neuroendocrinology 17:289-295.

Huang, H. H., S. Marshall and J. Meites. 1976 a. Induction of estrous cycles in old non-cyclic rats by progesterone, ACTH, ether stress of L-dopa. Neuroendocrinology 20:21-34.

Huang, H. H., S. Marshall and J. Meites. 1976 b. Capacity of old versus young female rats to secrete LH, FSH and prolactin. Biol. of Repro. 14:538-543.

Ingram, D. L. 1953. The effect of hypophysectomy on the number of oocytes in the adult albino rat. J. Endocrinol. 9:307-311.

Ingram, D. L., Z. M. Mandl and S. Zuckerman. 1958. The influence of age on litter size. J. Endocrinol. 17:230-285.

Ingram, D. L. 1959. The vaginal smear of senile laboratory rats. J. Endocrinol. 19:182-188.

Ito, A., P. Moy, H. Kaunitz, K. Kortwright, S. Clarke, J. Furth and J. Meites. 1972. Incidence and character of the spontaneous pituitary tumors in strain CR and W/Fu male rats. J. Natl. Cancer Inst. 49:701-711.

Jackson, G. L. 1972. Effect of actinomycin, D. on estrogen-induced release of LH in ovariectomized rats. Endocrinology 91:1284-1287.

Jacobs, L. S., P. J. Snyder, J. R. Wilber, R. D. Utiger and W. H. Daughaday. Increase in serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. J. Clin. Endocrinol. Metab. 33:996-998.

Janssen, P., C. Niemegeers, K. Schellekens, A. Dresse, F. Lenaerts, A. Pinchard, W. Schapper, J. VanNeuten and F. Verbruggen. 1968. Pimozide, a chemically novel, highly potent and orally long-acting neuroleptic drug. Arzneimittel-Forsch. 18:261-287.

Jecquir, E., D. S. Robinson, W. Lovenberg and A. Sjoerdsma. 1969. Furth studies on tryptophan hydroxylase in rat brainstem and beef pineal. Biochem. Pharmacol. 18:1971-1981.

Jones, E. C. and P. L. Krohn. 1961 a. The relationship between age, number of oocytes and fertility in virgin and multiparous mice. J. Endocrinol. 21:469-495.

Jones, E. C. and P. L. Krohn. 1961 b. Effect of hypophysectomy on age changes in the ovaries of mice. J. Endocrinol. 21:1020-1024.

Kalra, S. P., K. Ajika, L. Krulick, C. P. Fawcett, M. Quijada and S. M. McCann. 1971. Effects of hypothalamic and preoptic electrochemical stimulation on gonadotropin and prolactin release in proestrous rats. Endocrinology 88·1150-1158.

Kalra, P. S., S. P. Kalra, L. Krulich, C. P. Fawcett and S. M. McCann. 1972. Involvement of norepinephrine in transmission of the stimulatory influence of progesterone on gonadotropin release. Endocrinology 90:1168-1176.

Kamberi, I. A. 1976. Role of brain neurotransmitters in the secretion of hypothalamo-pituitary-gonadel principles. In: Neuro-endocrinology Regulation of Fertility, edited by T. C. Anand Kumar, pp. 141-154, S. Karger, Basel, Switzerland.

Kannwischer, R., J. Wagner and V. Critchlow. 1967. Effects of intrahypothalamic estrogen implants on puberty in female rats. Am. Assoc. Anatomists, (Abstract) pp. 268.

Kato, J. and C. A. Villee. 1967. Preferential uptake of estradiol by the anterior hypothalamus of the rat. Endocrinology 80: 567-575.

Kishimoto, S. and Y. Yamamur. 1973. Changes of antibody-forming cell precursors and antigen-reactive cells with aging. In: The Relation of Aging to Immunity, edited by R. L. Walford et al., pp. 26-31. MSS Information Corp., New York, N. Y.

Kalra, P. S. and S. M. McCann. 1975. The stimulatory effect on gonadotropin release of implants of estradiol or progesterone in certain sites in the central nervous system. Neuroendocrinology 19:289-302.

Kamberi, I., R. S. Mical and J. C. Porter. 1970 a. Prolactin-inhibiting activity in hypophyseal portal blood and elevation by dopamine. Experientia 26:1150-1151.

Kamberi, I. A., R. S. Mical and J. C. Porter. 1970 b. Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. Endocrinology 87:1-12.

Kamberi, I. A., R. S. Mical and J. C. Porter. 1971 a. Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88:1012-1020.

Kamberi, I. A., R. S. Mical and J. C. Porter. 1971 b. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88:1288-1293.

Knigge, K. M. 1962. Gonadotropic activity of neonatal pituitary glands implanted in the rat brain. Am. J. Physiol. 202:387-391.

Knigge, K. M. and A. J. Silverman. 1974. Anatomy of the endocrine hypothalamus. In: Handbook of Physiology, Vol. IV, edited by R. O. Greep, Sec. 7, Part I, pp. 1-34. American Physiological Society, Washington, D. C.

Kragt, C. L. and J. Meites. 1965. Stimulation of pigeon pituitary prolactin release by pigeon hypothalamic extract in vitro. Endocrinology 76:1169-1176.

Krohn, P. L. 1955. Tissue transplantation techniques applied to the problem of the aging of the organs of reproduction. In: CIBA Foundation Colloquia on Aging, Vol. I, p. 141, Little Brown, Boston, Mass.

