MORPHOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THE NEUROENDOCRINE REPRODUCTIVE AXIS ACCOMPANYING THE AGING PROCESS IN THE FEMALE HAMSTER

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MOLECULAR BIOLOGY IN THE GRADUATE SCHOOL OF TEXAS WOMAN'S UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

BY

JANICE JUSTICE SISSOM, B.S., M.S.

DENTON, TEXAS

MAY, 1980

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

ACKNOWLEDGMENTS

The development and successful completion of this research program has been made possible by the coordinated efforts of many people. I would like to express my graditude to the following people:

Dr. Eugene Hupp, the dissertation director, for allowing freedom in the selection of the research project and its research design, and for his support throughout the completion of the graduate program, research, and writing.

My program and research committee - Dr. Kenneth Fry, for his leadership and support; Dr. John Hines, for his support and guidance; Dr. Alan Cockerline, for his inspiration and encouragement; and Dr. Bernard Rubal, for his interest and suggestions.

My employers at Texas College of Osteopathic Medicine for the use of their equipment and their patience in allowing me to continue my graduate program during these years of my employment and especially to my supervisor, Dr. James Carnes, whose constant encouragement made it possible to do both.

Marilyn Smith, Dr. Louise Higgins, and Susan Allen for their technical assistance during the final phases of the work.

With my deepest appreciation, I would like to acknowledge the enduring patience, support, and encouragement of my husband, Ray, and my three daughters, Robin, Renae, and Sandee, without whose dedication and help the present study could not have been completed.

DEDICATION

I wish to dedicate this dissertation to my husband, Ray, whose faith and dedication have allowed the successful completion of the research and writing.

INTRODUCTION AND LITERATURE REVIEW

The idea that all things die when they become old is not new. We readily accept the universal characteristic that living organisms develop, mature, senesce, and then die. If one examines the literature carefully, it is possible to trace the concepts of aging as they emerged in antiquity, developed through history and progressed into the present. Even in the years B.C., Aristotle compared old age to youth with respect to psychological aspects and hypothesized that "the incapacity of old age is due to an affliction not of the soul but of its vehicle" (quoted in Griffin, 1950). In 1513, attention was directed at an attempt to avoid this inevitable aspect of life as Ponce de Leon announced a belief in the legend of a Fountain of Youth and searched for it in the Bahamas (Encyclopedia Britannica, 1972a). About this time careful observations on the anatomy of human aging were being recorded by Leonardo da Vinci who claimed to have dissected "more than 30 bodies" until stopped by Pope Leo X. Emphasizing how to draw, he took careful notes which included anatomical changes occurring with age (Encyclopedia Britannica, 1972b; Encyclopedia Britannica, 1972c). The cause of this slow death was deemed to be due to a lack of nourishment when

veins "thickened their tunics" (Belt, 1952). A physiological approach by Sir Francis Bacon explained that disease in old men's bodies was less curable because "old men's bodies do neither perspire well, nor assimilate well" (Strong, 1952).

Modern biology has attempted to explain aging in terms of whatever biological phenomena were popular at the moment. The amount of data regarding theories of aging has accumulated, entire editions have been written, and extensive bibliographies compiled (Shock, 1957), and yet no conceptual framework within which to argue these theories has been provided (Kohn, 1978). As curious as man is known to be, it is difficult to explain his general lack of true research on this phenomena unless scientists simply prefer to investigate why systems improve than to understanding why they fall apart. Goals of modern aging research are not so much to extend the life span as to increase the health, productivity, and creativity of life until death. Since famine and epidemic have been greatly overcome, greater numbers of people are experiencing increased life span and the phenomenon of aging has advanced more to the forefront. This interest has supplied the stimulus to study the problem in an attempt to increase the quality of these additional years.

There exists considerable problem in definition of the term aging. References to aging include both gross deterioration in living systems due to the passage of time and progressive, irreversible alterations common in nature after maturity (Kohn, 1978). But, if one must consider the passage of time as an innate aspect of the definition, then one must perceive time itself with definition. This progressive deterioration of the organism may involve physiological change with age or an increased frequency of pathology due to "old-age" and aging may manifest itself differently in various organ systems. The primary change is most likely to occur in nondynamic systems which are incapable of self-renewal where the rate of aging becomes greater than the rate of replacement. Thus, the metazoan must pay a special toll for the advantage of cell specialization when aging results in a decline in function to the interdependence of cells (Pearl, 1921). Therefore, one of the chief objectives in research concerning aging is a description in terms of structure and function of the nature of such changes that cause dysfunction and ultimately death.

A variety of theories and proposed basic mechanisms of aging have been neither established nor disproved and remain at the level of hypothesis or theory. An early

theory attempting to explain the cause of aging involved toxic products which were picked up by the blood from the intestines (Metchnikof, 1907). As early as 1943, advances in protein chemistry brought hydrolytic enzymes into the discussion as the cause of deteriorating organization at the supracellular level which resulted in dehydration of body colloids (Kohn, 1978). This was substantiated by the observation of an increased number of lysosomes in deteriorating areas and lead to the theory that degradative enzymes recognize age-altered molecules and selectively degrade such structures (Kohn, 1978). Soon after the discovery of hormones, it was noted that all hormones decrease secretion with age except where compensatory changes are noted and endocrine failure was blamed for initiating the aging processes (Birren, 1960). This led to the "monkey gland" era in which aging due to endocrine failure was believed to be postponed via injection of hormones and rejuvenation made possible. During the second and third decades of this country, the pituitary gland gained fame as a master gland and, with the fame, came the pituitary hypothesis of aging. It was believed that, since the pituitary is responsible for the onset of certain age-related phenomena (such as puberty), it may also secrete hormones which directly, or indirectly through target glands,

stimulate physiological aging and thus promote age-related pathology which ultimately shortens the life span.

Current theories include the belief that loss of pituitary function affects the ability of the body to maintain its homeostatic condition (Everitt, 1973), and that change is possibly due to abnormal pituitary response to hypothalamic regulation, loss of feedback and change in hypothalamic centers (Hafez, 1976).

Several approaches to the study of aging have been attempted. The general trend was originally to study the organism at the organ or tissue level. It has recently been suggested that one should study the fundamental process of aging by investigating the fundamental units through the study of cells (Bourne, 1961), organelles, and to ultimately connect these with specific biochemical events. As the organism deteriorates in a predictable fashion, the duty of the scientist is to strive for an understanding at the cellular and molecular levels (Cherkin et al., 1979). With the advent of molecular biology came a new and totally unique approach. Altered gene transcription and translation, somatic mutations, and immunologic reactions were given the role of the villain in the saga of aging and the molecular era was born. Explosive progress in the field of molecular biology yielded the "error theory" of aging which stated

that an accumulation of errors in protein synthesis was responsible such that altered protein molecules accumulated with age (Strehler, 1977; Ordy and Brizzee, 1975). Since hormones were known to regulate the expression of genes, they were once again indited (Everitt and Burgess, 1976).

All advances should now be re-examined in the light of new understandings in such fields as molecular biology and genetics. This molecular-genetic revolution, which was heralded by the discovery of DNA structure in the early 1950's (by Watson and Crick), the description of the characters involved in protein synthesis (by Zamecnek, Kornberg, and others) and the identification and verification of the code (by Nirenberg, Ochoa, Khorana, and others) yielded new insight into the aging process and opened the door for experimental testing. It became apparent that chemical aging depends upon which processes are permitted to occur and which are inhibited or have their consequences reversed (Strehler, 1977). As one looks at this interaction between the genetic program and the environment, one must carefully distinguish between facts and hypotheses.

Recently the causes of aging have been divided into two distinct classes. Primary, or physiological causes are unavoidable, are the primary cause of aging, and are present in all individuals of all species. The only one

which we know much about is the heredity factor. We understand secondary causes to a greater degree. These include all things of accidental nature which aggravate the process of aging (Korenchevsky, 1961). Most gerontologists are generally agreed that there is probably no single cause of aging and that whatever the causes may be, they do not produce similar changes, or similar rates of change, in each cell of an organism.

The effects of aging upon the reproductive axis have held the innate interest of man for years, especially since the first link of endocrinology to gerontology which occurred in 1889 when Brown-Sequard reported rejuvenation via extract from dog testis (Finch and Hayflick, 1977). Aging of the reproductive system is of interest to a multiphasic group. The social and psychological aspects involved with the dramatic changes occurring at puberty and menopause greatly effect society. Researchers, laymen, and physicians seek to understand the anatomic and physiological aspects (Birren, 1960). The mid-life onset of irregularity, especially in the female cycle, has been a well-accepted phenomena for many years but remains little understood. The ovary has advanced to the forefront as an important gerontological research tool since it has a pronounced life cycle and its senility precedes that of

other organs (Bourne, 1961). It is clear that follicles cease to develop and ovulation ceases but detailed information on ovarian aging in older mammals is scanty. Aging of the reproductive axis of lower animals induces such changes as a decrease in mean litter size (Talbert, 1968; Ingram, et. al., 1958; Adams, 1910) and frequency of birth and an increase of fetal abnormalities and mortality (Fugo and Butcher, 1971; Harmon and Talbert, 1970; Thorneycroft and Soderwall, 1969). Experimental gerontologists must also consider two aspects of relating aging to reproduction: (1) the effect of reproductive processes on biological aging and (2) the influence of biological aging on reproductive processes.

In order to better understand the mechanisms involved in female reproductive failure, one must investigate all available facts of normal female reproduction. These include ovarian factors as well as extraovarian (involving endocrine and neural factors). The fact that major, irreversible aging changes occur within the ovary has lead gerontologists into an analysis of female reproductive aging. The analysis of the mechanisms regulating these events has proven to be a challenging problem. More than three centuries ago, the process of ovulation was very accurately described by Regnier DeGraff (1672; trans., 1974). Since that time,

a mass of basic information has been published concerning the morphology of ovulation, and yet there exists great confusion, misunderstanding of terms, and lack of agreement on ovarian morphology and the control of ovulation.

Extraovarian factors contributing to these changes must not be overlooked and must include both the pituitary and the hypothalamus. The three point axis must be investigated in order to fully understand the changes. However, research on this neuroendocrine axis as related to aging changes is sparse. Changes in reproductive organs have been associated with degenerative changes in the pituitary and hypothalamus but the exact nature of the relationship remains hidden (Hafez, 1976). Any change in function as a consequence of aging of the pituitary-ovarian axis could conceivably result in the decreased fertility noted in lower animals. Since the exact site of aging had long been sought, the pituitary was an obvious choice once its control over the endocrine glands was recognized. Simmonds (1914) was the first to suggest that the anterior pituitary might accelerate aging via hormone deficiency or glandular hyperfunction.

It has been well established that development of follicles within the ovary and hormonal secretion by the ovaries is a direct result of stimulation by the gonadotropic hormones from the pars distalis of the pituitary (Jeffcoat

and Hutchinson, 1978; Turner and Bagnara, 1971; Guyton, 1971). It has been determined that cell type within this tissue varies with hormone production. Chromophils, those cells which contain granules that readily take up dye, secrete hormones and it is generally accepted that chromophobes, those whose granules do not stain greatly, do not secrete hormones (Martin, 1976). Chromophils can be farther subdivided into acidophils and basophils according to their staining characteristics.

The basophils secrete the gonadotropins FSH (follicle stimulating hormone) and LH (luteinizing hormone). These cells have been reported to undergo functional changes associated with physiological or pathological states and information on the relationship between morphological appearance and cell hormone content is becoming increasingly valuable as a one-cell, one-hormone hypothesis is evolving (Harris and Donovan, 1966; Young, 1961).

Attempts to subdivide these gonadotrophs further into specific folliculotrophs and interstitiotrophs have resulted in great confusion (Martin, 1976). Unless the animal has been subjected to some pretreatment, such as castration, that could influence function, the distinction is essentially non-existent. Antibody studies utilizing anti-FSH and anti-LH have demonstrated common binding sites on the same

cells (Kerdelhue' et al., 1975). The chemical nature of the hormone itself can vary with the endocrine statis of the animal. In the female, it is difficult to sort out pure LH actions because the release of FSH and LH is essentially simultaneous in most species. In spite of great interest in the area of hormone research, the exact relationship between estrogen feedback and gonadotropin release by the pituitary remains to be clearly defined. The first detailed aging study of the pituitary was done as early as 1925 (Cooper, 1925) and entire books have since been written; however, pituitary changes with age remain surrounded by a shroud of conflicting opinions on such matters as size and weight, invasion of the posterior lobe by basophils, and percentage of basophils (Andrew, 1971; Bourne, 1968; and Korenchevsky, 1961). The fact that reproduction can be influenced by such external factors as light or emotion has been accepted for centuries indicating that reproductive events such as libido or cycles must be subordinated to the brain. This involvement of the central nervous system was postulated as early as 1947 (Green and Harris, 1947) but the anatomical basis of such control remained obscure.

During the past three decades, physiological and morphological evidence has accumulated indicating that secretion of FSH and LH from the adenohypophysis is

ultimately connected to the central nervous system via stimulatory hormones from the hypothalamus which serves to receive stimuli, process the information, and transmit the signals (Martin, 1976; Schally, et al., 1973; Burgus and Gullemin, 1970; Schally, et al., 1968). The releasing factors are secreted by neurons of the arcuate nucleus and transported to the adenohypothesis by a portal vascular system (Martin, 1976; Harris and Donovan, 1966; Harris, 1955; Green, 1952; Green, 1951). Recently these releasing factors (RF) have been isolated and their structures determined, ushering in a new era of neuroendocrinology. The presence of LH-RF , which is thought to stimulate the release of both LH and FSH was first demonstrated in the 1960's (Campbell et al., 1964; Schally and Bowers, 1964; McCann, et al., 1960) and its isolation occurred in the early 70's (Amoss, et al., 1971; Schally, et al., 1971a; Schally, et al., 1971b), and was soon followed by the characterization of its decapeptide structure (Baba, et al., 1971; Matsuo, et al., 1971). The synthesis of this neurohormone, and the subsequent synthesis of acceptable analogs have opened a new field of research to gain knowledge of their mechanisms of action (Geiger, et al., 1971; Matsuo, et al., 1971; Sievertsson, et al., 1971).

Little evidence of morphological changes in the hypothalamus has been gathered that is specifically associated with change in reproductive capacity and those reported changes have been fragmentary and contain many points of controversy (Finch and Hayflick, 1977). The obvious importance of such changes lies in the fact that any change in the tissue will ultimately affect the pituitary-ovarian axis. Such changes as would result in age-associated changes of function within the reproductive system have not been thoroughly investigated.

The menopause has been associated with disturbances in the hypothalamo-pituitary-ovarian axis and the aging ovary looses its sensitivity to gonadotropins. Future research is vital not only to understand the changes in this axis itself, but also those changes of clinical importance occurring within the ovum that cause an increase in spontaneous abortions, congenital abnormalities, and intrauterine loss in the aging animal (Hafez, 1976).

Ovulation, both induced and natural, has been studied in a variety of animals. Reproductive biologists have searched for years for a natural phenomena exhibited by an animal by which ovulation could be readily detected. Efforts have included observation of menstruation (Koering, 1969), cervical mucous secretions (Ovadia, et al., 1971; Vickery

and Bennett, 1968), use of vaginal concretions (Alleva, et al., 1976), and the prediction of time of ovulation from hormone profiles (Barnes, et al., 1978). An ideal animal would have a short estrous cycle, readily detectable such that normal ovulation could be studied without the aid of an exogenously supplied hormone. This has been accomplished through the use of a variety of vaginal smear techniques, but is cumbersome for the experimentor (Schuchner and Stockert, 1974; Alleva, et al., 1971b; Kent and Mixner, 1945).

Most of the papers and reviews of animal care and breeding which have been published on the estrous cycle of the hamster have tended to ignore the external vaginal phenomena characteristically exhibited by the female hamster during each day of the four-day estrous cycle. Evidence will be gathered to examine the regularity of the hamster in expressing these characteristic phenomena for the four physiological estrous states (Orsini, 1961). Although the validity of the procedure has never been substantiated in the literature, several papers have been written with this phenomena used consistently as the basis for the research (Leavitt and Blaha, 1970; Lukaszewska and Greenwald, 1970).

This study will consist of light and ultrastructural observation of the hypothalamic-anterior pituitary-ovarian axis in old cycling and constant estrous female hamsters and compare with similar observations in 3- to 6-month-old cycling female hamsters on each day of the estrous cycle. Research objectives of the present study are to:

1. Investigate the reliability of vaginal phenomena as an indicator of the daily morphology of the ovary behavioral estrous, and of ovulation in the hamster
2. Describe the histological and ultrastructural morphology of the reproductive neuroendocrine axis (ovary, pituitary, and hypothalamus) representative of each cycle day for an animal of full reproductive age
3. Correlate daily changes with the process of ovulation
4. Investigate the ability of a post-reproductive, aging animal to express the characteristic vaginal phenomena
5. Describe the histological and ultrastructural reproductive neuroendocrine axis morphology of each cycle day in the aging hamster

The present study was undertaken in an attempt to describe observations on the hypothalamic, anterior

pituitary and ovarian morphology and related ultrastructural observations of aged hamsters, and compare them with those of young hamsters. Despite the widespread interest in the area of reproductive endocrinology, the precise relationship of morphological changes to the process of ovulation has yet to be clearly defined. Since "aging" is one of the most universal, inevitable consequences of nature, it is of benefit to seek an understanding of the process at the cellular level. If we understand these mechanisms more completely, we can better deal with human anxiety in such areas as infertility, psychosexual problems and menopause. Fundamental research in this area done on short-lived mammalian species could stimulate the transfer of a considerable body of new understanding to man and shift the emphasis from death and dying to more interest in increasing the quality of life throughout senescence.

METHODS AND MATERIALS

Animals

Although the hamster has a relatively short history as an experimental laboratory animal, the breeding habits have been studied extensively (Kent, 1968; Bond, 1945). The body of knowledge dealing with reproductive events in the hamster exceeds that of most other mammals. The species exhibits many advantageous characteristics which make it valuable in certain research phases, especially those involving reproduction. The attractiveness of the female hamster as a research animal in this aspect is enhanced by its availability, relatively short life span, and the fact that it is a polytocous animal. It is large enough to collect tissue, yet small enough to maintain in large numbers. The hamster will be exclusively used in the study due to a short quadradian (four-day), regular estrous cycle, easily characterized by external vaginal phenomena with a conspicuous post-ovulatory discharge on Day 2. It is an excellent laboratory animal due to its ability to do well on minimal care and space and to its relative freedom from disease. Animals will not be subjected to any pretreatment regime to influence function as it is the goal of the research to investigate natural

physiological aspects of the aging process upon the reproductive axis.

It is accepted that reproductive events in this animal are synchronized by one or more oscillators and that some events are under photoperiodic control. Therefore, two age groups of Texas Woman's University albino stock hamsters, each weighing approximately 130 g, were housed in thermostable animal rooms (temperature $20^{\circ} \pm 4^{\circ}$ C) and maintained on a photoperiod of 12:12 light:dark (8 a.m. to 8 p.m.) (Alleva, et al., 1971a; Alleva, et al., 1968). To insure that all animals were free of infection a closed colony was maintained during the entire study. Littermates were caged together in plastic shoe box cages with shredded corncob bedding and the bedding changed weekly (San-i-cell, Paxton Processing Co., Inc., Illinois). Animals were individually caged for the final phase of each program. Diet consisted of Purina Laboratory Chow and tap water ad libitum.

Experimental Design (Tables 1 and 2)

Hamsters were divided into two groups. The first group (group A) were 2- to 6-months old and considered to be of prime reproductive status. Group B animals were 18- to 24-months old and considered to be post-reproductive. Hamsters were divided into subgroups, with each of the subgroups in Phase 1 containing a minimum of three animals;

TABLE 1

Experimental Design: Correlation of Vaginal Phenomena with Ovarian Morphology and Behavioral Estrous.*

Type of Study	Group	Subgroup	Day of Cycle	Number of animals
Phase 1 - Histological determination of ovulation				
Light histology and scanning electron microscopy	A	1	1	3
	A	2	2	3
	A	3	3	3
	A	4	4	3
Phase 2 - Behavioral estrous				
Placed with male for 15 minutes. Record litters and number born per litter.	A	5	1	20
	A	6	2	20
	A	7	3	20
	A	8	4	20
Placed with male for 1 week. Record litters and number born per litter.	A	9	disregarded	20
	B	10	disregarded	20

*Group A represents sexually-mature female hamsters (2- to 6- months-old)
Group B represents aged female hamsters (18- to 24- months-old)

TABLE 2

Phase 3

Experimental Design: Histological and Ultrastructural Analysis

Group	Subgroup	Day of Cycle	Number of Animals
A	11	1	4
A	12	2	4
A	13	3	4
A	14	4	4
B	15	1	4
B	16	2	4
B	17	3	4
B	18	4	4
B	19	acyclic	4

The hypothalamus, pituitary and ovaries were collected from each animal. Each tissue was analyzed by means of light microscopy. One ovary from each animal was analyzed by means of scanning electron microscopy and transmission electron microscopy was utilized to evaluate the pituitary.