Krohn, P. L. 1962. Review lectures on senescence. II. Heterochronic transplantation in the study of aging. Proc. Roy. Soc. (London) B 157:128-147.

Krulich, L., A. P. S. Dhariwal and S. M. McCann. 1968. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology 83:783-790.

Labhsetwar, A. P. 1970. The role of oestrogens in spontaneous ovulation: evidence for oestrogen positive feedback in the 4-day oestrous cycles. J. Endocrinol. 47:481-493.

Labhsetwar, A. P. 1969. Age dependent changes in the pituitary-gonadal relationship. II. A study of pituitary FSH and LH content in female rat. J. Reprod. Fertil., 20:21-28.

Lane, C. E. and R. O. Greep. 1935. The follicular apparatus of the ovary of the hypophysectomized immature rat and the effect of hypophyseal gonadotropic hormones on it. Anat. Record. 63: 139-146.

Lansing, W. and J. M. Wolfe. 1942. Changes in the fibrillar tissue of the anterior pituitary of the rat associated with advancing age. Anat. Rec. 83:355-365.

Lawton, I. E. 1970. Effect of ovarian and adrenal hormones on cyclic LH release. Physiologist (Wash.) 13:246.

Lauson, H. D., J. B. Golden and E. I. Sevringhaus. 1939. The gonadotropic content of the hypophysis thought the life cycle of the normal female rat. Am. J. Physiol. 125:396-404.

Lawson, D. M. and R. R. Gala. 1975. The influence of adrenergic, dopaminergic, cholinergic and serotonergic drugs on plasma prolactin levels in ovariectomized, estrogen treated rats. Endocrinology 96:313-318.

Lazarus, L. and C. J. Eastman. 1976. Assessment of hypothalamic pituitary function in old age. In: Hypothalamus, Pituitary and Aging, edited by A. V. Everitt and J. A. Burgess, pp. 97-122, Charles C. Thomas, Springfield, Ill.

Legan, S. J., C. A. Coon and F. J. Karsch. 1975. Role of estrogen as initiator of daily LH surges in the ovariectomized rat. Endocrinology 96:50-56.

Levitt, M., S. Spector, A. Sjoerdsma and S. Udenfriend. 1965. Elucidation of the rate limiting step in norepinephrine biosynthesis using the perfused guinea pig heart. J. Pharmacol. Exp. Ther. 148:1-8.

Levitt, M., J. W. Gibb, J. W. Daly, M. Lipman and S. Udenfriend. 1967. A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition in vivo. Biochem. Pharmacol. 16:1313-1321.

Lisk, R. D. 1960. Estrogen-sensitive centres in the hypothalamus of the rat. J. Exp. Zool. 145:197-208.

Lisk, R. D. 1962. Testosterone-sensitive centres in the hypothalamus of the rat. Acta. Endocrinol. 41:195-204.

Lisk, R. D. 1965. Reproductive capacity and behavioral estrus in the rat bearing hypothalamic implants of sex steroids. Acta. Endocrinol. 48:209-219.

Long, J. A., and H. M. Evan. 1922. The estrus cycle in the rat and its associated phenomena. Mem. Univ. Calif. 6:1-148.

Lu, K. H., and J. Meites. 1971. Inhibition by L-dopa and monoamine oxidase inhibitors of pituitary prolactin release: stimulation by methyldopa and DA-amphetamine. Proc. Soc. Exp. Biol. Med. 137:480-483.

Lu, K. H., and M. Meites. 1972. Effects of L-dopa on serum prolactin and PIF in intact and hypophysectomized, pituitary-grafted rats. Endocrinology 91:868-872.

Lu, K. H., and Meites, J. 1973. Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology 93:152-155.

Lu, K. H. and H. H. Huang, H. T. Chen, M. Kurcz, R. Mioduszewski, and J. Meites. 1977. Positive feedback by estrogen and progesterone on LH release in old and young rats. Proc. Soc. Exp. Biol. Med. 154:82-85.

MacIndoe, J. H., and R. W. Turkington. 1973. Stimulation of human prolactin secretion by intravenous infusion of L-trypotphan. J. Clin. Invest. 52:1972-1978.

MacLeod, R. M. 1969. Influence of norepinephrine and catecholamine-depleting agents on the synthesis and release of prolactin and growth hormone. 1969. Endocrinology 85:916-923.

MacLeod, R. M., and J. E. Lehmeyer. 1974. Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinology 94:1077-1985.

Makinodan, T., E. H. Perkins, and M. G. Chen. 1971. Immulogic activity of the aged. In: Advances in Gerontological Research Vol. 3, edited by B. Strehler, pp. 171-196. Academic Press, New York, N. Y.

Mandl, A. M., and M Shelton. 1959. A quantitative study of oocytes in young and old nulliparous laboratory rats. J. Endocrinol. 18:444-450.

Mandl, A. M., and S. Zuckerman. 1951. The relation of age to numbers of oocytes in young and old multiparous laboratory rats. J. Endocrinol. 7:190-194.

Mangili, G., M. Motta, and L. Martini. 1966. Control of adreno-corticotropic hormone secretion. In: Neuroendocrinology, Vol. I, edited by L. Martini and W. F. Ganong, p. 297. Academic Press, New York, N. Y.

Marchlewska-Koj, A., and L. Krulich. 1975. The role of central monoamines in the stress induced prolactin release in the rat. Fed. Proc. 34:252 (abstract 191).

Markee, J. E., J. W. Everett, and C. H. Sawyer. 1952. The relationship of the nervous system to the release of gonadotropin and the regulation of the sex cycle. Recent Prog. Horm. Res. 7:139-163.

Markee, J. E., C. H. Sawyer, and W. H Hollinshead. 1948. Adrenergic control of the release of luteinizing hormone from the hypophysis of the rabbit. Recent Prog. Horm. Res. 2:117-131.

Mattingly, R. F., and W. Y. Huant. 1969. Steroidogenesis of the menopausal and post-menopausal ovary. Am. J. Obstet. Gynecol. 103:679-693.

McBride, J. M. 1957. Estrogen excretion levels in the normal post-menopausal woman. J. Clin. Endocrinol. 17:1440-1447.

McCann, S. M. 1962. Effect of progesterone on plasma luteinizing hormone activity. Am. J. Physiol. 202:601-604.

McCann, S. M. 1971. Mechanisms of action of hypothalamic hypophyseal stimulating and inhibiting hormones. In: Handbook of Physiology, Vol. IV, edited by R. O. Greep, Sec. 7, Part II, pp. 489-517. American Physiological Society, Washington, D. C.

McCann, S. M., and A. P. S. Dhariwal. 1966. Hypothalamic releasing factors and the neurovascular link between the brain and the anterior pituitary. In: Neuroendocrinology, Vol. I., edited by L. Martini and W. F. Ganong, pp. 261-296. Academic Press, New York, N. Y.

McCann, S. M., P. S. Kalra, A. O. Donoso, W. Bishop, H. P. G. Schneider, C. P. Fawcett, and K. Krulich. 1976. In: Brain-Endocrine Interaction: Median Eminence: Structure and Function, edited by K. M. Knigge, D. E. Scott, and A. Weindle, pp. 224-235. S. Karger, Basel, Switzerland.

McCann, S. M., and R. L. Moss. 1976. Putative neurotransmitters involved in discharging gonadotropin-releasing neurohormones and the action of LH-releasing hormone on the CNS. Life Science 16:833-852.

McCann, S. M., and S. R. Ojeda. 1973. Evidence for participation of a catecholaminergic mechanism in the post-castration rise in plasma gonadotropins. Neuroendocrinology 12:295.

McCann, S. M., and J. C. Porter. 1969. Hypothalamic pituitary stimulating and inhibiting hormones. Physiol. Rev. 49:240-284.

McCann, S. M., and V. D. Ramirez. 1964. The neuroendocrine regulation of hypophyseal luteinizing hormone secretion. Recent Prog. Horm. Res. 20:131-181.

McCann, S. M., S. Taleisnik, and H. M. Friedman. 1960. LH releasing activity in hypothalamic extracts. Proc. Soc. Exper. Biol. Med. 104:432-434.

McDermont, W. V., E. S. Fry, J. R. Brobeck, and C. N. H. Long. 1951. Mechanism of control of adrenocorticotropin hormones. Yale J. Biol. Med. 23:52-65.

McGreer, E. G., H. C. Fibeger, P. L. McGeer, and V. Wickson. 1971. Aging and brain enzymes. Exp. Gerontol. 6:391-396.

McKay, D. G., and R. Robinson. 1947. Observations on the fluorescence, birefringence and histochemistry of the human ovary during the menstrual cycle. Endocrinology 41:378-394.

Meites, J. 1967. Control of prolactin secretion. Archives d'Anatomie Microscopique et de Morphologie Experimentale 56:516-529.

Meites, J., and J. A. Clemens. 1972. Hypothalamic control of prolactin secretion. Vitamins and Hormones 30:165-221.

Meites, J., K. H. Lu, W. Wuttke, C. W. Welsch, H. Nagasawa, and S. K. Quadri. 1972. Recent studies on function and control of prolactin secretion in rats. Recent Prog. Horm. Res. 28:471-516.

Meites, J., and C. Nicoll. 1966. Adenohypophysis: Prolactin. In: Annual Review of Physiology, Vol. 28, pp. 57-88. Stanford University Press, Palo Alto, CA.

Meites, J., J. Simpkins, J. Bruni, and J. Advis. 1977. Role of biogenic amines in control of anterior pituitary hormones. IRCS J. of Medical Science 5:1-7.

Meyer, R. K., and C. E. McCormack. 1967. Ovulation in immature rats treated with ovine follicle-stimulating hormone: facilitation by progesterone and inhibition by continuous light. J. Endocrinol. 38:187-94.

Minaguchi, H., and J. Meites. 1967. Effects of suckling on hypothalamic LH-releasing factor and prolactin-inhibiting factor, and on pituitary LH and prolactin. Endocrinology 80:603-607.

Moir, A. T. B., and D. Eccleston. 1968. The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J. Neurochem. 15:1083-1108.

Moore, C. R., and D. Price. 1932. Gonad hormone functions and the reciprocal influence between gonads and hypophysis, with its bearing on sex hormone antagonism. Am. J. Anat. 50:13-71.

Motta, M., G. Fraschini, Giuliani, and L. Martini. 1968. The central nervous system, estrogen and puberty. Endocrinology 83:1101-1107.