* Group A represents sexually mature female hamsters

Group A represents aged female hamsters

in phase 2, 20 animals; and phase 3, 4 animals. Care was taken to avoid placing littermates in the same subgroups.

Determination of cycle day (table 3)

Female hamsters were carefully observed for the purpose of determining cycle day via vaginal characteristics (Table 3). Vaginal phenomena were recorded daily between 8:00 and 10:00 a.m., and only those animals which displayed three consecutive cycles were used as subgroups 5-18.

Determination of estrous (table 1, phase 2 and table 2, phase 3)

Phase 2 was done in order to confirm the identification of stages of the estrous. Eighty 2- to 6-month-old female hamsters were observed for two cycles and vaginal phenomena recorded. Twenty females were selected to represent each of the four days of the cycle. They were introduced into a cage occupied by a male between 5:00 and 6:00 p.m. and observed for 15 minutes. The male and female were then separated and copulation recorded. The percentage of litters obtained and the size of the litters were recorded. Another group of 20 2- to 6-month-old female hamsters without the benefit of vaginal reading were introduced into a cage occupied by a male and left together

TABLE 3

Daily vaginal phenomena of the female hamster

Estrous cycle day	Characteristics
1 "estrous"	Thick mucous Opaque, cream-colored in appearance
2	Very viscous, wax-like discharge Forms well-rounded plug Strings out on contact Has distinct, musky odor
3	Waxy, convoluted plug If plug is lost prior to examination, vagina will be slightly distended and dry
4	Delicate non-mucous, translucent secretion Forms fine cobweb on contact

Source: Orsini, 1961

for one week. The male and female were then separated and percentage of litters obtained and size of litters recorded.

Histological and ultrastructural comparison (table 2)

The purpose of phase 3 was to describe the histological and ultrastructure morphology of the reproductive axis which is representative of each day of the four day estrous cycle in a normal, sexually mature female hamster. This reproductive axis includes primary reproductive organs (ovaries), endocrine tissues (anterior pituitary) and neuroendocrine tissue (ventromedial hypothalamus).

Analysis was accomplished by utilizing light microscopy, and scanning and transmission electron microscopy. Electron microscopy reveals details in greater depth than light microscopy such as size, shape, and numbers of secretory granules, and morphology of subcellular organelles, but possesses two innate drawbacks; much time and skill is required for preparation and only a small fraction of the tissue is examined. Therefore, this study utilized both light and electron microscopy techniques and an attempt was made to correlate the alteration in fine structure with cytological studies. These daily changes were correlated with the process of ovulation and the day of ovulation identified.

The aged-animal study (phase 3, table 2) was done to investigate the ability of a post-reproductive aging animal to express the vaginal phenomena representative of each day and to accomplish ovulation. The histology and ultrastructure of the ovarian-pituitary-hypothalamic axis was examined and changes correlated with the process of aging. Litters born and litter size was investigated in phase 2 (table 1).

Sampling of tissue

Hamsters were killed between 10 and 12 a.m. on the desired day of the cycle after three consecutive estrous cycles had been demonstrated to obtain tissue for histological and ultrastructural analysis. They were anesthetized with an intraperitoneal injection of 15 mg of pentobarbitol sodium solution. A mid-abdominal incision was made to facilitate removal of both ovaries. The dorsal skin folds were clipped from the neck and the cranial cavity entered from the base of the skull with bone forceps. The brain was carefully removed, trimmed anteriorly at the optic chiasma and posteriorly at the mammillary bodies. The pituitary was removed from the sella turcica, and the anterior protion trimmed and fixed.

Ultrastructural procedures

Scanning electron microscopy was done on one ovary from each animal involved in this study. Tissue was fixed in .2 M cacodylate buffered glutaraldehyde and prepared for SEM according to accepted procedures (Hayat, 1978; Hayat, 1975). The procedure is outlined below:

1. Ovary was obtained from animal and cleaned of all extraneous membranes and fat
2. Tissue was fixed and stored until use in .2 M cacodylate buffered glutaraldehyde
3. Tissue was dehydrated through a graded series of alcohols
4. Tissues were subjected to critical point drying with CO_2
   a. Flush 3 times with CO_2
   b. Allow to remain in CO_2 for 15 min
   c. Dry
5. Tissueswere then mounted on an aluminum stub with double-sided tape
6. Tissueswere coated with a layer of gold in a Bomar Vacuum machine
7. Tissues were examined and photographed on an AMR scanning electron microscope

The ripest follicle of each ovary and a small piece of anterior pituitary were obtained from each animal, fixed in .2 M cacodylate buffered glutaraldehyde and prepared for transmission electron microscopy according to accepted procedures (Weakley, 1972). The procedure is outlined below:

1. After removal, the tissue was fixed and stored until use in .2 M cacodylate buffered glutaraldehyde
2. Tissue was fixed in .2 M cacodylate buffered OsO_4 for 45 min
3. Tissue was rinsed in .2 M cacodylate buffer
4. Tissue was dehydrated through a graded series of alcohols
5. Preparation of epon
   a. Mixture A = 62 ml epon 812 + 100 ml dodecenylsuccinic anhydride
   b. Mixture B = 100 ml epon + 89 ml nadic methyl anhydride
   c. Final mixture = 30 ml mixture A + 20 ml mixture B + .75 ml DMP-30
6. Tissue was placed sequencially in propylene oxide; 50% propylene oxide; 50% epon; epon
7. Tissue was placed in epon in numbered beem capsules and placed in an oven at 70° C overnight

8. Beem capsules were sectioned on an ultramicrotome and mounted on copper grids
9. Sections were stained with a saturated solution of uranyl acetate followed by Reynolds' lead citrate
10. Sections were rinsed well and allowed to drain dry
11. Sections were examined and photographed on a Siemens Model III transmission electron microscope

Light microscopy

Tissues were fixed approximately 24 hours in FAA and prepared for light microscopy by accepted methods of dehydration and embedded in paraffin (Humason, 1972). Transverse sections 5μ in thickness were cut from paraffin blocks on a Spencer "820" microtome. Hypothalamus, ovary, and a sample of each of the anterior pituitary tissues were stained with hematoxylin and eosin (Humason, 1972). There exists great confusion in the literature with respect to staining of pituitary cells to characterize and differentiate the gonadotrophic cells of the anterior pituitary. Different systems of nomenclature range from names for cell types based on hormone secretion to morphological characteristics of the cells themselves. Pituitaries from different species

may react with varied color specificities. Herland methods of staining basophilic cells are highly recommended by Humason, but several variations are included and the difficulty of separation of chromophobes from chromophils may be compounded by transectional stages or the state of secretion of a particular cell. Therefore, several of these methods were utilized in an attempt to differentiate the gonadotrophic cells. As other investigations have noted, these methods were unsuccessful in subclassifying basophilic cells (Thompson, 1960; Serber, 1958).

RESULTS

I. Assignment of vaginal phenomena to cycle day

Vaginal phenomena as shown in Table 3 were very consistent with cycle day. Animals cycled regularly through each of the four days. The cycle days were arbitrarily numbered with Day 1 corresponding to behavioral estrous (Orsini, 1961). Day 1 animals showed a thick mucous just inside the vagina. It is opaque, cream-colored, and tends to string out when touched. There is no characteristic odor (Fig. 1). Day 2 animals display a viscous, cream-colored discharge with a characteristically musky odor. This becomes more solid during the day and may take on wax-like characteristics by mid-afternoon. The musky odor is prevalent throughout the day (Fig. 2). In Day 3 animals, the wax-like discharge has solidified into a much-convoluted, easily lost plug. Often examination will dislodge the plug, leaving the vagina dry and distended with no secretion (Fig. 3). Day 4 secretion is often almost dry and may be overlooked by a casual approach. Upon close observation, a slight, transleucent, non-mucous secretion may be detected. There is no odor associated with this day (Fig. 4).

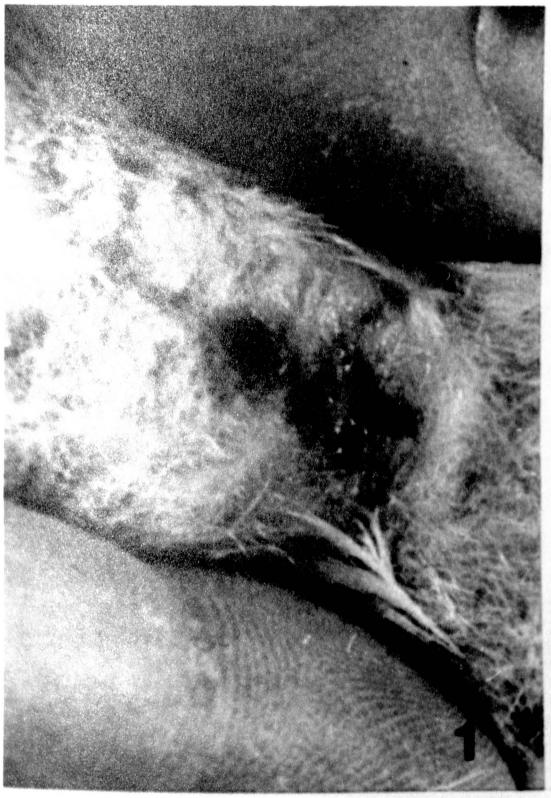
Figures 1-4. Vaginal phenomena observed in each day of the hamster estrous cycle are seen by photography.

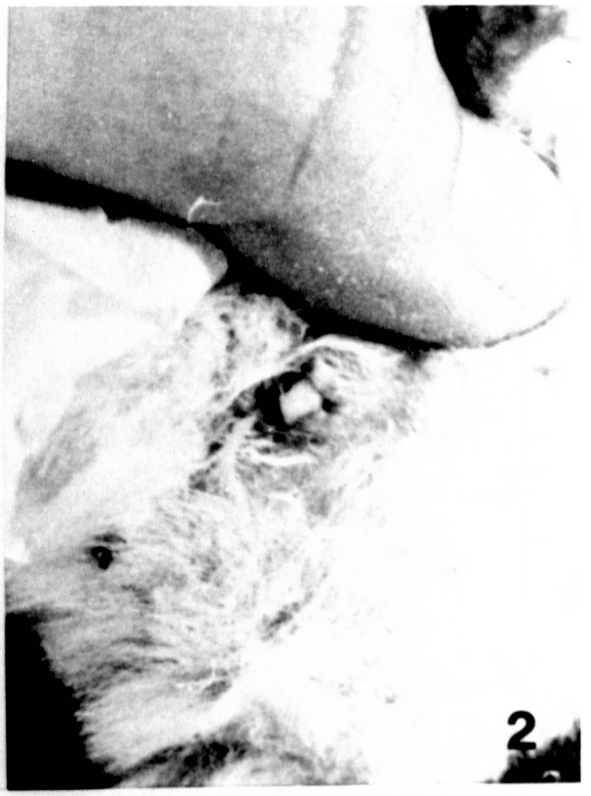
Figure 1. Day 1 (Estrous). The vagina secretes a thin, opaque mucous on the morning when the female is in heat.

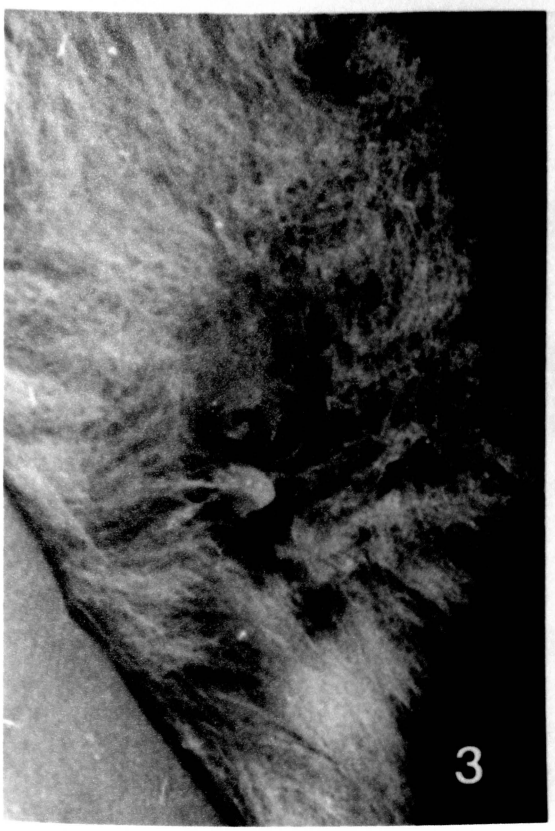
Figure 2. Day 2. Post-estrous discharge is easily distinguished. It has become thick, cream-colored, and has a distinct musky odor.

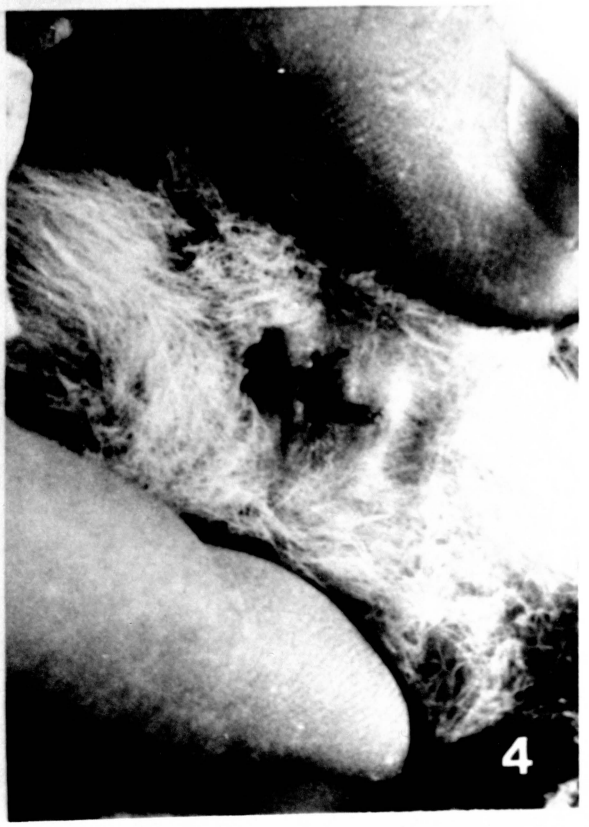
Figure 3. Day 3. Discharge is waxy and more solid. It may be convoluted or may have been lost, leaving a distended dry vagina.

Figure 4. Day 4. The sparse Day 4 discharge is translucent, nonmucous and has no odor.


1


2


3


4

II. Determination of behavioral estrous and its correlation to cycle day

For the determination of estrous, female hamsters were observed for three cycles and vaginal phenomena recorded. 20 females were selected to represent each of the four days of the cycle (subgroups 5-8). They were introduced into a cage occupied by a male between 5:00-6:00 p.m. and observed 15 minutes. The male and female were then separated and copulation recorded. Animals which had displayed Day 1 phenomena at 10:00 a.m. were the only females to ever accept a male in the afternoon of the same day (Table 4). The 20 females selected to represent each of the other 3 days of the cycle refused the males and often displayed aggitation and aggressive behavior in response to the advances of the male. Nineteen out of 20 females displaying Day 1 phenomena (95%) did accept the male and 100% of these 19 litered with an average litter size of 11.4. The one female that was unresponsive displayed Day 2 postovulatory discharge at 10:00 a.m. on the morning of the following day.

III. Morphological observation of ovaries from young animals

Due to the fact that ovulation occurs early on the morning of Day 2, Day 3 is considered first such that

TABLE 4

Behavioral Estrous and Litter Data

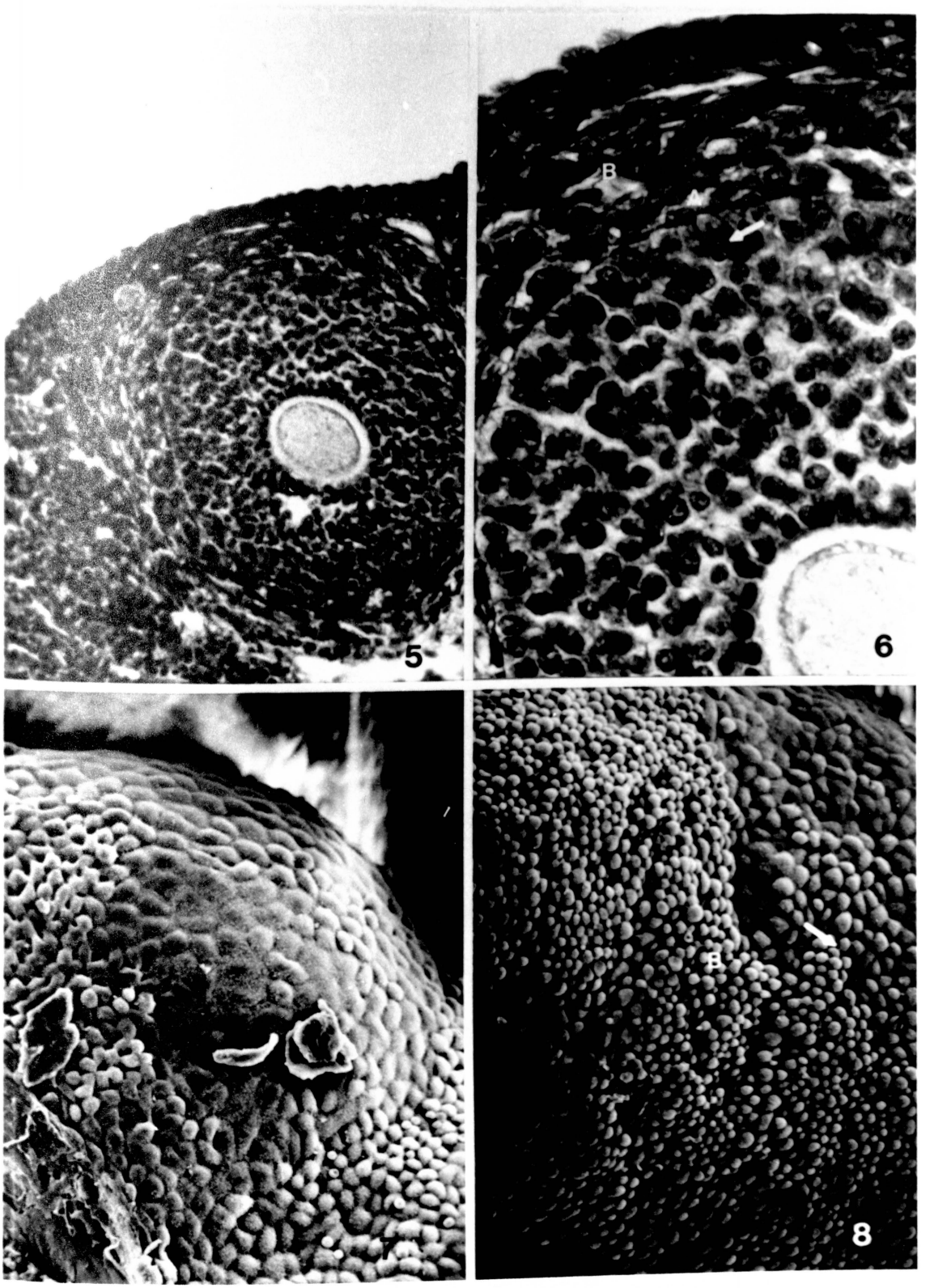
Group	Age	Cycle Day	Percent to accept male	Percent to litter	Average litter size
A	Young	1	95	95	11.4
A	Young	2	0	0	0
A	Young	3	0	0	0
A	Young	4	0	0	0
A	Young	disregarded	not observed	85	9.1
B	Senescent	disregarded	not observed	10	3

the events leading up to ovulation may be considered. This investigator considers these results to be representative of each cycle day and to have been displayed by most of the animals, however; frequency of occurrence for each phenomenon was not recorded. Ovaries from sexually mature animals displaying Day 3 vaginal phenomena (Figs. 5 and 6) showed several early, immature follicles developing within the ovarian cortex. The epithelial layer was continuous, a poorly developed zona pellucida was present, granulosa cell layers were 8-10 cells in thickness but were diffusely scattered. Scanning electron microscopy revealed immature follicles of Day 3 animals (Figs. 7 and 8) showing relatively smooth ovarian surfaces covered by polyhedral cells with numerous microvilli.

Ovaries from animals displaying Day 4 phenomena (Figs. 9 and 10) had begun to show a separation of the follicle cells into two layers. As the oöcyte grew to full size, the antrum appeared and the granulosa cells proliferated. The zona pellucida became distinct, and was surrounded by 4 or 5 layers of granulosa cells. Blisterlike projections were apparent on ovarian surfaces from Day 4 (Figs. 11-12) animals. The lateral walls had become elongated and the apex cells had flattened.