Moy, P., H. Kaunitz, K. Kortwright, S. Clarke, J. Furth, and J. Meites. 1972. Incidence and character of the spontaneous pituitary tumors in strain CR and W/Fu male rats. J. Nat. Cancer Inst. 49: 701-711.

Mueller, G. P., H. J. Chen, and J. Meites. 1973. In vitro stimulation of prolactin release in the rat by synthetic TRH. Proc. Soc. Exp. Biol. Med. 144:613-615.

Mueller, G. P., J. Simpkins, J. Meites, and K. E. Moore. 1976. Differential effects of dopamine agonists and haloperidol on release of prolactin, thyroid stimulating hormone, growth hormone, and luteinizing hormone in rat. Neuroendocrinology 20:121-135.

Nagatsu, T., M. Levitt, and S. Udenfriend. 1964. Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910-2917.

Neil, J. L. 1972. Sexual differences in the hypothalamic regulation of prolactin secretion. Endocrinology 90:1154-1159.

Neil, J. D., and M. S. Smith. 1974. Pituitary-ovarian interrelationship in the rat. In: Current Topics in Experimental Endocrinology, Vol. II, edited by V. H. James, and L. Martini. Academic Press, New York, N. Y.

Nequin, L. G., and N. B. Schwartz. Adrenal participation in the timing of mating and LH release in the cyclic rat. Endocrinology 88:323-325.

Netter, F. H. 1968. The Hypothalamus, Suppl. to Vol. I, Nervous System, The CIBA Collection of Medical Illustrations. CIBA Pharmaceutical Products, Inc., Summit, N. J.

Nicoll, C. S. 1965. Neural regulation of adenohypophyseal prolactin secretion in tetrapods: indication from in vitro studies. J. Exp. Zool. 158:203-210.

Nicoll, C. S., R. P. Fiorindo, C. T. McKennee, and J. A. Parsons. 1970. Assay of hypothalamic factors which regulate prolactin secretion. In: Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry, edited by J. Meites, Williams and Wilkins, Baltimore, MD.

Nicoll, C. S., and J. Meites. 1962. Prolactin secretion in vitro. Comparative aspects. Nature 195:606-607.

Nikodijevic, B., S. Senoh, J. W. Daly, and Creveling. 1970. Catechol-o-methyltransferase II. A new class of inhibitors of catechol-o-methyltransferase; 3,5-dihydroxy-r-methoxybenzoic acid and related compounds. J. of Pharmacol. Exp. Ther. 174:83.

Niswender, G. D., A. R. Midgley, Jr., J. E. Monroe, and L. E. Reichert. 1968. Radioimmunoassay of rat luteinizing hormone with anti-ovine LH serum and ovine LH^{131}I. Proc. Soc. Exp. Biol. Med. 28:807-811.

Orgel, L. E. 1963. The maintenance of the accuracy of protein synthesis and its relevance to aging. Proc. Nat'l. Acad. Sci. (U.S.A.) 49:517-521.

Paesi, F. J. A. 1949. The influence of hypophysectomy and subsequent treatment with chorionic gonadotropin on follicles of different size in the ovary of the rat. Acta. Endocrinol. 3:89-104.

Paesi, F. A. 1952. The effect of small dose of estrogen on the ovary of the immature rat. Acta. Endocrinol. 11:251-268.

Palkovits, M., M. J. Brownstein, J. M. Saavedra, and J. Axelrod. 1974. Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Research 77:137-149.

Palkovits, M., A. Arimura, M. Brownstein, A. V. Schally, and J. M. Saavedra. 1974. Luteinizing hormone-releasing hormone (LH-RH) content and hypothalamic nuclei in rat. Endocrinology 96:554-558.

Parson, R. J. 1935. Medical Papers Dedicated to Henry Astbury Christian, pp. 366. Waverly Press, Inc., Baltimore, MD.

Paschkis, K. E., and A. E. Rakoff, A. Canatarow, and J. Rupp. Clinical Endocrinology, 3rd ed. Harper and Row Pub., New York, N. Y.

Paulsen, C. A., R. B. Leach, H. Sandberg, S. Sheinfeld, and W. O. Maddock. Function of the post-menopausal ovary: comparison of urinary estrogen and gonadotropin excretion and response to administration of FSH in post-menopausal and ovariectomized women. J. Am. Geriatr. Soc. 6:803-813.

Peng, M. T., and H. H. Huang. 1972. Aging of hypothalamic pituitary-ovarian function in the rat. Fertility and Sterility 23:535-542.

Peng, M. T., and Y. M. Peng. 1973. Changes in the uptake of triated estradiol in the hypothalamus and adenohypophysis of old female rats. Fertility and Sterility 24:534-539.

Pincus, G. 1956. Aging and urinary steroid excretion. In: Hormones and the Aging Process, edited by E. T. Engle, and G. Pincus. Academic Press, New York, N. Y.

Popa, G. T., and U. Fielding. 1930. A portal circulation from the pituitary to the hypothalamus. J. Anat. (London) 65:88-91.

Procope, B. J. 1969. Studies on urinary excretion, biological effects and origin of estrogens in post-menopausal women. Acta. Endocrinol., Suppl. 135.

Quadri, S. K., G. D. Kledzik, and J. Meites. 1973. Reinitiation of estrous cycles in old constant estrous rats by central acting drugs. Neuroendocrinology 11:248-255.