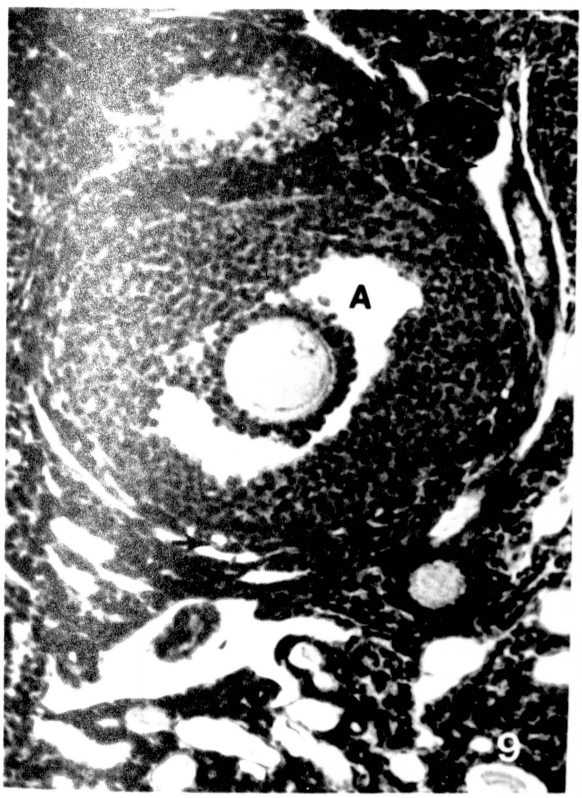
Figures 5-6. Light micrographs reveal characteristics of Day 3 ovary. (Fig. 5) The cortex of the ovary contains early stages of developing secondary follicles. The epithelial cell layer is continuous. Ovum is oval shaped and is surrounded by zona pellucida. A thick granulosa layer 8-10 cells thick surrounds the ovum. X 252. (Fig. 6) Higher magnification of cell layers shown in Fig. 5 shows a high number of cells in mitosis. The arrow indicates a cell in mitosis. Note the overall compactness of the theca interna (A) and the theca externa (B) areas and the continuity of cubodial cells of the germinal epithelium. A layer of connective tissue called the tunica albuginea is apparent. X 750.

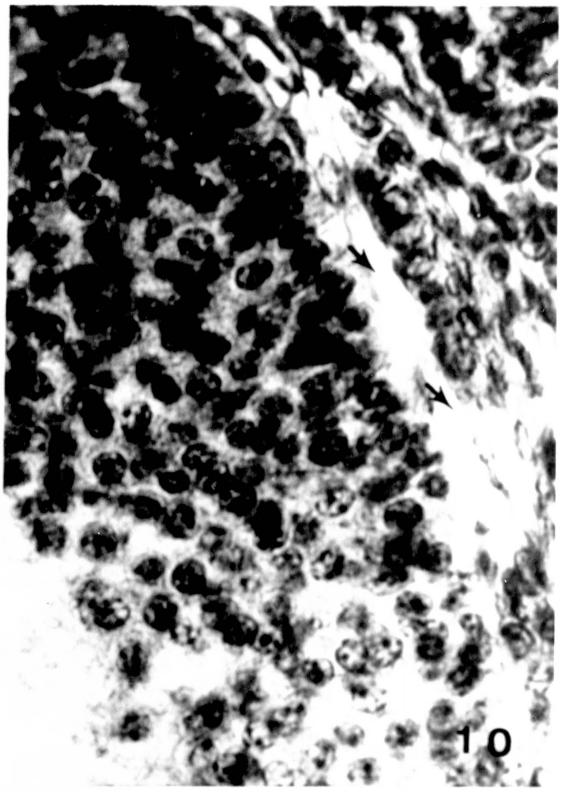
Figures 7-8. Scanning electron micrographs of Day 3 ovary show early follicular growth. (Fig. 7) An early follicle develops and begins to distend the outer cell layer of the ovary. X 500. (Fig. 8) Higher magnification of cells shown in Fig. 7 shows that basal cells (B) are polyhedral and exhibit many extensions between cells (arrow). Apical cells are flattened and lack microvilli. X 500.

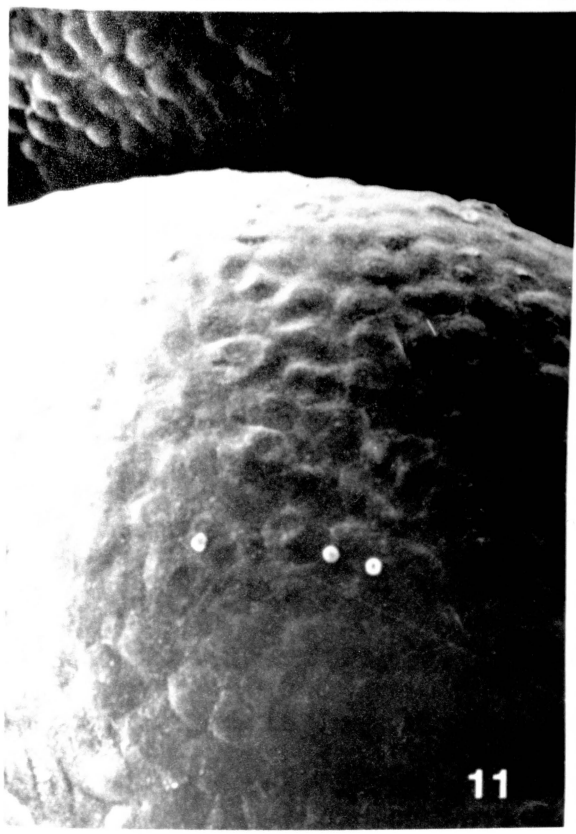

B
5
6
B
8

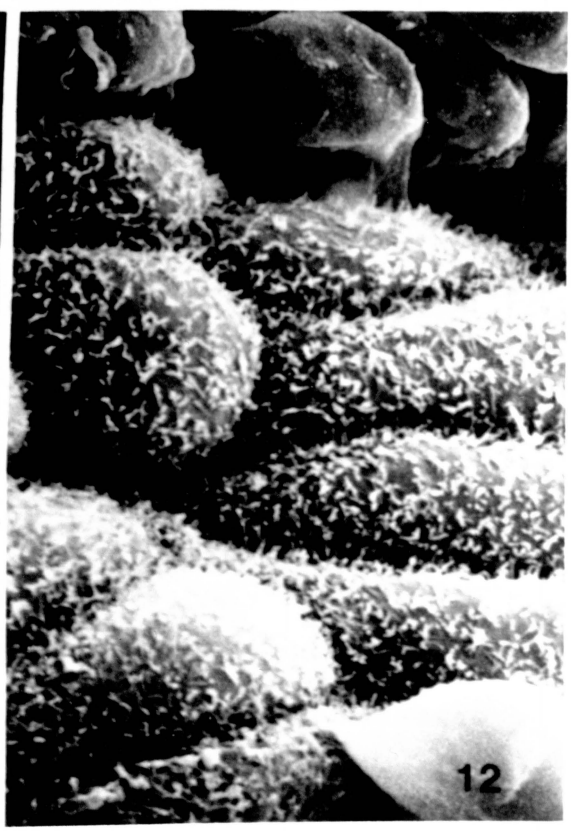
Figures 9-10. Light micrographs illustrate changes in the Day 4 ovary. (Fig. 9) Fluid-filled spaces have appeared among the granulosa cells and coalesced to contribute to the early formation of the crescent-shaped antrum (A) in the stratum granulosa of the follicle. Edema (arrows) is becoming apparent just beneath the epithelial layer. X 302. (Fig. 10) Slight hemorrhage can be seen at higher magnification and the edema (arrows) is conspicuous resulting in a slight separation of the granulosa layer from the theca interna. X 900.

Figures 11-12. Scanning electron micrographs of Day 4 ovary show developing follicles. (Fig. 11) Two developing follicles bulge markedly from the ovarian surface. X 480. (Fig. 12) The lateral wall cells become stretched and distorted. Cell to cell contact disintegrates. X 3000.


A
9


10


11


12

Follicle cells had separated into two layers and were prevalent in the bulging follicles of the Day 1 animals (Figs. 13 and 14) in the formation of a fluid-filled antrum. Pronounced edema was seen just beneath the surface epithelium and hemostasis was seen within the granulosal area. The cumulus oöphorus became elevated and undercut. Ovaries from Day 1 animals (Figs. 15 and 16) showed large areas of cellular disruption resulting in a sloughing off of cells exposing connective tissue and allowing fluid-like substances to ooze from the cells below and out onto the surface (Fig. 16).

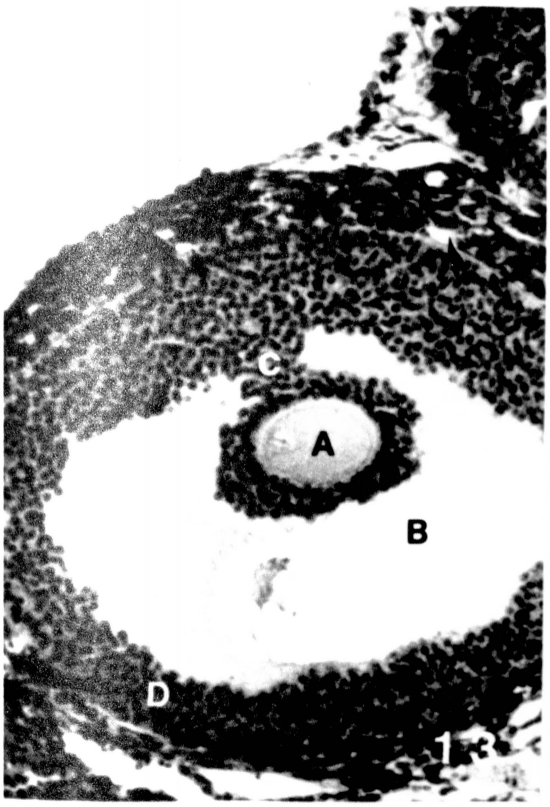
Animals with Day 2 phenomena (Figs. 17 and 18) showed collapsed follicles and evolution of early corpora lutea. Day 2 animals (Figs. 19 and 20) showed cells remaining in the area of the ruptured follicle that had become smooth, devoid of all microvilli and had begun to hypertrophy.

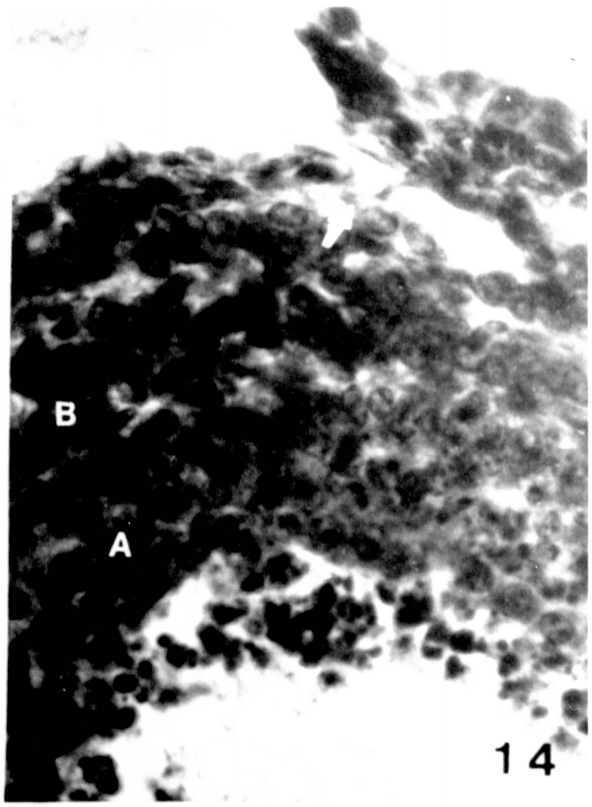
IV. Morphological observation of ovaries from senescent animals

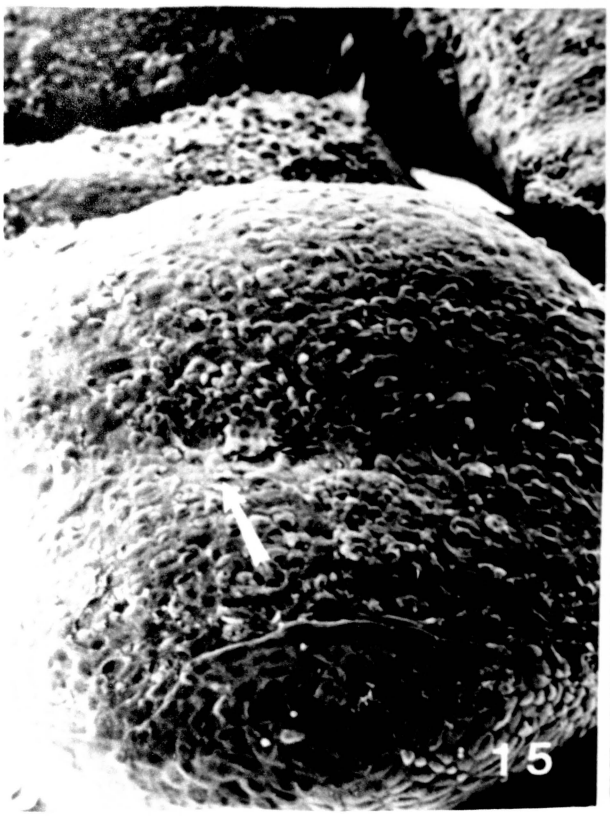
Due to the fact that ovulation is expected to occur in the morning of Day 2, the initial day of the cycle to be considered is Day 3. Ovaries from senescent hamsters contained greater numbers of atretic follicles than those

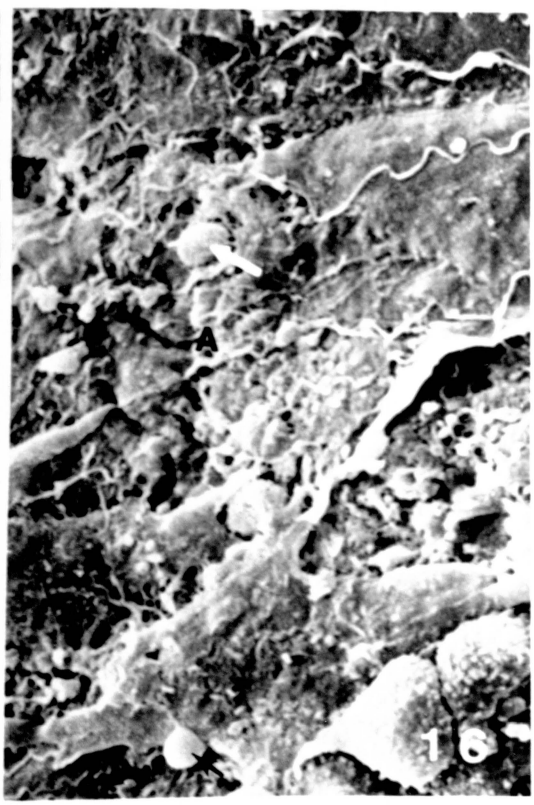
Figures 13-14. Light micrographs of Day 1 ovary show maturation of the antrum. (Fig. 13) A young Graafian follicle displays both ovum (A) and antrum (B). The cumulus oöphorus (C) is more compact than that of the previous day and the oöcyte has become eccentrially situated within the antrum. The antrum enlarges and fills with fluid. Mural granulosa has formed (D). Edema (arrows) has become more prevalent, extending throughout the theca externa and theca interna. X 315. (Fig. 14) Granulosal cell layer is now 8-10 cells in thickness and the cells are smaller and more compact. Epithelial layer is seen sloughing off (arrow). Delicate basement membrane can be seen separating the mural granulosa cells (A) from the theca interna (B). X 945.

Figures 15-16. Scanning electron micrographs of Day 1 ovary reveal large bulges on the ovarian surface. (Fig. 15). Apical area of bulge shows degenerative nature of the epithelial area (arrows) and loss of contact as cells slough off. X 250. (Fig. 16) A greater magnification of the area exposes the connective tissue (A) where epithelial cell loss has occurred. Droplets of fluid (arrow) ooze from beneath and are visible on the surface. X 2500.


C
A
B
D
13

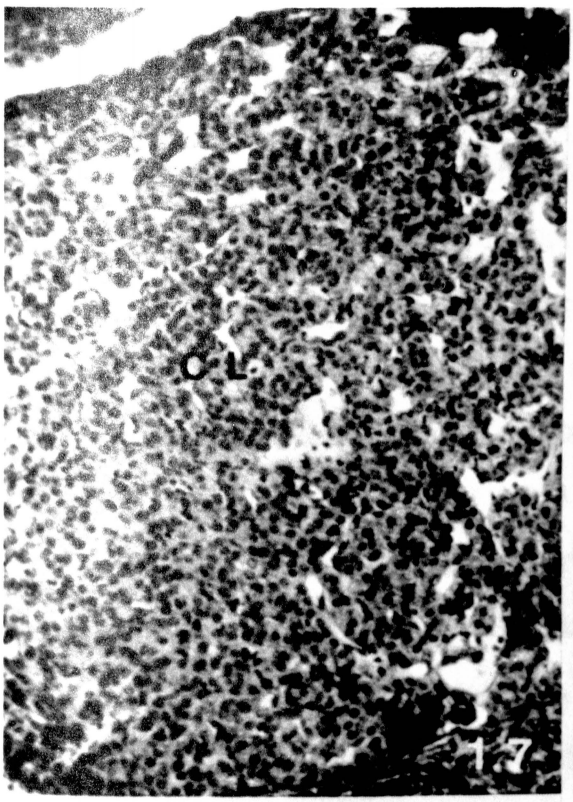

B
A
14

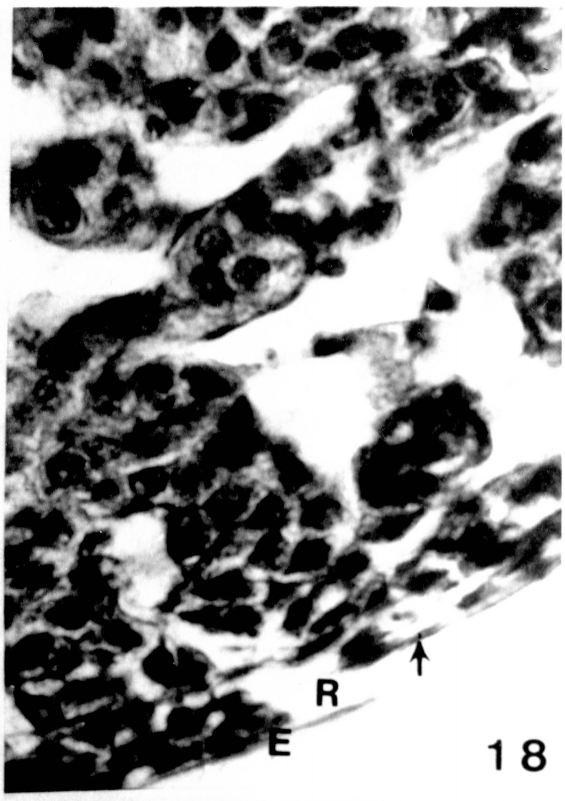

15

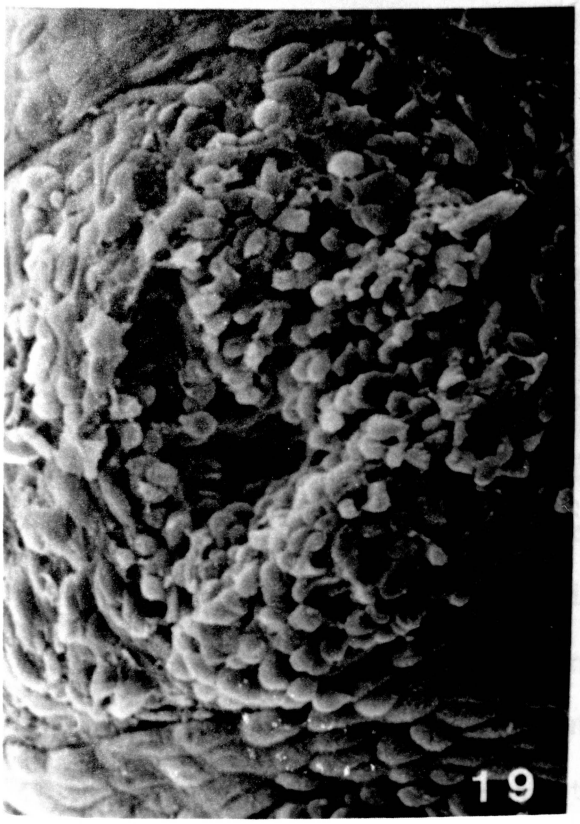

A
16

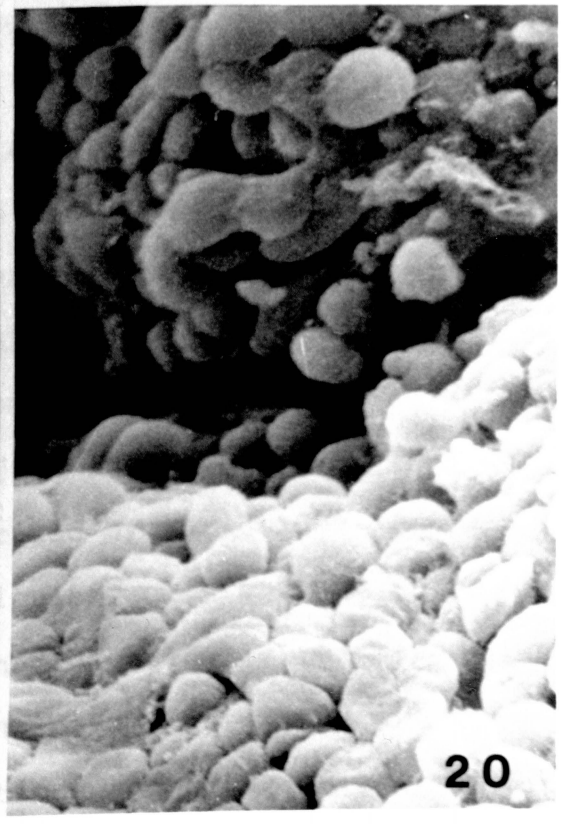
Figures 17-18. Light micrographs of Day 2 ovary show that ovulation has occurred. (Fig. 17) Early corpus luteum (C.L.) is easily distinguished. X 335. (Fig. 18) Area of rupture can be seen (R) with large area of epithelial cell layer disrupted (E). X 1005.

Figures 19-20. Scanning electron micrographs of Day 2 show the site of rupture. (Fig. 19) An early corpus luteum has formed. Area of sloughed epithelial cells is visible. X 532. (Fig. 20) Luteal cells become smooth, completely void of microvilli, and swollen. X 1330.


CL
17


R
E
18


19


20

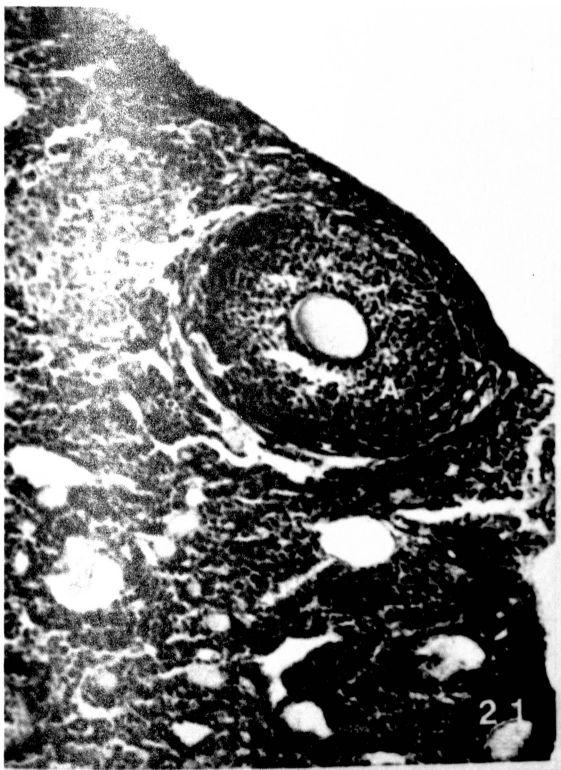
from young animals. Ovaries from acyclic aged females contained very few non-atretic follicles and many degenerated follicles at all early stages of development. Animals representing all cycle days possessed ovaries exhibiting shrunken oöcytes with irregular contour, flocculent or condensed cytoplasm, broken zona pellucida, and disrupted granulosa cell pattern.

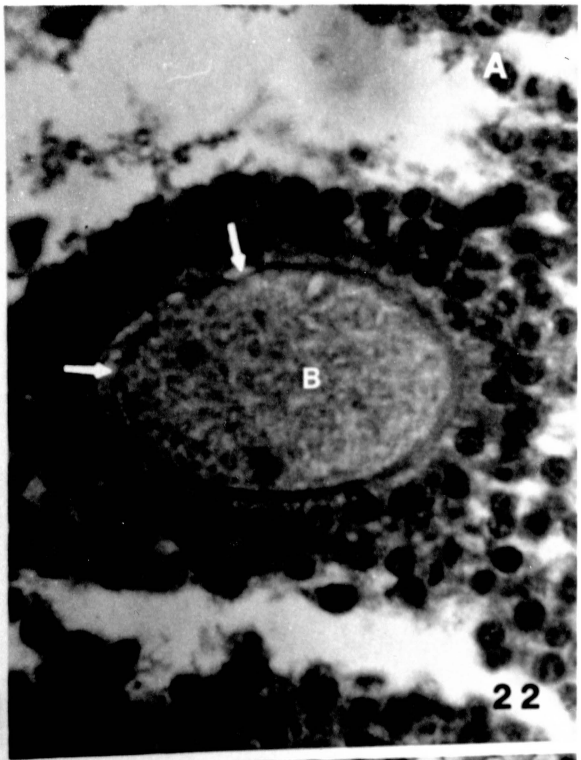
Animals displaying Day 3 vaginal phenomena contained ovaries with secondary follicles but having no Graaffian follicles present (Fig. 21). Usually the developing follicles present showed signs of early atresia (Fig. 22) that included disruption of granulosa cell pattern, irregular contour of oöcyte, disrupted zona pellucida, and flocculent oöcyte cytoplasm. SEM revealed the ovarian surface to be smooth and devoid of any large distinctive bulges or corpora lutea (Fig. 23 and 24).

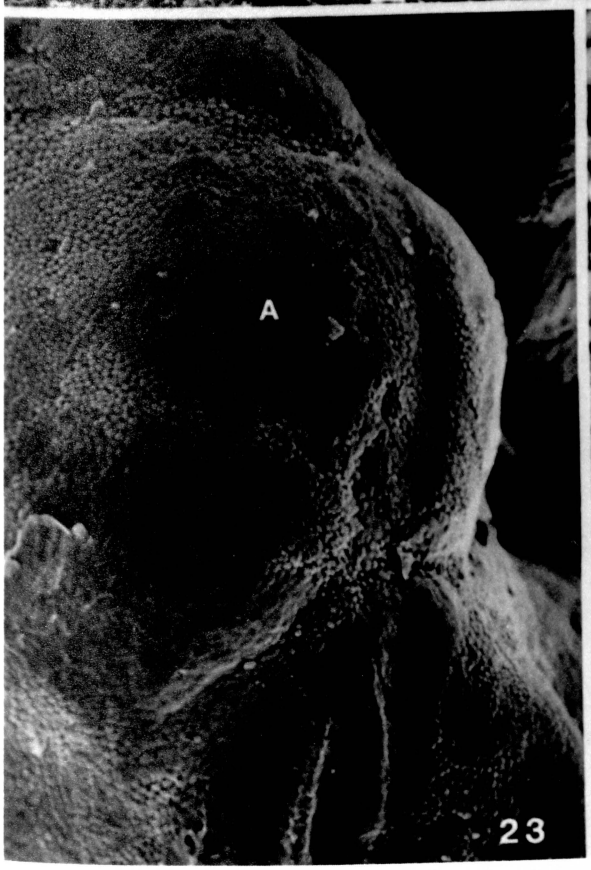
Ovaries from animals displaying Day 4 vaginal phenomena exhibited many superficial, greatly developed follicles and several Graaffian follicles (Fig. 25). Most of these were characterized by variations in the thickness of the mural follicular cells and the zona pellucida. The theca internal was usually thickened and the follicular fluid condensed (Fig. 26). SEM revealed blisterlike projections appearing on the ovarian

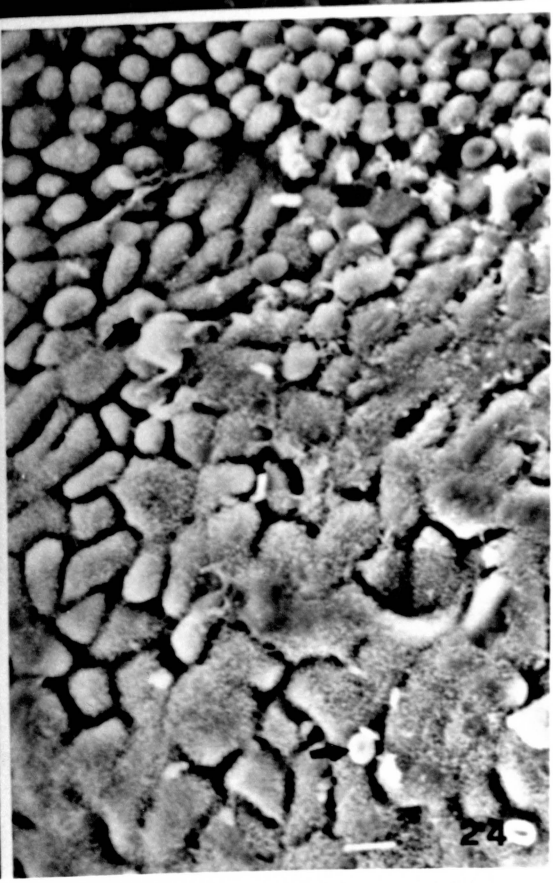
Figures 21-22. Light micrographs of Day 3 ovary from a senescent hamster show atresia. (Fig. 21) Many follicles can be seen, most in various stages of atresia. A large secondary follicle (A) displays slightly flocculent cytoplasm. X 538. (Fig. 22) Higher magnification of such follicles reveals a disrupted pattern of granulosa cells (A). The cytoplasm of the degenerating oocyte stains deeply (B) and the contour becomes irregular as shrinkage occurs. The zona pellucida shows signs of being slightly disrupted (arrows). X 1720.

Figures 23-24. Scanning electron micrographs of Day 3 ovaries from senescent hamsters reveal surface characteristics. (Fig. 23) Smooth surfaces are interrupted by slight bulges resulting from enlarging follicles. Note the exceptional smoothness at the apex (A). X 200. (Fig. 24) Greater detail of the apex area. Many erythrocytes (arrows) are visible. The microvilli are less distinct. X 1000.


A
21

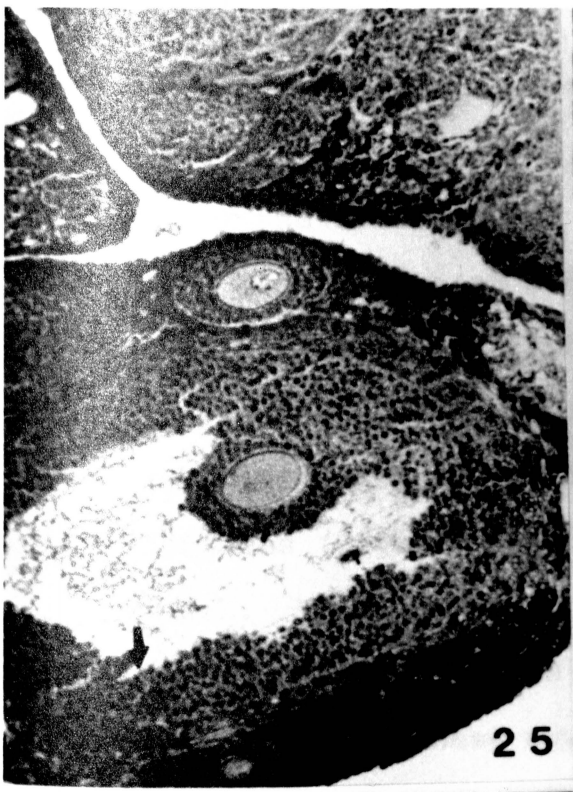

A
B
22

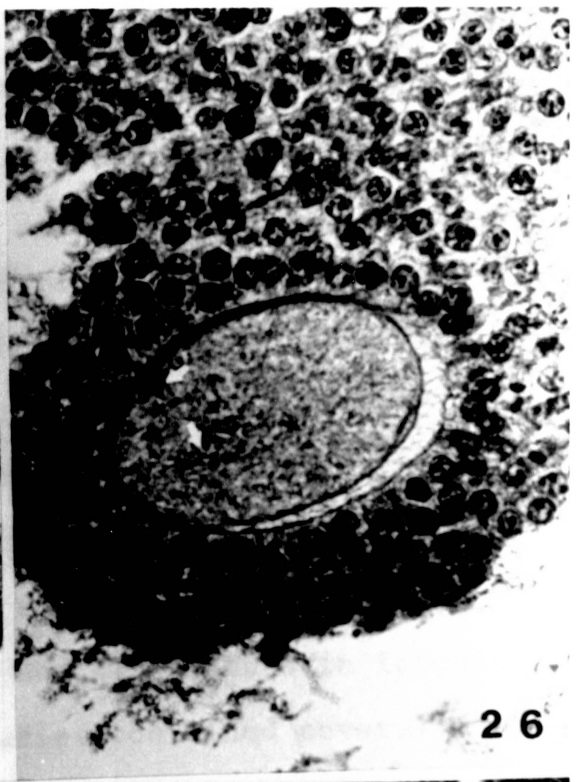

A
23

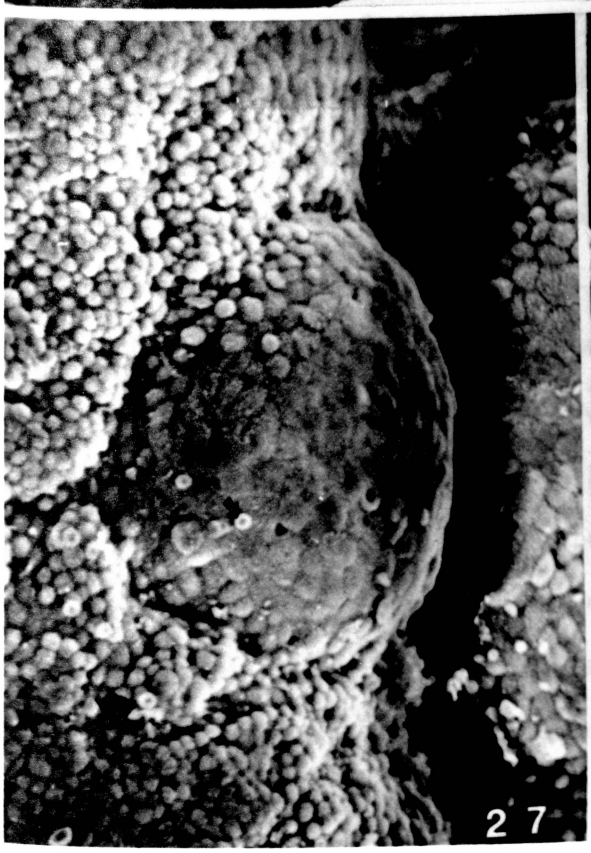

24

Figures 25-26. Light micrographs of Day 4 ovary from senescent hamster illustrate advanced atresia. (Fig. 25) Superficially located follicles in a more advanced stage. A greater degree of disruption in the pattern of granulosa cells is obvious (arrow). The mural cells vary greatly in thickness and display a loss of continuity. The theca interna is thickened. X 538. (Fig. 26) The follicular fluid appears condensed and stains unevenly (arrow). X 1720.

Figures 27-28. Scanning electron micrographs of Day 4 ovaries from senescent hamsters reveal surface deposits. (Fig. 27) The blister-like projections on the ovarian surface have enlarged and the smoothness of the apex has extended down the walls. Erythrocytes (arrows) are apparent both free on the surface and slightly embedded in the deposit on the apex. X 500. (Fig. 28) Greater magnification reveals deposits of collagen and fibrin (A) which have trapped a large number of erythrocytes (arrows) that add to the build up on the external surface of the ovary. X 1000.


25


26


27

28

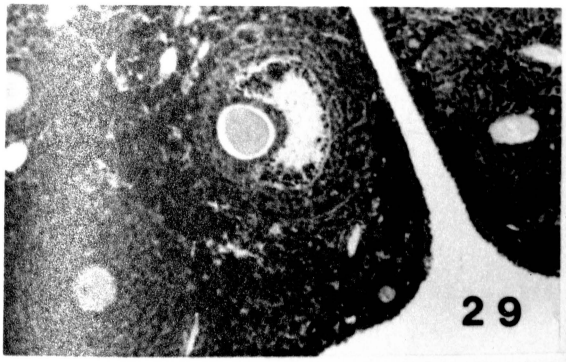
surface (Fig. 27) with a deposition of erythrocyte - containing fibrin around the base (Fig. 28). The surfaces of the apex and sides of the projections were extremely smooth and coated with an additional layer of substance of unknown make-up and origin (Figs. 27, 53, and 54).

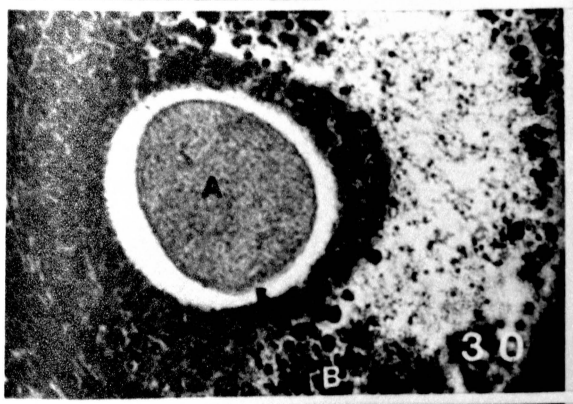
Ovaries from animals displaying Day 1 vaginal phenomena were shown by SEM to possess large bulges on their surfaces (Fig. 32) which were no longer smooth at the apex. This roughened surface was due to the accumulation of erythrocytes and fibroblasts in large numbers (Fig. 33). These ovaries contained several greatly advanced follicles (Fig. 29) and the majority of the follicles showed advanced atresia. The zona pellucida was often observed to be irregular in width. A proliferation of the germinal epithelia and thickening of the tunica albuginea resulted in a stratified appearance (Figs. 31, 50, and 52). Connective tissue increased in amount and the antrum was invaded by macrophages, erythrocytes, fibroblasts, and cell fragments (Fig. 30).

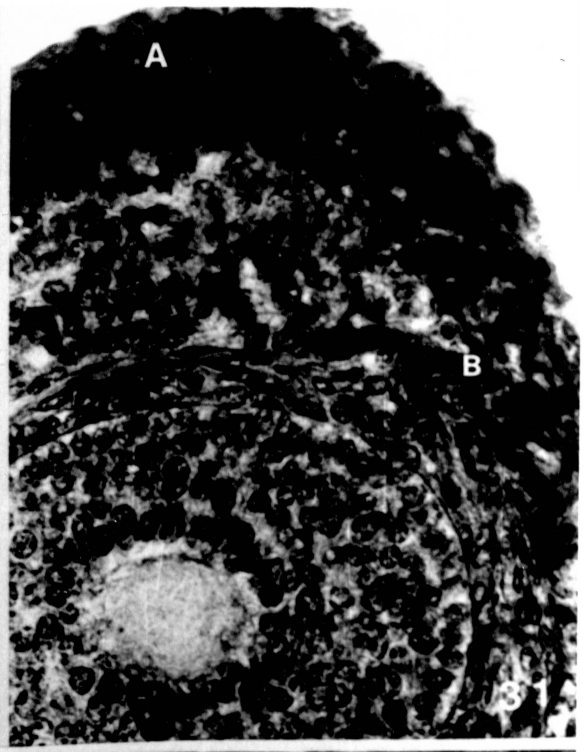
SEM revealed that ovaries from animals displaying Day 2 vaginal phenomena were characterized by large projections which were invaginated at the apex (Figs. 38 and 39). Light microscopy showed that these bulges contained Graaffian follicles in almost total atresia

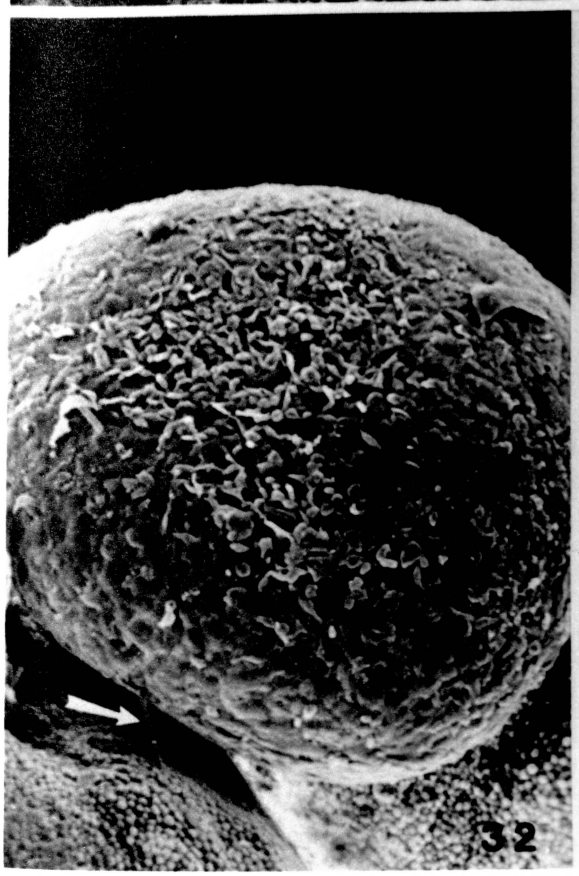
Figures 29-31. Light micrographs of Day 1 ovaries of senescent hamster show advanced atresia. (Fig. 29) Advancing atresia of primary, secondary, and Graaffian follicles. X 206. (Fig. 30) An ovum which is irregular in outline (A). This distortion is indicative of shrinkage. The antrum of the follicle contains cell fragments and macrophages. The zona pellucida (arrow) has separated and become irregular in thickness. The mural granulosa (B) is irregular in thickness. The follicular fluid appears condensed and contains darkly-staining bodies resulting from hemorrhage. X 825 (Fig. 31) Extensive proliferation of the germinal epithelia (A) results in a stratified appearance. The tunica albuginea (B) appears thicker and contains a large amount of connective tissue. The degenerating cytoplasm of the ovum stains deeply and the contour is irregular. X 1665.