Ramaley, J. A., and R. A. Gorski. 1967. The effect of hypothalamic deafferentation upon puberty in the female rat. Acta. Endocrinol. 59:661-674.

Ramirez, V. D., and S. M. McCann. 1964. Induction of prolactin secretion by implants of estrogen into the hypothalamo-hypophysial region of female rats. Endocrinology 75:206-214.

Ramirez, V. D., R. Abrams, and S. M. McCann. 1964. Effect of estradiol implants in the hypothalamo-hypophysial region of the rat on secretion of LH. Endocrinology 75:243-248.

Ratner, A., and J. Meites. 1964. Depletion of prolactin-inhibiting activity of rat hypothalamus by estradiol or suckling stimulur. Endocrinology 75:315-319.

Ratner, A., P. K. Talwalker, and J. Meites. 1965. Effect of reserpine on prolactin-inhibiting activity of rat hypothalamus. Endocrinology 77:315-319.

Ratner, A. 1970. Stimulation of luteinizing hormone release in vitro by dibutyryl-cyclic-amp and theophylline. Life Science 9:1221-1226.

Reichlin, S., R. Saperstein, I. M. D. Jackson, A. E. Boyd, III, and Y. Patel. 1976. Hypothalamic hormones. Ann. Rev. of Physiol. 38:389-423.

Resko, J. A. 1969. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164:70.

Reigle, G. D. 1973. Chronic stress effects on adrenocortical responsiveness in young and aged rats. Neuroendocrinology 11:1-10.

Riegle, G. D., and G. D. Hess. 1972. Chronic and acute dexamethasone suppression of stress activation of the adrenal cortex in young and aged rats. Neuroendocrinology 9:175-187.

Robinson, D. S., A. Nies, J. Davis, W. E. Bunney, J. M. Davis, R. W. Colburn, H. R. Bourne, D. M. Shaw, and A. J. Coppen. 1972. Aging monoamines and monoamine oxidase levels. Lancet 1:290-291.

Rose, S., and J. E. Nelson. 1957. The direct effect of estradiol on the pars distalis. Australian J. Exp. Biol. Med. Sci. 35:605-610.

Ryan, K. J., Z. Petro, and J. Kaiser. 1968. Steroid formation by isolated and recombined ovarian granulosa and theca cells. J. Clin. Endocrinol. Metab. 28:335-358.

Saffran, M., and A. V. Schally. 1955. Release of corticortropin by anterior pituitary tissue in vitro. Canadian J. Biochem. 33:408-415.

Saxton, J. A. 1941. The relation of age to the occurance of adenoma-like lesions in the rat hypophysis and to their growth after transplantation. Cancer Res. 1:227-282.

Sawyer, C. H. 1952. Stimulation of ovulation in the rabbit by the intraventricular injection of epinephrine or norepinephrine. Ann. Rec. 112:385-392.

Sawyer, C. H. 1964. Control of secretion of gonadotropins. In: Gonadotropins, edited by H. H. Cole, pp. 113-159. W. H. Freeman and Co., San Francisco, CA.

Sawyer, C. H. 1975. First Geoffrey Harris Memorial Lecture. Some recent developments in brain-pituitary-ovarian physiology. Neuroendocrinology 17:97-124.

Sawyer, C. H., J. Hilliard, S. Kanematsu, R. Scaramuzzi, and C. A. Blake. 1974. Effects of intraventricular infusions of norepinephrine and dopamine on LH release and ovulation in the rabbit. Neuroendocrinology 15:328-337.

Sawyer, C. H., and M. Kawakami. 1961. Interactions between the central nervous system and hormones influence ovulation. In: Control of Ovulation, edited by C. A. Vilee, pp. 79-100. Pergamon Press, New York, N. Y.

Sawyer, C. H., J. E. Markee, and J. F. Townsend. 1949. Cholinergic and adrenergic components in the neurohumoral control of the release LH in the rabbit. Endocrinology 44:18-37.

Schally, A. V., A. Arimura, C. Y. Bowers, A. J. Kastin, J. Sawano, and T. W. Redding. 1968. Hypothalamic neurohormones regulating anterior pituitary function. Recent Prog. Horm. Res. 24:497-581.

Schally, A. V., A. Arimura, and A. J. Kastin. 1973. Hypothalamic regulatory hormones. Science 179:341-359.

Schally, A. V., C. Y. Bowers, T. W. Redding, and J. F. Barrett. 1966. Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus. Biochem. Biophys. Res. Comm. 25:165-169.

Schally, A. V., A. Dupont, A. V. Arimura, J. Takahara, T. W. Redding, J. Clemens, and C. Shaar. 1976. Purification of a catecholamine rich fraction with prolactin release inhibiting factor (PIF) activity from porcine hypothalamic. Acta. Endocrinol. 82:1-14.

Scharrer, E. 1928. Die lichtempfindlichkeit blinder erlritzen (untersuchungen über das zwischenhirn der fische I). Z. Vergleich. Physiol. 7:1-38.

Scharrer, E. 1930. Über sekretorisch tätige zellen in thalamus von fundulus heteroclitus L. ntersuchungen über das zwichenhirn der fische II). Z. Vergleich. Physiol. 11:767-773.

Scharrer, E. 1932. Die sekretproduktion im zwischenhirn einiger fische (untersuchengen über das zwischenhirn der fische. III). Z. Vergleich. Physiol. 17:491-509.