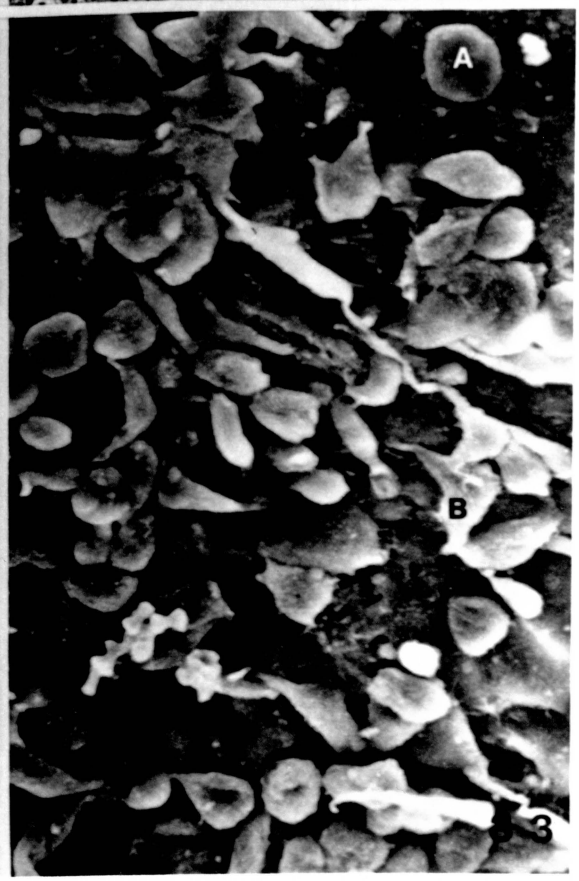
Figures 32-33. Scanning electron micrographs of Day 1 ovaries from senescent hamsters reveal roughness on the surface. (Fig. 32) Large areas of roughness at the apex of the bulging follicles are evident. Papillary growth (arrow) can be seen extending from one area of the ovary to another. X 200. (Fig. 33) Greater magnification of the area reveals the presence of many erythrocytes (A) and fibroblasts (B). X 1000.


29


A
B
30

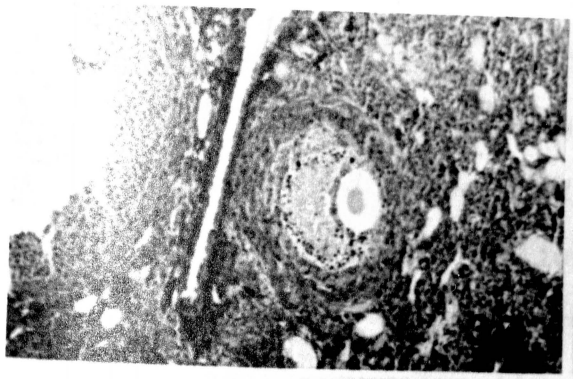

A
B
31

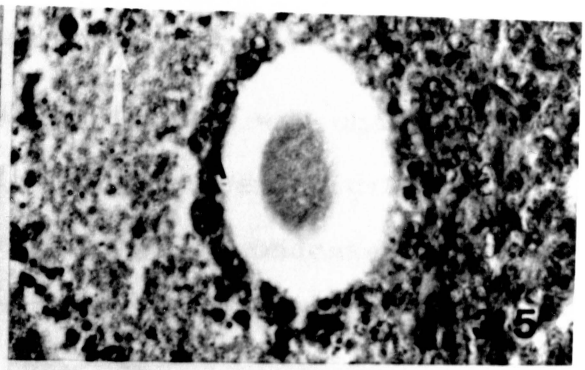

32

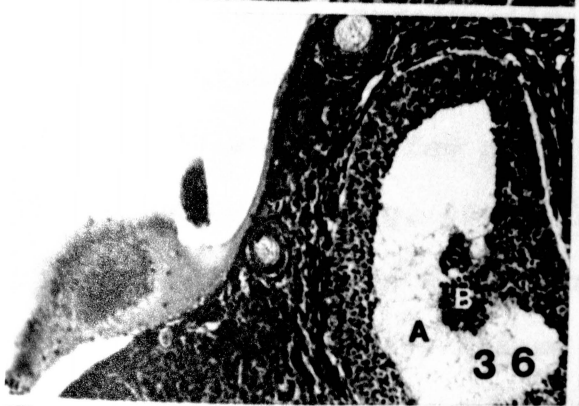

A
B
33

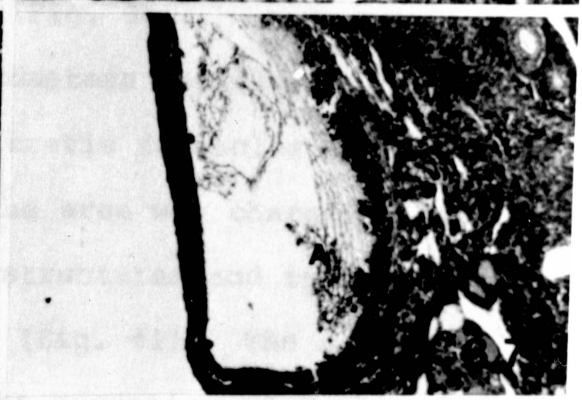
Figures 34-37. Light micrographs of Day 2 ovary of senescent hamster show Graaffian follicles in a state of advanced atresia. (Fig. 34) The advanced degeneration is indicated by the almost complete absence of granulosa and the greater variation in thickness of the mural granulosa. X 206. (Fig. 35) Cell fragments and macrophages (arrow) are present in the antrum (A) and clear areas with the follicular fluid. X 825. (Fig. 36) As atresia progresses, Graaffian follicles shrink and become deformed. The follicular fluid (A) is extremely condensed. The corona radiata cells (B) that once surrounded the oöcyte are disorganized and loosely attached to the mural granulosa. X 206. (Fig. 37) The mural cells have been reduced in places to 1-2 cells in thickness. The follicle collapses. The antrum is infiltrated with connective tissue (A). X 206.

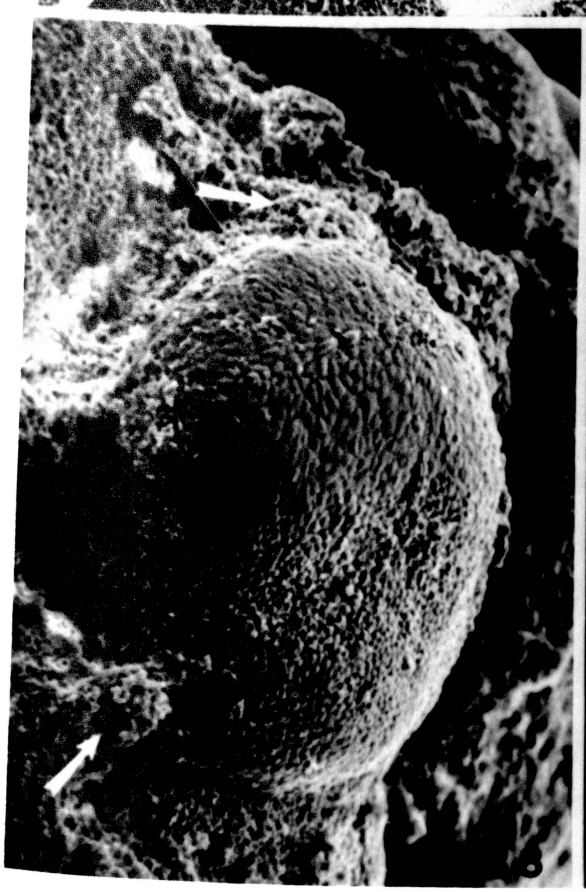
Figures 38-39. Scanning electron micrographs of Day 2 ovary from senescent hamster. (Fig. 38) reveal the surface of a Graaffian follicle. No signs of eruption are present. Note the areas of large accumulations of fibrin which contain many erythrocytes and fibroblasts (arrows). X 2000. (Fig. 39) The greatly magnified apex of the follicle shown in figure 38 appears to be sunken. The germinal epithelium invaginates in the areas of atretic Graaffian follicles (A). X 500.

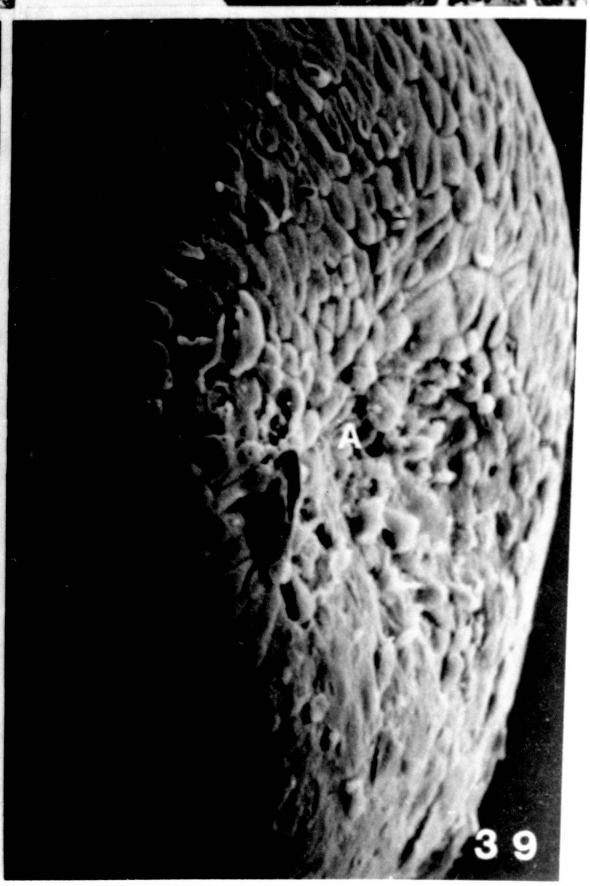





A
B
36






A
39

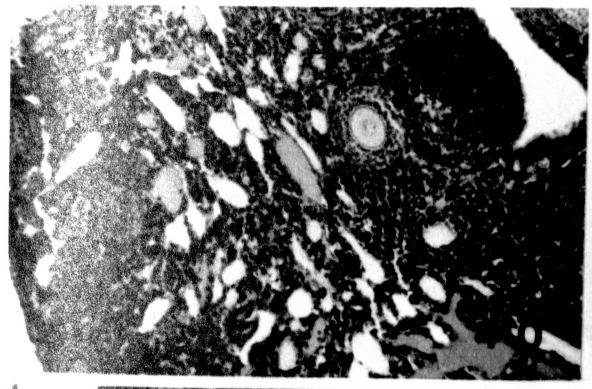
(Figs. 34 and 35). The advanced atresia was characterized by decreased numbers of granulosa cells, great variation in the mural cells (Fig. 37), condensed follicular fluid, and a disrupted corona radiata loosely attached to the mural cells (Fig. 36).

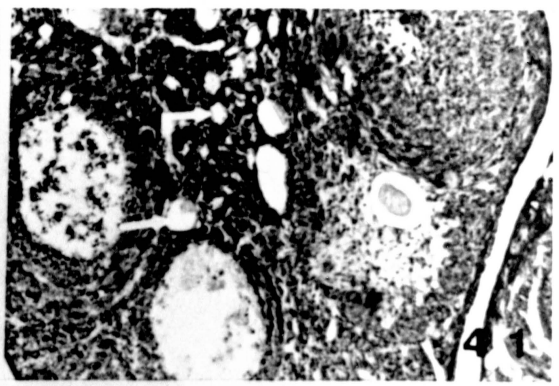
Ovaries from acyclic hamsters contained no Graaffian follicles and very few non-atretic follicles in any stage of development (Fig. 40). The area was characterized by a great increase in atretic structures and increasing amounts of connective tissue (Fig. 41). The surfaces were smooth (Fig. 44) with the exception of a thin layer of erythrocyte-containing fibrin (Fig. 45). The germinal epithelium was continuous and showed no visible signs of disruption (Fig. 42) and an increase in stratification (Fig. 43).

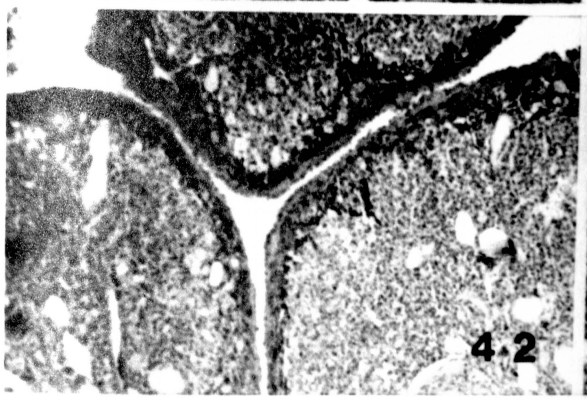
Ovaries from all senescent hamsters showed papillary-like outgrowths of the germinal epithelium on their surfaces (Figs. 46-52). These were present in large numbers with each ovary containing 10-12 of them (Fig. 47). These projections usually extended across to another lobe of the ovary where they became continuous with the epithelium of that lobe (Figs. 46 and 48). These were shown by SEM to be actual extension of the germinal epithelia covering the ovary (Figs. 51 and 52) and often carrying follicles

Figures 40-43. Light micrographs of ovary from acyclic senescent hamster reveal a decrease in advanced follicular stages. (Fig. 40) Total absence of advanced secondary or Graaffian follicles is noted. X 206. (Fig. 41) Many residual structures (arrows) appear in the stroma and cortex. X 206. (Fig. 42) Germinal epithelia appears greatly thickened, stratified, and continuous. X 206. (Fig. 43) Greater magnification of Figure 43 details stratification. X 825.

Figures 44-45. Scanning electron micrographs of ovary from acyclic senescent hamster reveal that surfaces are greatly lobed. (Fig. 44) Smooth surfaces are devoid of large bulging areas. X 200. (Fig. 45) Large areas of fibrin deposition (A) and thickening on the surface of the ovary are present. X 400.

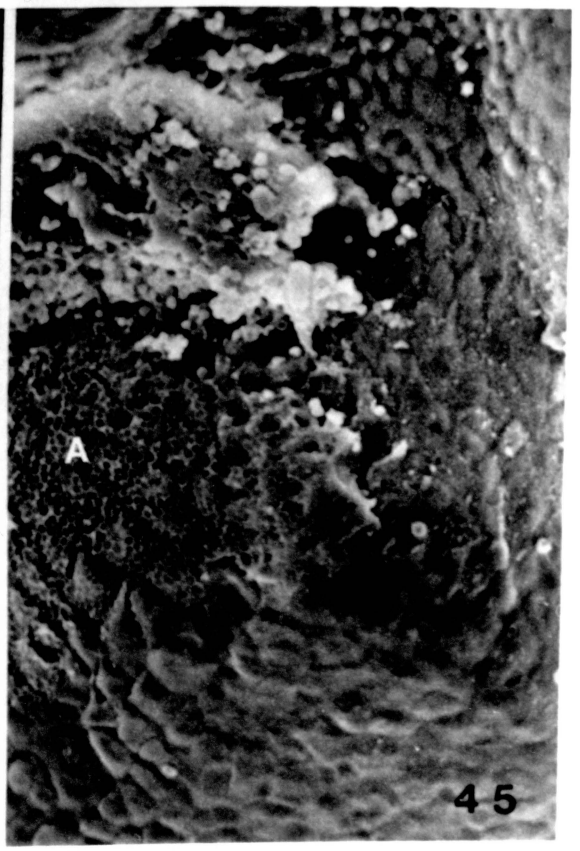



4.1


4.2


4.3

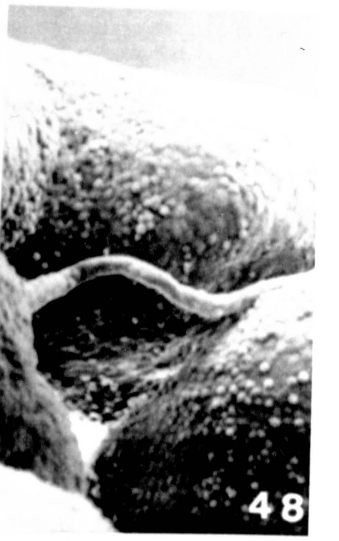

4.4

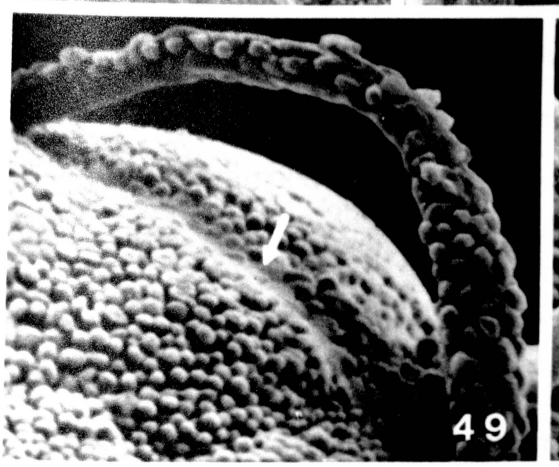

A
4.5

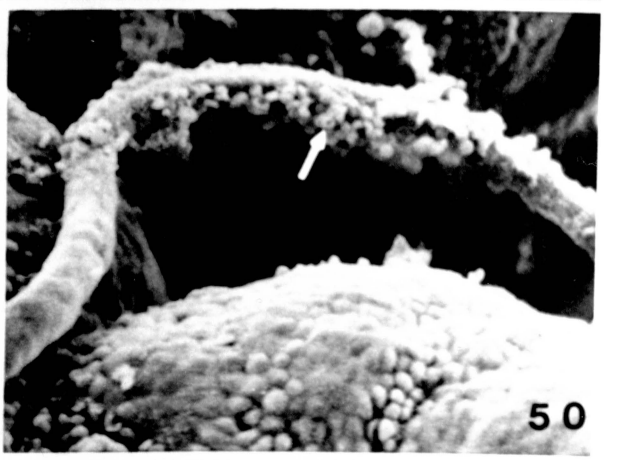
Figures 46-52. Scanning electron micrographs of ovaries from senescent hamsters show papillary outgrowths. (Fig. 46) Papillary outgrowth of epithelia extends from one lobe to another of a Day 4 ovary. X 220. (Fig. 47) Low magnification reveals that many papillary growths (arrows) are present on Day 4 ovary. X 55. (Fig. 48) A papillary outgrowth can be seen bridging lobe to lobe on a Day 3 ovary. It is obvious that it is attached in some fashion at each end. X 220. (Fig. 49) Greater magnification reveals that this papillary outgrowth on Day 2 ovary consists of epithelial cells similar to those covering the ovarian surface. An indentation (arrow) can be seen indicating that the length of this growth might once have been in contact with the surface. X 500. (Fig. 50) Papillary growths on a Day 1 ovary were often observed covered with material similar to that covering the apex of follicles. Erythrocytes (arrow) can be seen clinging to the surface of the growth. X 550. (Fig. 51) High magnification of a Day 2 ovary reveals that the base of such outgrowths is continuous with the epithelia and covered by the same erythrocyte-invaded material. Erythrocytes can be seen (arrow). X 1100. (Fig. 52) High magnification of a Day 3 ovary reveals the continuity between papillary growth and germinal epithelia. X 550.

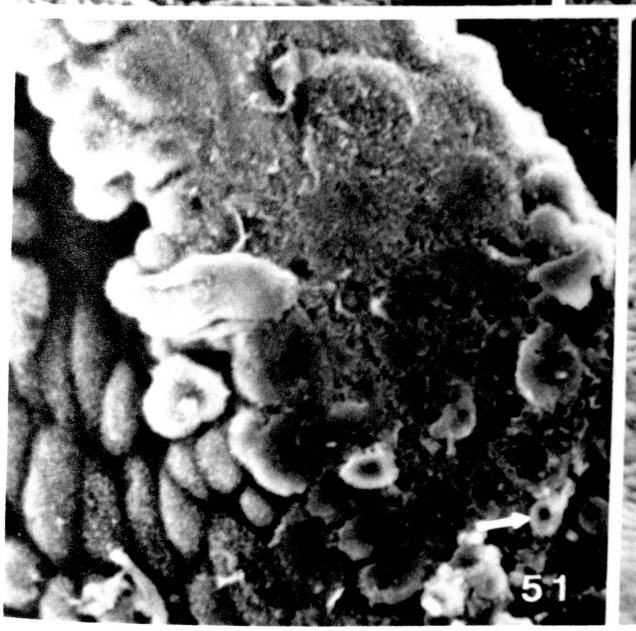

46

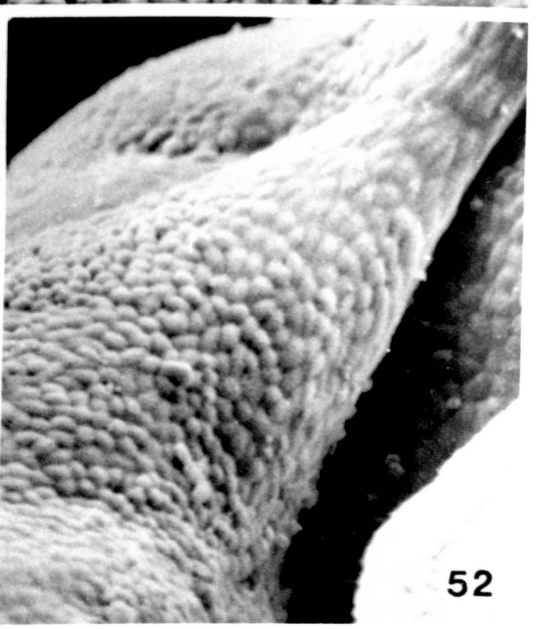

47


48


49


50


51


52

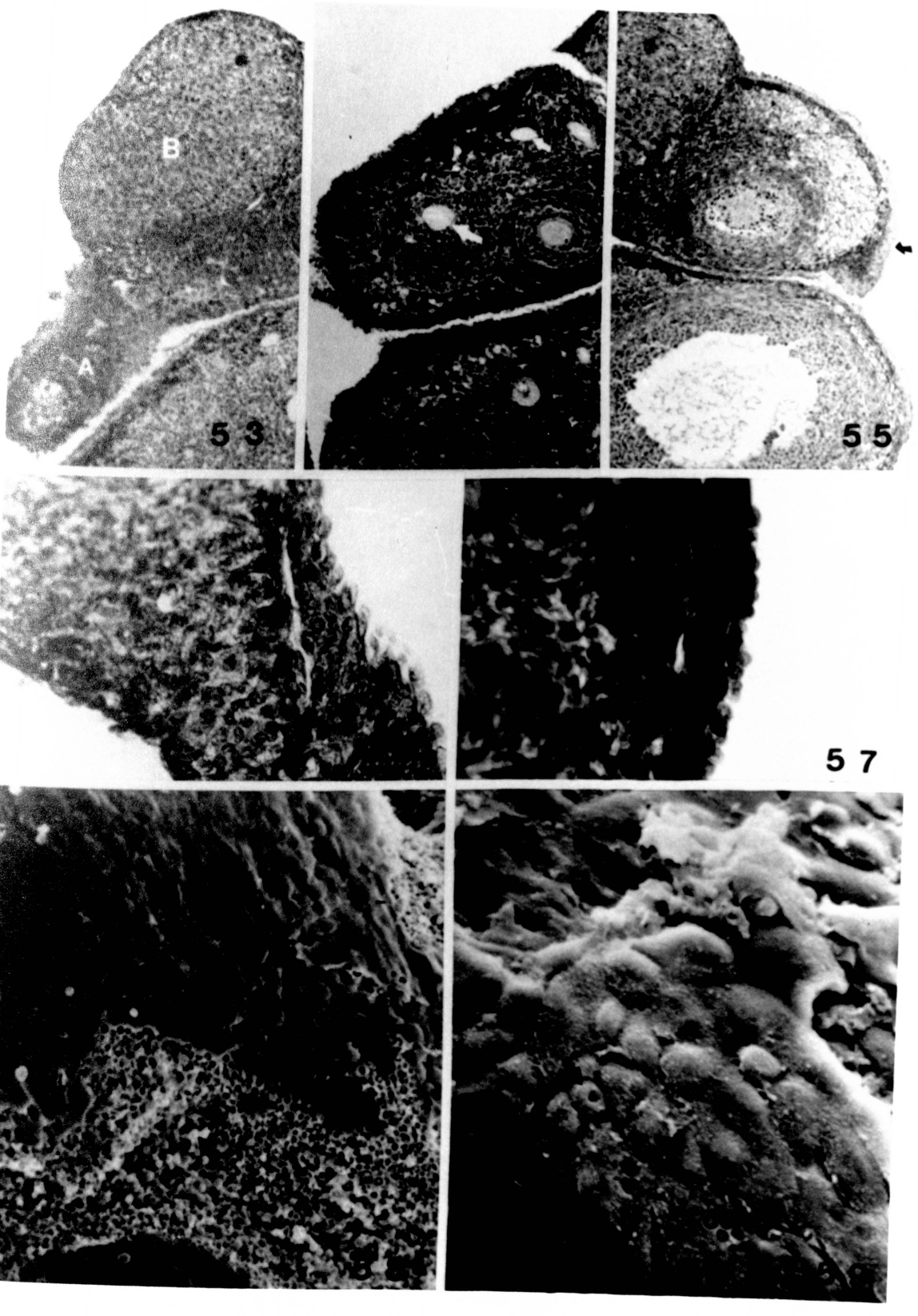
or corpora lutea within them (Figs. 53, 54, and 55). The blood vessels within the ovaries of senescent hamsters appeared hyalinized and showed signs of arteriosclerosis (Figs. 60-63) and the stroma and cortex were characterized by depositions of connective tissue and fibrin containing fibroblasts and macrophages (Figs. 64, 65 and 66).

V. Morphological observations of hypothalamic areas of young sexually mature hamsters

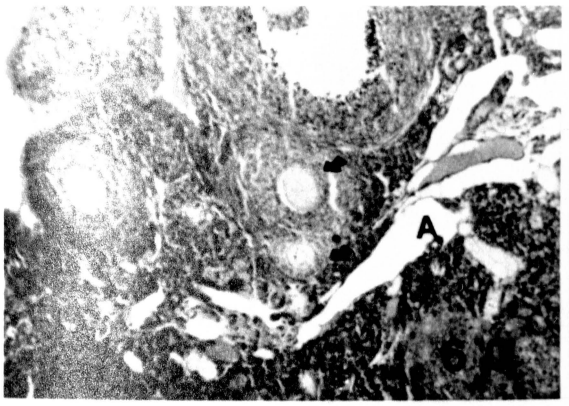
The anatomy of the brain becomes complicated and confusing in the area of the hypothalamus. The region consists of several ill-defined cell masses which are diffusely arranged into bilaterally paired cell groups known as nuclei with indistinct boundary areas of cell-poor zones (Fig. 67). Low magnification pictures provide a basic orientation of the area and its structural aspects. The size, shape, and internal contents of the cell bodies can be seen at higher magnifications and may provide information on the possible cyclic function of these cells in the female hamster (Fig. 68 and 70).

The cells which produce the neurohormones that control the release of LH and FSH are located within one of these cell masses called the arcuate nucleus (or infundibularis). In coronally sectioned samples, the

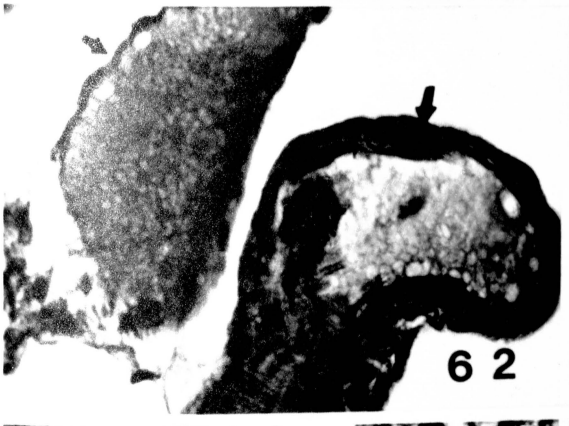
Figures 53-59 Light micrographs show surface germinal epithelium and papillary growth on ovaries from senescent hamsters. (Fig. 53) Papillary extension (A) is shown originating from an ovarian lobe containing a large, corpus luteum (B). X 206. (Fig. 54) A beginning papillary growth is shown carrying with it several follicles in various stages of atresia (arrows) and one undisturbed secondary follicle. X 206. (Fig. 55) A lobe of a Day 4 ovary is shown with a distinct proliferation of the germinal epithelium (arrow). This could indicate the early formation of a papillary growth X 206. (Fig. 56) Two papillary growths extend across one another still in close proximity to the ovarian surface. X 825. (Fig. 57) A considerable proliferation of germinal epithelial cells rests against the surface in a manner closely reminescent of that in Figure 44. X 825. (Fig. 58) Scanning electron microscopy of ovary from senescent hamster reveals surface characteristics. High magnification reveals the roughened appearance at the base of the follicles to be due to an accumulation of fibrin (A) and trapped erythrocytes (arrows). X 400. (Fig. 59) The apexes of such follicles are covered by a substance of unknown make-up in which erythrocytes lay buried (arrows). X 1000.

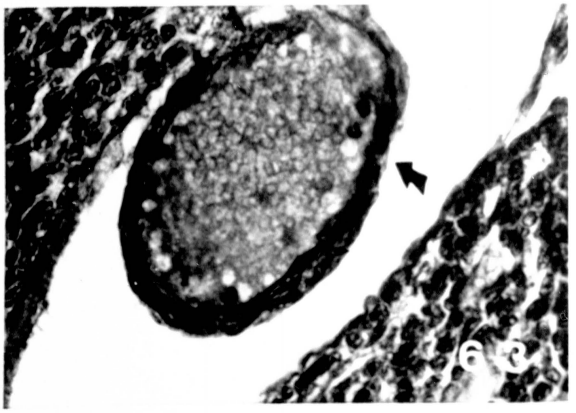

B
A
5 3
5 5
5 7

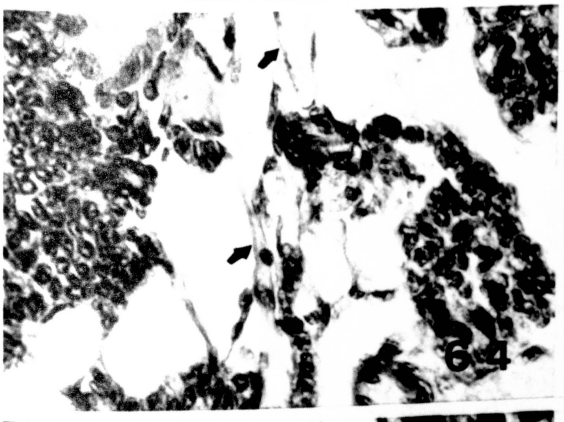
Figures 60-66. Light micrographs of ovaries from senescent hamsters reveal an accumulation of connective tissue and hyalination of blood vessels. (Fig. 60) Low magnification of an ovary from a senescent animal reveals separation of tissue (A), several follicles in various stages of atresia (arrows), and increased thickness of walls of blood vessels. X 227. (Fig. 61) This ovary shows a increase in connective tissue (A), many atretic follicles (arrows) and a greatly increased thickness of blood vessels. X 227. (Fig. 62) Greater magnification of the area in Figure 61 details cellular deposition within the vessel walls (arrows) and erythrocytes inside the vessels. X 550. (Fig. 63) Higher magnification reveals the stratified appearance of blood vessel (arrow). X 550. (Fig. 64) Increase in connective tissue (arrows) is apparent at higher magnification. X 550. (Fig. 65) Hemostasis occurs within the follicle as fibrin (A) is deposited, and erythrocytes and fibroblasts are trapped (arrows). X 227. (Fig. 66) This micrograph reveals the increase in connective tissue (A), erythrocyte entrapment and fiberblast formation (arrow). X 923.

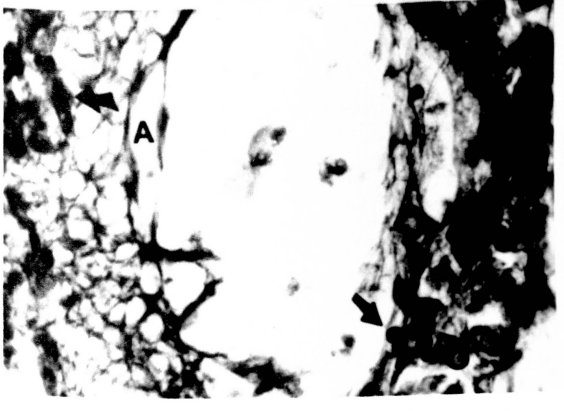

A

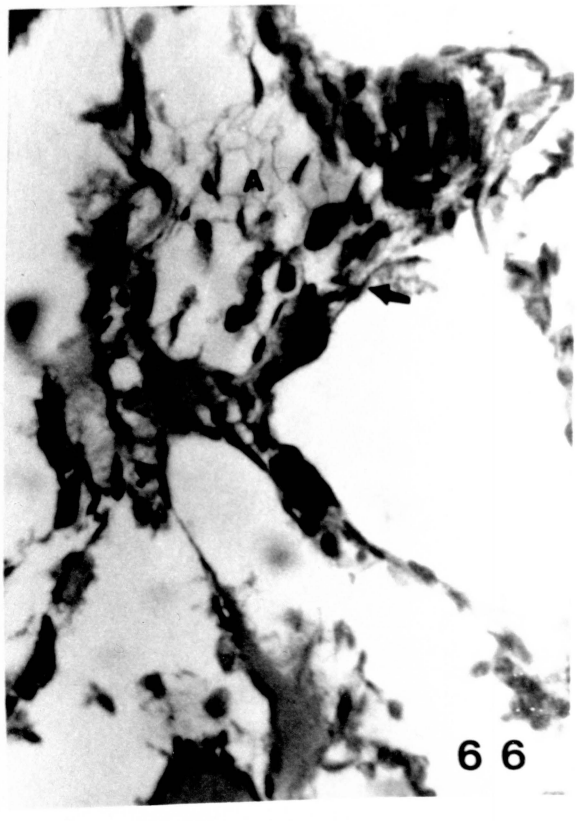

A
6 1


6 2


6 3

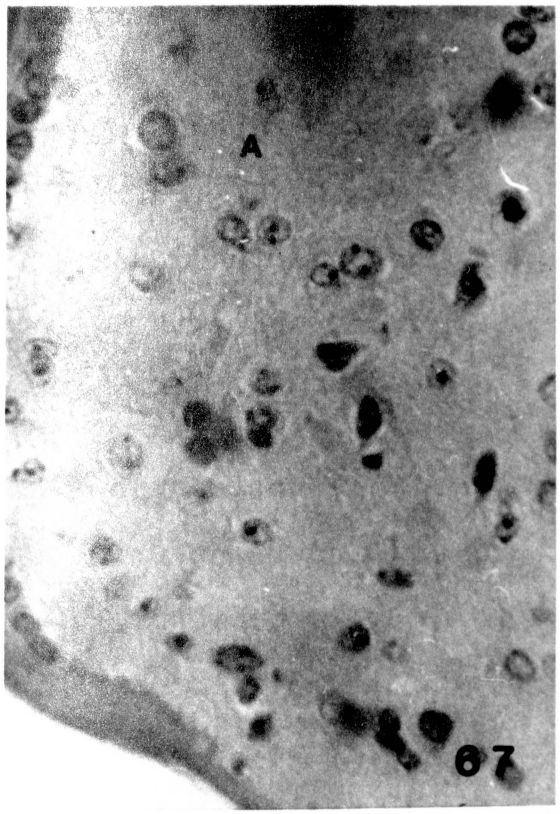

6 4

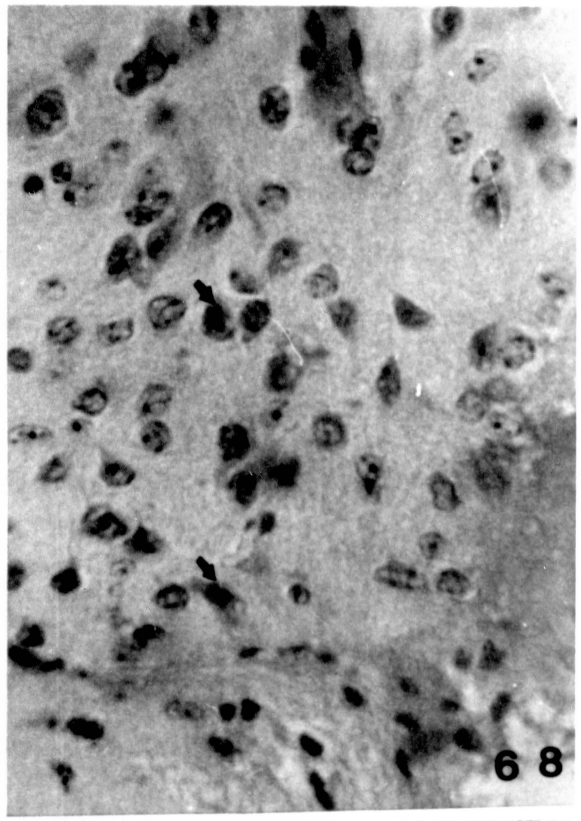

A

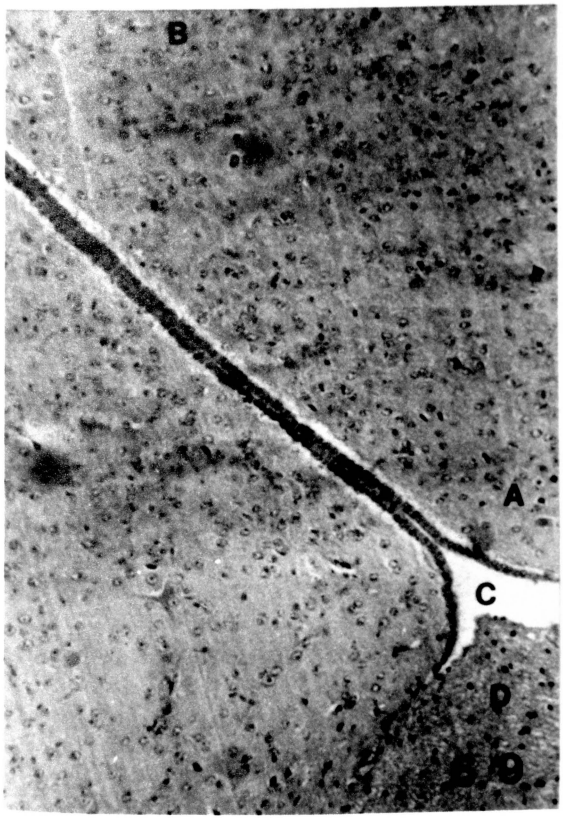

A
6 6

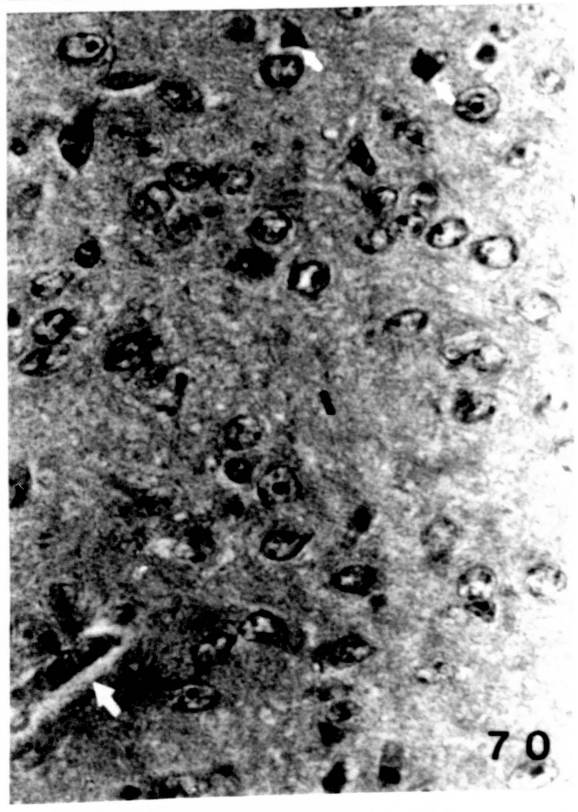
Figures 67-68. Light micrographs show cellular organization of hypothalamic area of the brain from sexually mature female hamster exhibiting Day 2 vaginal phenomena (coronally sectioned) (Fig. 67) Hypothalamic area is composed of diffusely scattered cell masses separated by areas of cell poor zones (A). X 823. (Fig. 68) Greater magnification of Figure 67 in the arcuate nucleus. (Fig. 68) A coronally sectioned speciman reveals that the secretory cells of the arcuate nucleus appear round or oval. Secretory material can be seen in and around the cells (arrows) X 1665.