Scharrer, E. 1952. The general significance of the neuro-secretory cell. Scientia (Milan) 46:177.

Scharrer, E., and B. Scharrer. 1963. Neuroendocrinology, pp. 289. Columbia University Press, New York, N. Y.

Schneider, D. P. G., and S. M. McCann. 1969. Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. Endocrinology 85:121-132.

Schneider, H. P. G., and S. M. McCann. 1970. Mono- and indolamines and control of LH secretion. Endocrinology 86:1127-1133.

Schreider, V., A. Eckertova, Z. Franc, J. Koci, M. Ryback, and V. Kmentova. 1961. Effects of a fraction of bovine hypothalamic extract on the release of TSH by rat adenohypophyses in vitro. Experientia 17:264-265.

Schwartz, N. B. 1964. Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. Amer. J. Physiology 207:1250-1259.

Schwartz, N. B. 1969. A model for the regulation of ovulation in the rat. Recent Prog. Horm. Res. 25:1-55.

Schwartz, N. B., and C. E. McCormack. 1972. Reproduction, gonadal function and its regulation. Ann. Rev. Physiol. 34:425-472.

Shaar, C. J., and J. A. Clemens. 1974. The role of catecholamines in the release of anterior pituitary prolactin in vitro. Endocrinology 95:1202-1212.

Shaar, C. J., J. S. Euker, G. D. Riegle, and J. Meites. 1975. Effects of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. J. Endocrinol. 66:45-51.

Shelton, M. 1959. A comparison of the population of oocytes in nulliparous and multparous senile laboratory rats. J. Endocrinol. 18:451-455.

Shirley, B., J. Wolinsky, and N. B. Schwartz. 1968. Effect of a single injection of an estrogen antagonist on the estrous cycle of rat. Endocrinology 82:959-968.

Simpkins, J. W., G. P. Mueller, H. H. Huang, and J. Meites. 1976. Changes in metabolism of dopamine, norepinephrine and serotonin and relation to gonadotropin secretion in aging male rats. The Physiologist 19:368.

Smalstig, E. B., and J. A. Clemens. 1974. Pharmacological characterization of rat pituitary receptor mediating the inhibition of prolactin. Endocrinology Suppl. A-185.

Smalstig, E. B., B. D. Sawyer, and J. A. Clemens. 1974. Inhibition of rat prolactin release by apomorphine in vivo and in vitro. Endocrinology 95:123-129.

Smith, P. E. 1930. Hypophysectomy and a replacement therapy in the rat. Am. J. Anat. 45:205-274.

Smith, E. R., and J. M. Davidson. 1968. Role of estrogen in the cerebral control of puberty in female rats. Endocrinology 82: 100-108.

Smith, V. G., and E. M. Convey. 1975. TRF stimulation of prolactin release from bovine pituitary cells. Proc. Soc. Exp. Med. 149: 70-74.

Sobel, H., and J. Marmorston. 1956. The possible role of the gel-fiber ratio of connective tissue in the aging process. J. Gerontol. 11:2-7.

Sokal, R. R., and F. J Rohlf. 1969. Single classification of analysis of variance. In: Biometry, pp. 204-252, W. H. Freeman and Co., San Francisco, CA.

Solomon, D. H., and N. W Shock. 1950. Studies of adrenal cortical and anterior pituitary function in elderly men. In: Proceedings of the First ACTH Conference, edited by J. R. Mote, pp. 44-51. Blakiston, Philadelphia, PA.

Spagnoli, H. H., and H. A. Charipper. 1955. The effects of aging on histology and cytology of the pituitary gland of golden hamster (cricetus auratus) with brief references to simultaneous changes in the thyroid and testis. Anat. Rec. 121:117-139.

Spiegel, P. M. 1972. Theories of aging. In: Developmental Physiology and Aging, edited by P. S. Timira, pp. 564-580. The Macmillan Co., New York, N. Y.

Steger, R. W., H. H. Huang, G. Kuppe, J. Meites, E. S. E. Hafez, and H. Ludwig. 1976 a. Effects of age on uterine surface ultrastructure. Scanning Electron Microscopy, Part VI: 359-366.

Steger, R. W., J. J. Peluso, H. H. Huang, E. S. E. Hafez, and J. Meites. 1976 b. Gonadotropin-binding sites in the ovary of aged rats. J. Reprod. Fert. 48:205-207.

Stochard, C. R., and G. N. Papanicolaou. 1917. The existence of a typical oestrous cycle in the guinea pig -- with a study of its histology and physiological changes. Am. J. Anat. 22:225-283.

Stumpf, W. 1968. Estradiol-concentrating neurons: topography in the hypothalamus by dry-mound autoradiography. Science 162:1001-1003.

Sulkin, N. M , and P. Srivanij. 1960. The experimental production of senile pigments in the nerve cells of young rats. J. Gerontol. 2:15-21.

Sulkin, B. M. 1961. Aging of the Nerve Cell in Structure Aspect of Aging, edited by H. B. Geoffrey, pp. 291-301. Hafner Publishing Co., New York, N. Y.

Swerdloff, R. S., H. H. Jacobs, and W. D. Odell. 1972. Synergistic role of progesterone in estrogen induction of LH and FSH surge. Endocrinology 90:1529-1536.

Szentagothai, J. 1969. The synaptic architecture of the hypothalamo-hypophyseal neuron systems. Acta. Neurol. Psychiat. (Belgium) 69:453-468.

Szentagothai, J., B. Flerko, B. Mess, and B. Halasz, (editors). 1968. Hypothalamic Control of the Anterior Pituitary, 3rd ed. Akademiai Kiado, Budapest.

Szentagothai, J., and B. Halasz. 1964. Regulation des endokrinen systems uber hypothalamus. (Vortag auf der jahresversammlung, Deut. Akad. Naturforscher Leopoldina, Halle/Saale, 1963). Nova Acta. Leopoldina 28:227-248.

Szilard, L. 1959. On the nature of the aging process. Proc. Nat'l Acad. Sci. (U.S.A.) 45:30-45.

Talbert, G. B. 1968. Effect of maternal age on reproductive capacity. Am. J. Obstet. Gynecol. 102:451-477.

Takahara, J., A. Arimura, and A. V. Schally. 1974. Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophyseal portal vessel. Endocrinology 95:462-465.

Takahara, J., A. Arimura, and A. V. Schally. 1974. Stimulation of prolactin and growth hormone release by TRH infused into hypophyseal portal vessels. Proc. Soc. Exp. Biol. Med. 146:831-835.

Tapper, C. M., F. Naftolin, and K. Brown-Grant. 1972. Influence of the reproductive state at the time of operation on the early response to ovariectomy in the rat. J. Endocrinol. 53:47-57.

Taubenhaus, M., and S. Soskin. 1941. Release of luteinizing hormones from anterior hypophysis by an acetycholine-like substance from the hypothalamic region. Endocrinology 29:958-964.

Tessaurd, G. 1952. La senescence de l'hypophyse. Comptes Rendus de la Societe Francoise de Gynedologie 22(4):188-214.

Thung, P. J., L. M. Boot, and O. Muhlbock. 1956. Senile changes in the estrous cycle and in ovarian structure in some imbred strains of mice. Acta. Endocrinol. (Copenh.) 23:8-32.

Timiras, P. S. 1972. Developmental Physiology of Aging, pp. 502-541. MacMillan, New York, N. Y.

Torok, B. 1954. Lebendbeobactung des hypophysenkreislaufes an hunden. Acta. Morphol. Acad. Sci. (Hungary) 4:83-89.

Uemura, H., and H. Kobayashi. 1971. Effects of dopamine implanted in the median eminence on the estrous cycle of the rat. Endocrinology (Japan) 18:91-100.

Ungerstedt, U. 1971. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. (Scand.) 367:1-48.

Vale, W., P. Brazeau, C. Rivier, M. Brown, B. Boss, J. Rivier, R. Burgus, N. Ling, and R. Guillemin. 1975. Somatostatin. Rec. Prog. Horm. Res. 31:365-397.

Vogt, M. 1954. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. (London) 123:451-481.

Voogt, J. L., and J. Meites. 1971. Effects of an implant of prolactin in median eminence of pseudopregnant rats on serum and pituitary LH, FSH, and prolactin. Endocrinology 88:286-292.

Wagner, J. W. 1968. Luteinizing of ovarian transplants in PMS-primed immature male rats. Am. Assoc. Anatomists, p. 445.

Walford, R. L. 1962. Auto-immunity and aging. J. Gerontol. 17: 281-285.

Walford, R. L. 1966. Generalizing biological hypotheses and aging: An immunologic approach. In: Topics in the Biology of Aging, edited by P. L. Krohn. Wiley, New York, N. Y.

Watkins, B. E., J. Meites, and G. D. Riegle. 1975. Age-related changes in pituitary responsiveness to LHRH in female rats. Endocrinology 97:543-548.

Weiss, J., and A. I. Lansing. 1953. Age changes in the fine structure of anterior pituitary of the mouse. Proc. Soc. Exp. Biol. 82: 460-466.

Welch, Philip J. 1967. Somatic mutations and the aging process. In: Advance in Gerontological Research, Vol. II, edited by B. Strehler, pp. 1-35. Academic Press, New York, N. Y.

Wislocki, G. B. and L. S. King. 1936. The permeability of the hypophysis and hypothalamus to vital dyes, with a study of the hypophyseal vascular supply. Am. J. Anat. 58:421-427.

Wilson, C. A. 1974. Hypothalamic amines and the release of gonadotropins and other anterior pituitary hormones. Advances in Drug Research 8:119-205.

Wilson, D. L. 1974. The programmed theory of aging. In: Theoretic Aspects of Aging, edited by M. Rockstien, pp. 11-21. Academic Press, New York, N. Y.

Woessner, J. F., Jr. 1963. Age related changes of the human uterus and its connective tissue framework. J. Gerontol. 18:220-226.

Wolfe, J M. 1943. The effects of advancing age on the structure of the anterior hypophysis and ovaries of female rats. Am. J. Anat. 72:361-383.

Wolfe, J. M., R. Bryan, and A. W. Wright. 1938. Histological observation on the anterior pituitary of old rats with particular reference to the spontaneous apparition of pituitary adenomata. Am. J. Cancer 34:352-372.

Worthington, W. C., Jr. 1955. Some observation on the hypophyseal portal system in the living mouse. Bull. Johns Hopkins Hosp. 97:343-357.

Wurtman, R. J. 1970. Control of the synthesis of melatonin and other methoxyindoles in the mammalian pineal organ. In: Neurochemical Aspects of Hypothalamic Function, edited by L. Martini and J. Meites, pp. 135-140. Academic Press, New York, N. Y.