Figures 69-70. Light micrographs of hypothalamic area of the brain from sexually mature female hamster exhibiting Day 3 vaginal phenomena (coronally sectioned) illustrate orientation of the arcuate nucleus. (Fig. 69) The hypothalamic area is seen. The dorsolateral border of the arcuate nucleus (A) can be seen separated from the ventromedial nucleus (B) by an area of low cell concentration. Infundibulum recess is distinct; ventrally a portion of the median eminence (D) can be seen. X 416. (Fig. 70) Greater magnification of cells within the arcuate nucleus of Figure 69 shows secretory substances (arrows) within the cells and extracellularly in the space around them. Note the erythrocyte-containing capillary in the lower left corner (arrow). X 1665.


A
67


68


B
A
C
D


70

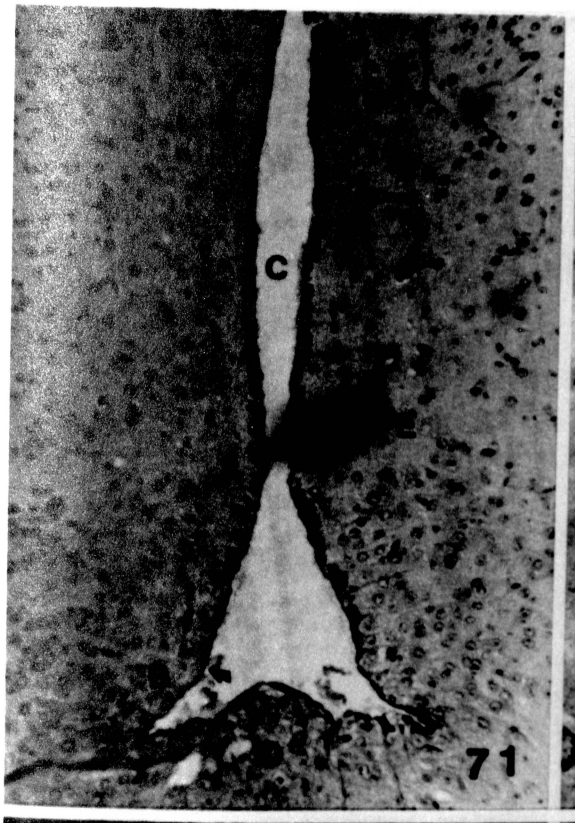
cells of the arcuate nucleus appeared round or oval and could often be observed to contain secretory material (Figs. 68, 70, 72 and 74). This secretory material was present both intracellular and extracellularly (Fig. 70) on all days but becoming reduced in amount in Day 4 animals (Fig. 72).

VI. Morphological observations of the hypothalamic areas of senescent hamsters

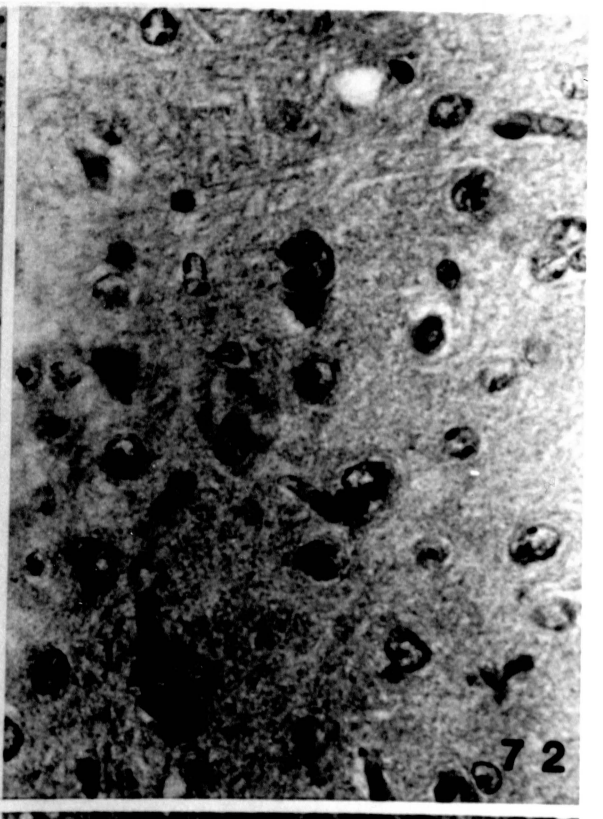
The morphological study of the hypothalamic area in older animals revealed a decreased regularity of cell arrangement, especially of the glial cells which line the walls of the third ventricle (Figs. 75 and 76). The tissue tended to retract from the glial cells and was observed totally separated (Fig. 81). Tissue also tended to become retracted from the blood vessels and such extracellular vacuolization was visible in tissues from animals representing all four days of the estrous cycle (Figs. 75, 78, 81, and 85). Cellular deposits could be seen on the walls of the vessels indicating an early sclerotic condition. The cells of the arcuate nucleus tended to be less compact (Figs. 80, 83 and 84) than those seen in younger animals and a greater diversity of size could be observed (Figs. 77, 79, and 82).

Figures 71-72. Light micrographs of the brain from sexually mature female hamster exhibiting Day 4 vaginal phenomena show hypothalamic area. (Fig. 71) Hypothalamic area. The ventromedial nucleus is (A) located dorsolaterally from the arcuate nucleus (B). The third ventricle (C) is shown. Note the internal glial layer of the ventricle wall is missing (arrows) in the area bordering the arcuate nucleus. Median emenence (D) is located ventrally and the dorsomedial nucleus dorsally (E). X 416. (Fig. 72) Greater magnification of cells within the area of the arcuate nucleus reveals secretory product in many of the cells. X 1665.

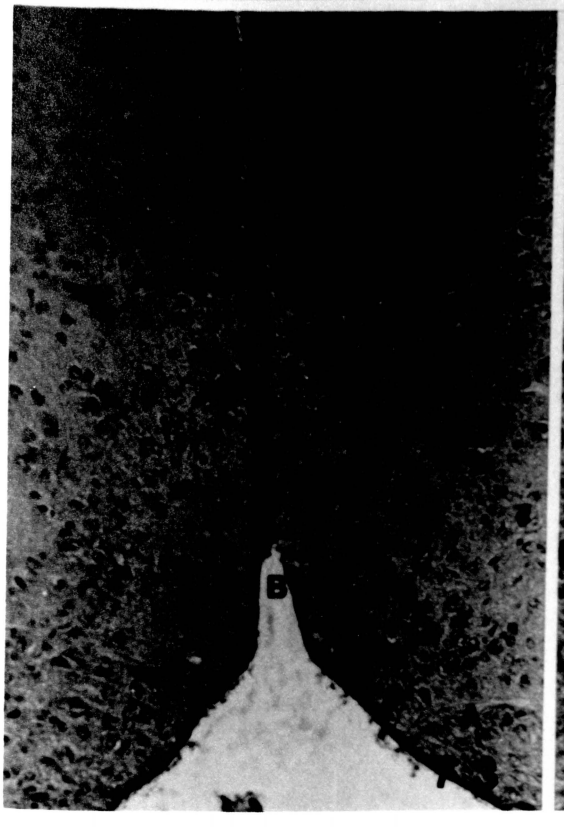
Figures 73-74. Light micrographs show hypothalamic area of the brain from sexually mature female hamster exhibiting Day 1 vaginal phenomena. (Fig. 73) Heavy concentrations of cells can be seen in the arcuate nucleus (A) with the glial cells which line the walls of the third ventricle (B) disappearing in this area (arrow). Sparceness of cells between the arcuate nucleus and the ventromedial nucleus (C). X 416. (Fig. 74) Higher magnification shows the cells of the arcuate nucleus to be similar in size and shape to those of the other three days but having less accumulated secretory product. X 1665.



71



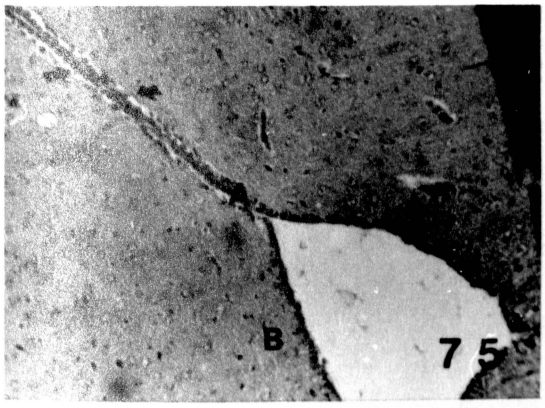
72

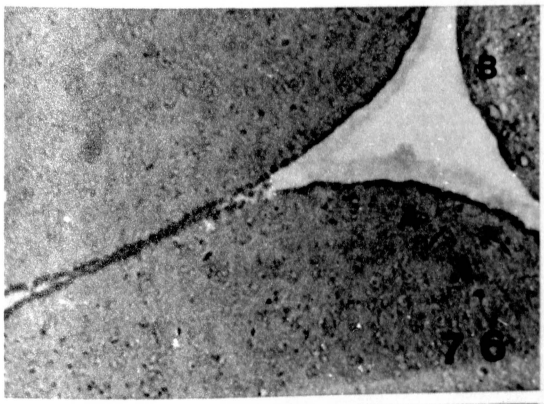


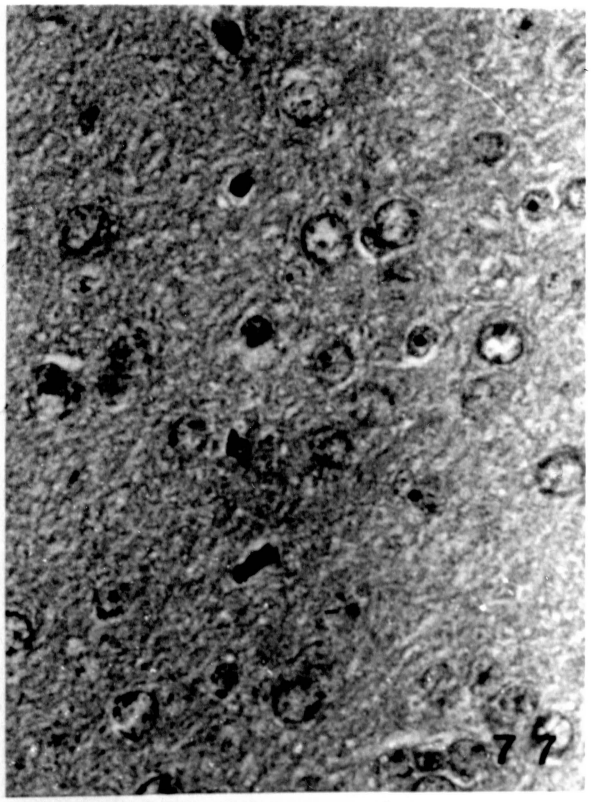
74

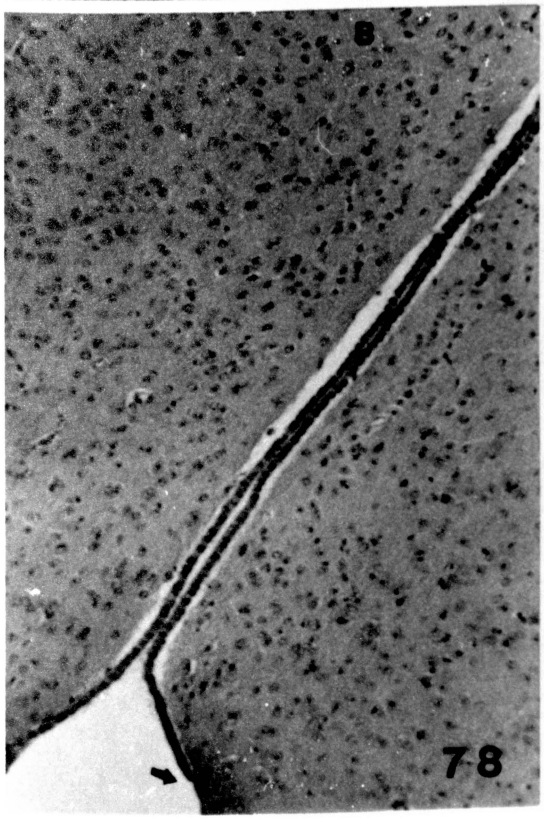
Figures 75-77. Light micrographs from Day 2 senescent hamster show hypothalamic area. (Fig. 75) Glial cells (A) can be seen retracting from their close association with the walls of the third ventricle. Vascular areas tend to loose contact with tissue surrounding them (arrows). Arcuate nucleus (B) is separated from the ventromedial nucleus by a cell poor area. X 206. (Fig. 76) The area of the arcuate nucleus is shown revealing the absence of glial cells (A). The median eminence (B) is located ventral to the third ventricle (C). X 206. (Fig. 77) Greater magnification reveals cells of the arcuate nucleus to be less compact than those of young animals but containing secretory material within them. Very little secretory material can be seen extracellularly and cells are variable in shape. X 1665.

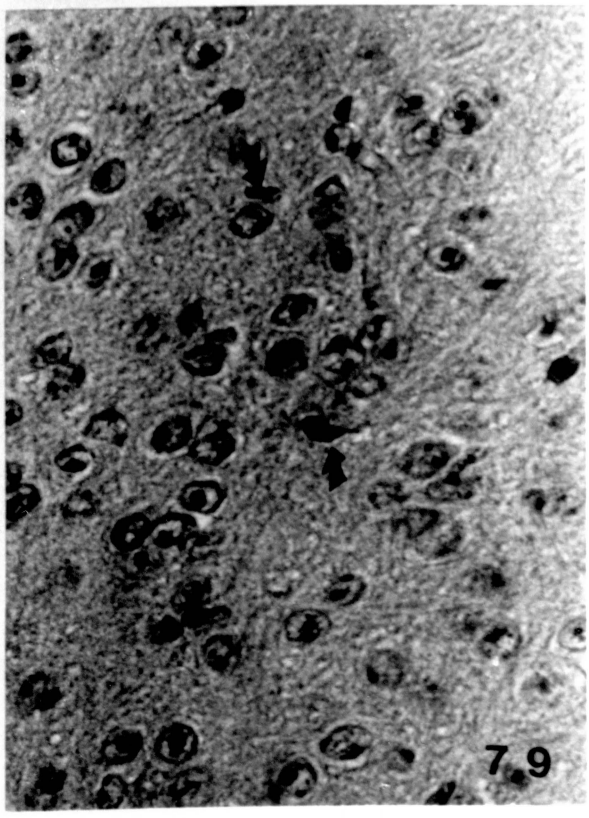
Figures 78-79. Light micrographs from Day 3 senescent hamster show hypothalamic area. (Fig. 78) The glial cells are retracted from the wall of the hypothalamic tissue and can be seen ending abruptly (arrow) at the arcuate nucleus (A). Blood vessels are retracted from the tissue. The cells of the ventromedial nucleus are apparent (B). X 416. (Fig. 79) Greater magnification shows secretory material (arrows) within the cells of the arcuate nucleus. X 1665.


B
75


B
76

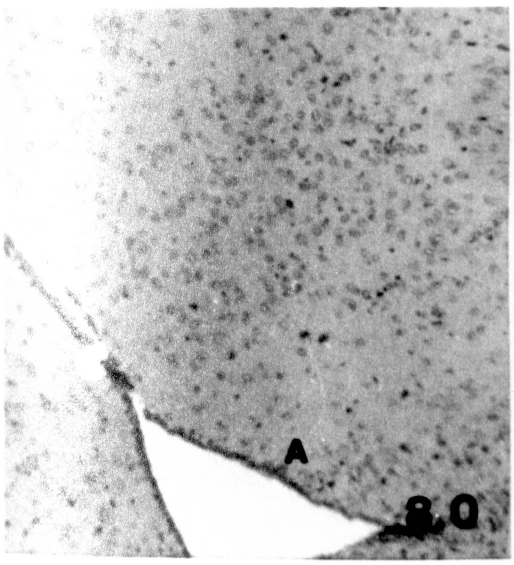

77

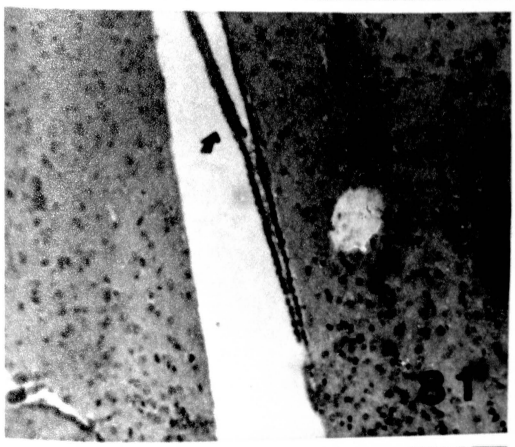

B
78

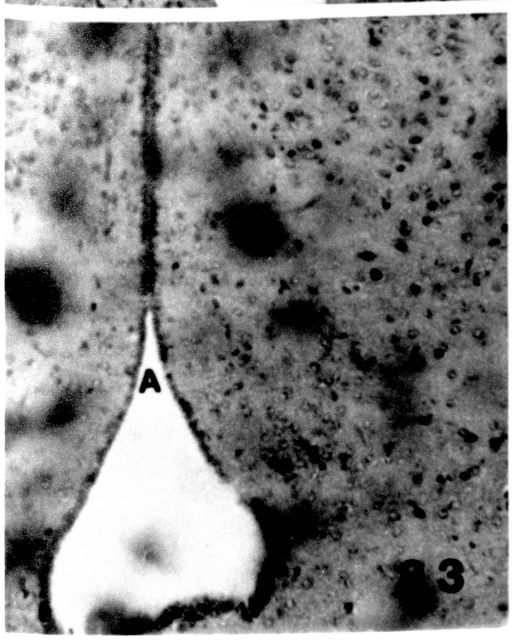

79

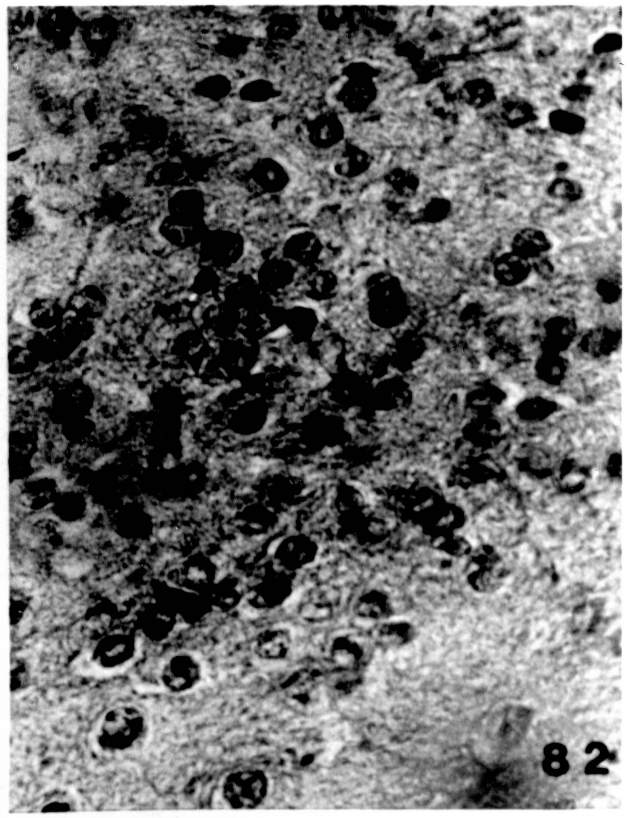
Figures 80-82. Light micrographs from Day 4 senescent hamster show hypothalamic area. (Fig. 80) Glial cells can be seen separating from hypothalamic tissue. The cells of the arcuate nucleus (A) are not as distinct as those seen previously. The cells of the ventromedial nucleus are apparent. X 206. (Fig. 81) Several areas of vascular retraction from tissue can be seen (arrow). Glial cells lie within the third ventricle. X 206. (Fig. 82) Extracellular deposits of secretory material can be seen at higher magnification. Note the amount of secretory material. X 1665.

Figures 83-84. Light micrographs from Day 1 senescent hamster show hypothalamic area. (Fig. 83) The third ventricle (A) is clearly demarcated. Darkly-stained cells of the arcuate nucleus can be seen even at low magnification (arrow). X 3000. (Fig. 84) Much extracellular secretory material can be seen. A blood vessel retracting from tissue is visible (arrow). X 1665.