Wurtman, R. J. 1971. The role of brain and pineal indoles in neuroendocrine mechanisms. In: Integration of Endocrine and Nonendocrine Mechanisms in the Hypothalamus, edited by L. Martini. Academic Press, New York, N. Y.

Wurtman, R. J. 1972. Brain monoamines and endocrine function. Neuroscience Res. Symp. Summ. 6:171-297.

Wurtman, R. J., and J. D. Fernstrom. 1971. L-tryptophan and L-tyrosine, and the control of brain monoamine synthesis. In: Perspectives of Neuropharmacology, edited by S. H. Snyder. Oxford University Press, New York, N. Y.

Wuttke, W. E., E. Cassell, and J. Meites. 1971. Effects of ergocornine on serum prolactin and LH, and on hypothalamic content of PIF and LRF. Endocrinology 88:737-741.

Wuttke, W., M. Gelato, and J. Meites. 1971. Mechanism of pentabarbital actions on prolactin release. Endocrinology 89:1191-1194.

Wuttke, W., and J. Meites. Effects of electrochemical-stimulation of medial preoptic area on prolactin and luteinizing hormone release in old female rats. Pflügers Arch. Ges. Physiol. 341:1-6.

Xuereb, G. P., M. M. L. Prichard, and P. M. Daniel. 1954. The hypophyseal portal system of vessels in man. Quart. J. Exp. Physiol. 39:219-230.

Yamamoto, M., N. L. Diebel, and E. M. Bogdanove. 1970. Analysis of initial and delayed effects of ovariectomy and orchidectomy on pituitary and serum LH levels in adult and immature rats. Endocrinology 86:1102-1111.

Yen, S. S. C., B. L. Lasley, C. F. Wang, H. Leblanc, and T. M. Siler. 1975. The operating characteristics of the hypothalamic-pituitary system during the menstrual cycle and observations of biological action of somatostatin. Recent Prog. Horm. Res. 31:321-357.

Zeilmaker, G. H. 1966. The biphasic effect of progesterone on ovulation in the rat. Acta. Endocrinol. (Copenh.) 51:461-468.

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1. Peng, M. T., and Huang, H. H.: Aging of hypothalamic-pituitary-ovarian function in the rats. Fertil and Steril. 23: 535-542, 1972.

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3. Huang, H. H., and Meites, J.: Reproductive capacity of aging female rats. Neuroendocrinology 17: 289-295, 1975.

4. Huang, H. H., Marshall, S., and Meites, J.: Induction of estrous cycles in old non-cyclic rats by progesterone, ACTH, ether stress or L-dopa. Neuroendocrinology 20: 21-34, 1976.

5. Huang, H. H., Marshall, S., and Meites, J.: Capacity of old versus young female rats to secrete LH, FSH and Prolactin. Biology of Reproduction 14, 538-543, 1976.

6. Meites, J., and Huang, H. H.: Relation of the neuroendocrine system to loss of reproductive functions in aging rats. In: Neuroendocrine Regulation of Fertility, pp. 264-258, Int. Sym. Simla, 1974. (Karger, Basel 1976).

7. Meites, J., Huang, H. H., and Riegle, G. D.: Relation of the hypothalamo-pituitary-gonadal system to decline of reproductive functions in aging female rats. In: Hypothalamus and Endocrine Functions, ed. by Labrie, F., Meites, J., and Pelletier, G. pp. 3-19. Plenum. Publ. Co., N. Y., 1976.

8. Steger, R. W., Huang, H. H., Kuppe, G., Meites, J., Hafez, E. S. E., and Ludwig, H.: Effect of age on uterine surface ultrastructure. Scanning Electron Microscopy, Proceedings of the workshop on SEM in Reproductive Biology, ed. by Zohari, O. IIT Research Institute, Chicago, Ill. pp. 359-366, 1976.

9. Steger, R. W., Peluso, J., Huang, H. H., Hafez, E. S. E., and Meites, J.: Gonadotropin-Binding sites in the ovary of aged rats. J. Reprod. Fert. 48: 205-207, 1976.

10. Bruni, J. F., Marshall, S., Huang, H. H., Chen, H. J., and Meites, J.: Serum prolactin, LH and FSH in old and young male rats. IRCS Med. Sci. Reprod. Obst. and Gynec., 4265, 1976.

11. Lu, K. H., Chen, H. T., Huang, H. H., Grandison, L., Marshall, S., and Meites, J.: Relation between prolactin and gonadotropin secretion in post-partum lactating rats. J. Endocr. 68: 241-250, 1976.

12. Lu, K. H., Huang, H. H., Chen, H. T., Kurcz, M, Mioduszewski, R., and Meites, J.: Positive feedback of estrogen and progesterone on LH release in old and young rats. Proc. Exp. Biol. Med. 154: 82-85, 1977.

13. Huang, H. H., Simpkins, J. W., and Meites, J.: Hypothalamic norepinephrine and dopamine turnover and relation to LH, FSH and prolactin release in old female rats. Endocr. Soc. Program and Astracts, pp. 331, 1977.

14. Simpkins, J. W., Mueller, G. P., Huang, H. H., and Meites, J.: Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats: possible relation to gonadotropin secretion. Endocrinology 100: 1672-1678, 1977.