A
80


81


A
83


82

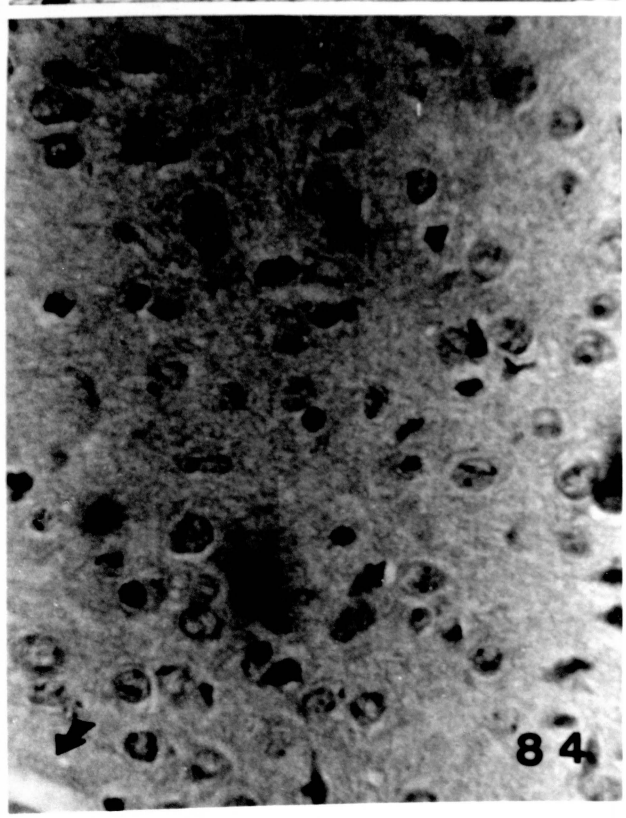
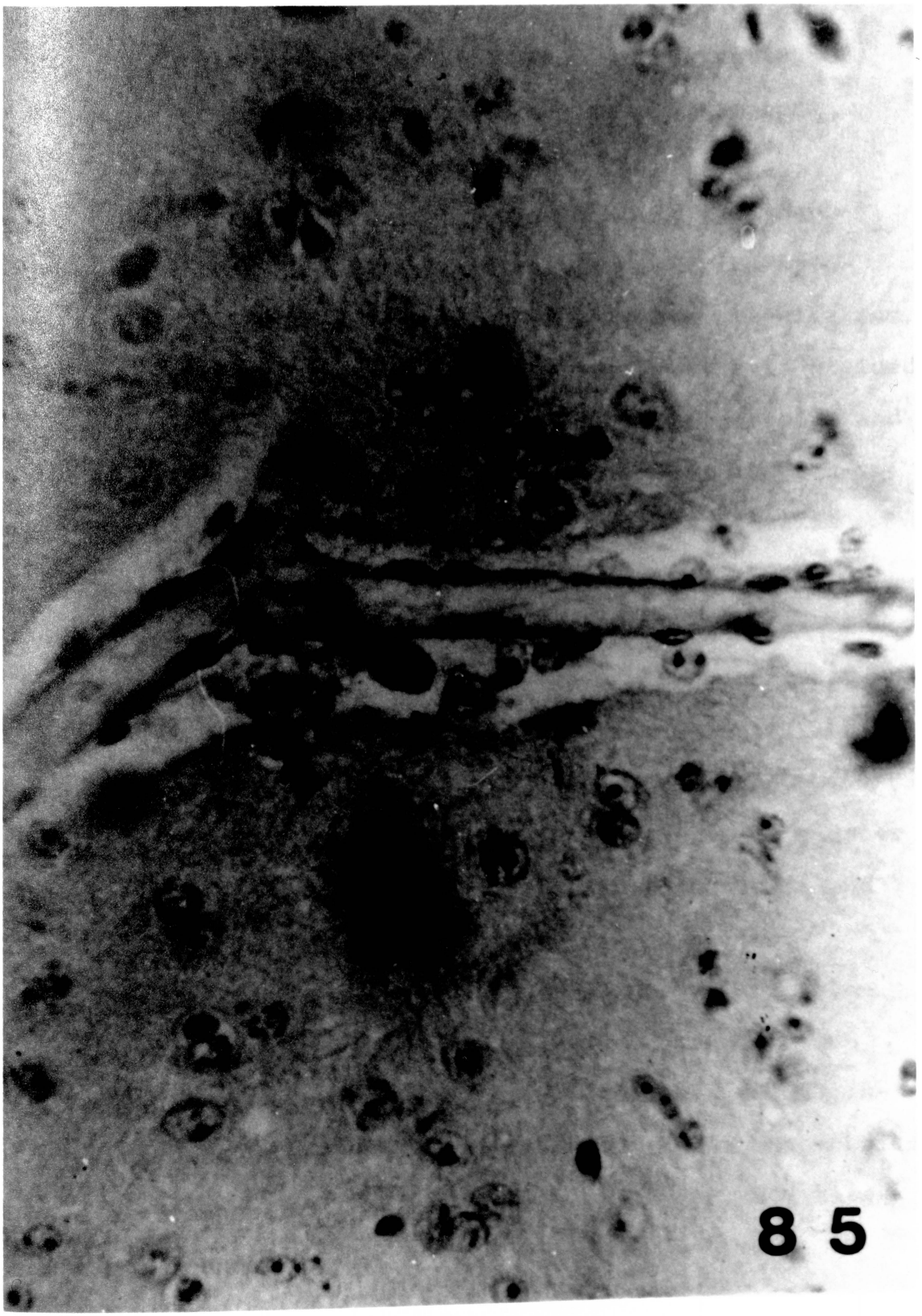

84

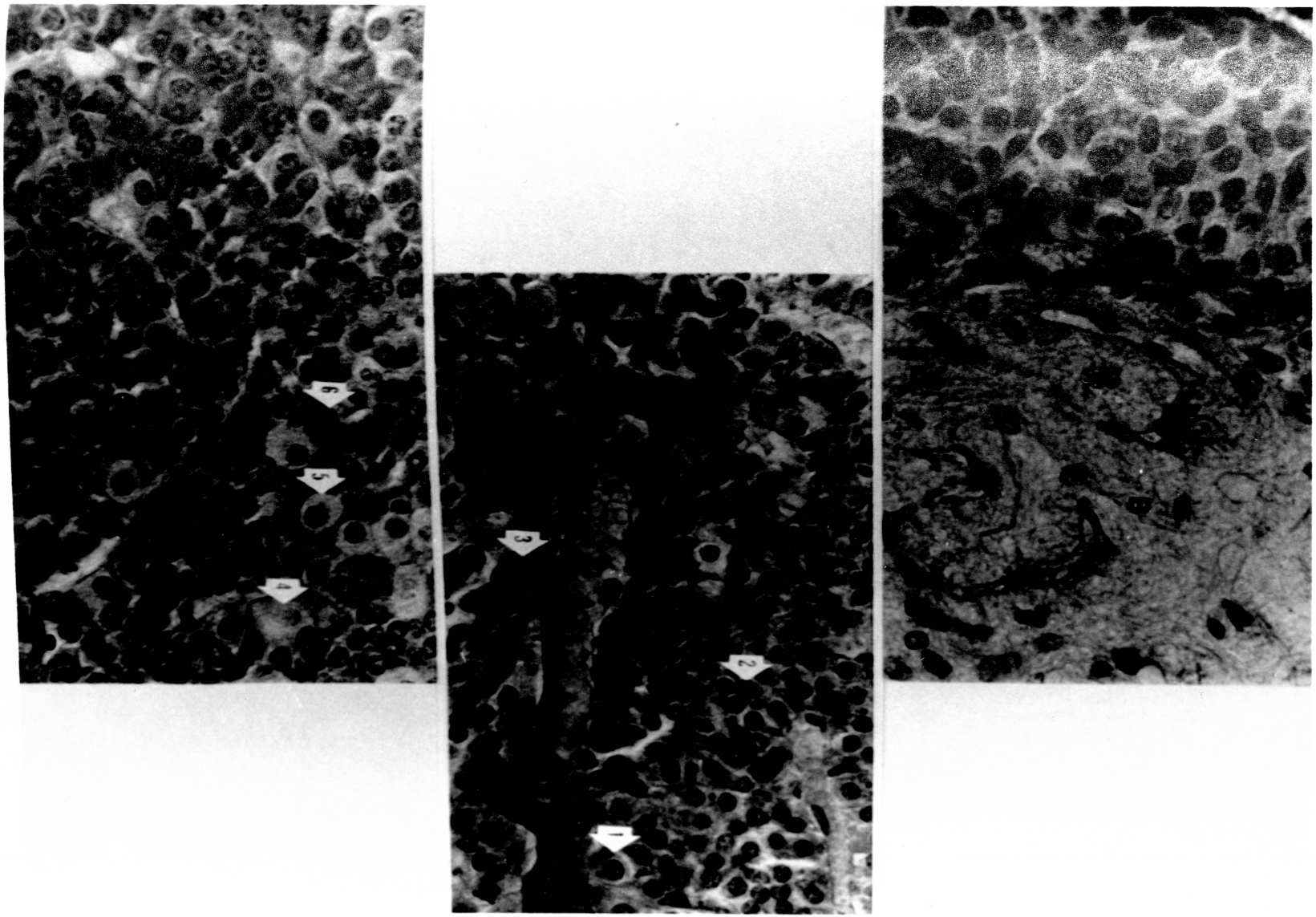
Figure 85. The tissue surrounding a branched capillary is revealed by greater magnification to retract away from the vessel. Secretory cells of the arcuate nucleus (arrows) remain behind. Note cells adhering to the outer surface of the vessel. X 1555.


85

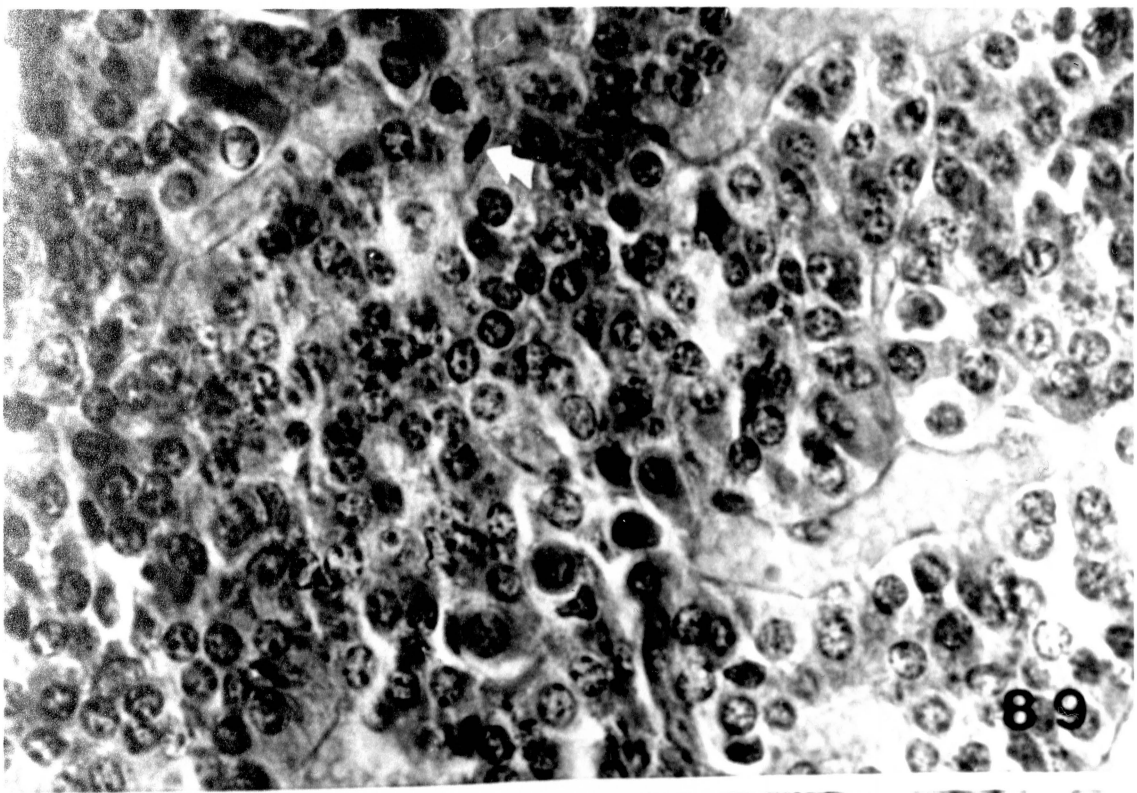
VII. Morphological and ultrastructural observation of the adenohypophysis from young sexually mature female hamsters

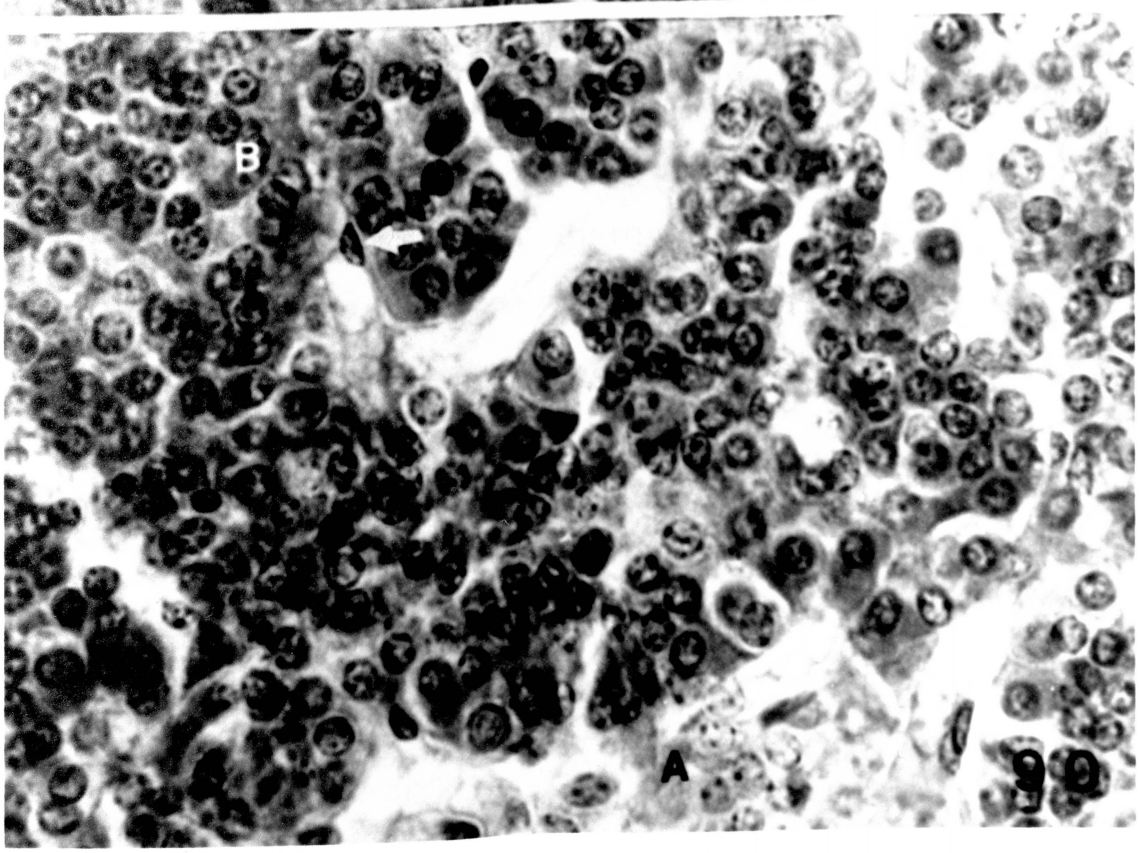
Before describing changes produced in the adenohypophysis by aging, it is necessary to describe cytological features seen in young female hamster pituitaries. The hypophysis (pituitary gland) of the hamster is divided into the glandular adenohypophysis which appears pink and the white, fibrous neurohypophysis which contains non-myelinated nerve fibers (Fig. 86). The pars distalis consists of short cords abundantly supplied with capillaries and sinusoids (Fig. 87). This area contains two classical cell types (Fig. 88). The chromophobes are small, closely packed cells with very little cytoplasm and no granules which are visible by light microscopy. They possess a weak staining affinity and tend to cluster in the connective tissue stroma away from sinusoids and capillaries (Fig. 89). These cells represent the degranulated, inactive phase of chromophils. The cytoplasm is sparse, causing the clusters to appear to be closely packed nuclei and making it difficult to distinguish between individual cells (Fig. 90). The chromophils have a stronger staining affinity, are larger, and have distinct boundaries (Fig. 88). These cells have a

Figures 86-88. High magnification photomicrographs of sections of hypophysis of young, sexually mature female hamsters are stained with H and E. (Fig. 86) The hypophysis cerebri of a section extends from the highly cellular adenohypophysis (A) to the smaller fibrous neurohypophysis (B). The difference in the glandular and the nervous portions of this gland is striking. Pituicytes can be observed intermingled with the non-mylinated nerve fibers. X 1665. (Fig. 87) A region of the pars distalis from a Day 1 sexually mature hamster shows a large number of basophils (arrow 1) clustering around a sinusoid (A). Heavy vascularization of the area can be seen. The area also contains acidophils (arrow 2), chromophobes (arrow 3), and sparse stromal elements. X 1665. (Fig. 88) Three cell types can be distinguished in this photomicrograph taken of the pars distalis from a Day 2 hamster. The chromophobes (arrow 4) can be distinguished by their general lack of stain. The basophils (arrow 5) are slightly larger than the acidophils (arrow 6) and the cytoplasm stains darkly. X 1665.


5
4
3
2
1

Figures 89-90. These high magnification photomicrographs of sections of the pars distalis of the hypophysis from young sexually mature female hamsters are stained with H and E. (Fig. 89) The unusual amount of the vascularity of the pars distalis is seen in this section taken from a Day 3 hamster. Elongated nuclei (arrow) are visible and probably belong either to cells of connective tissue or to the endothelial cells. The chromophobes are clustered. X 2148. (Fig. 90) Numerous basophils are visible grouped tightly and clustered around a large blood sinusoid (A). This section was cut from the pars distalis of a Day 4 animal. An elliptical nucleus of a sinusoid cell is visible (arrow). Chromophiles (B) appear close due to their scanty cytoplasm and relatively large nuclei. X 2148.

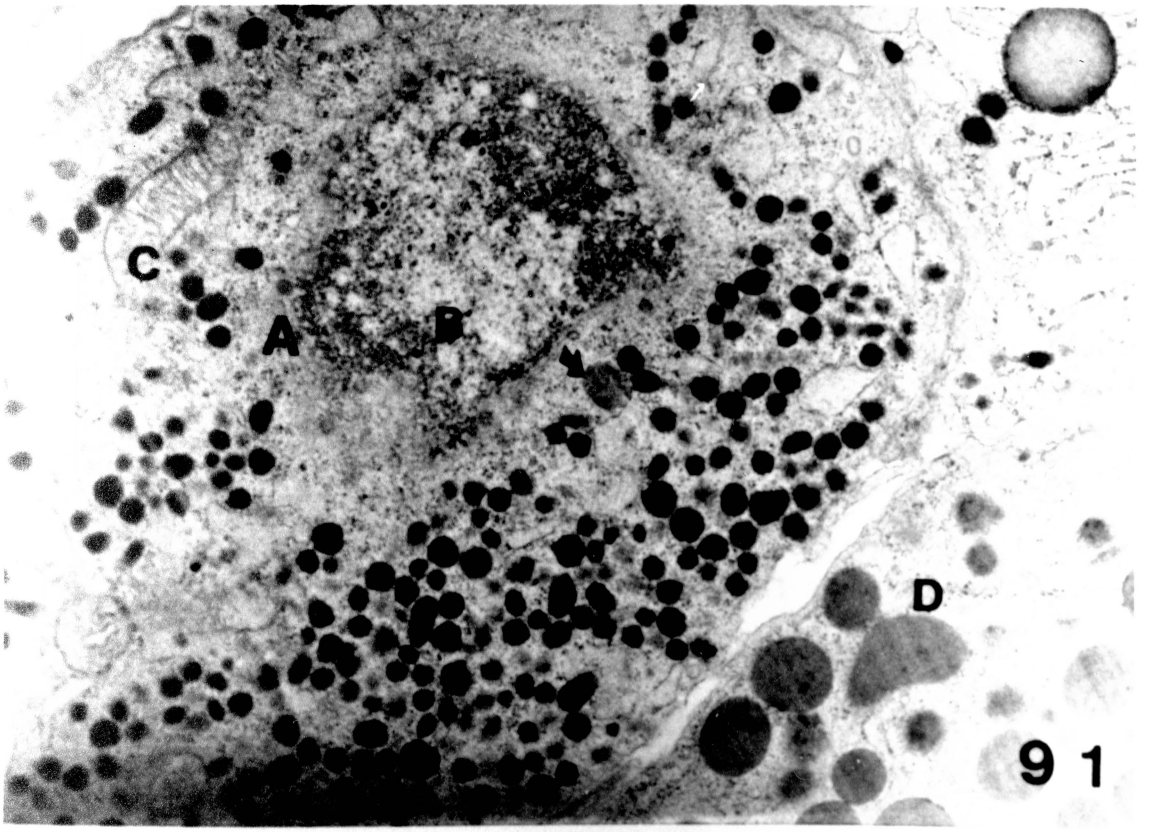

89


B
A
90

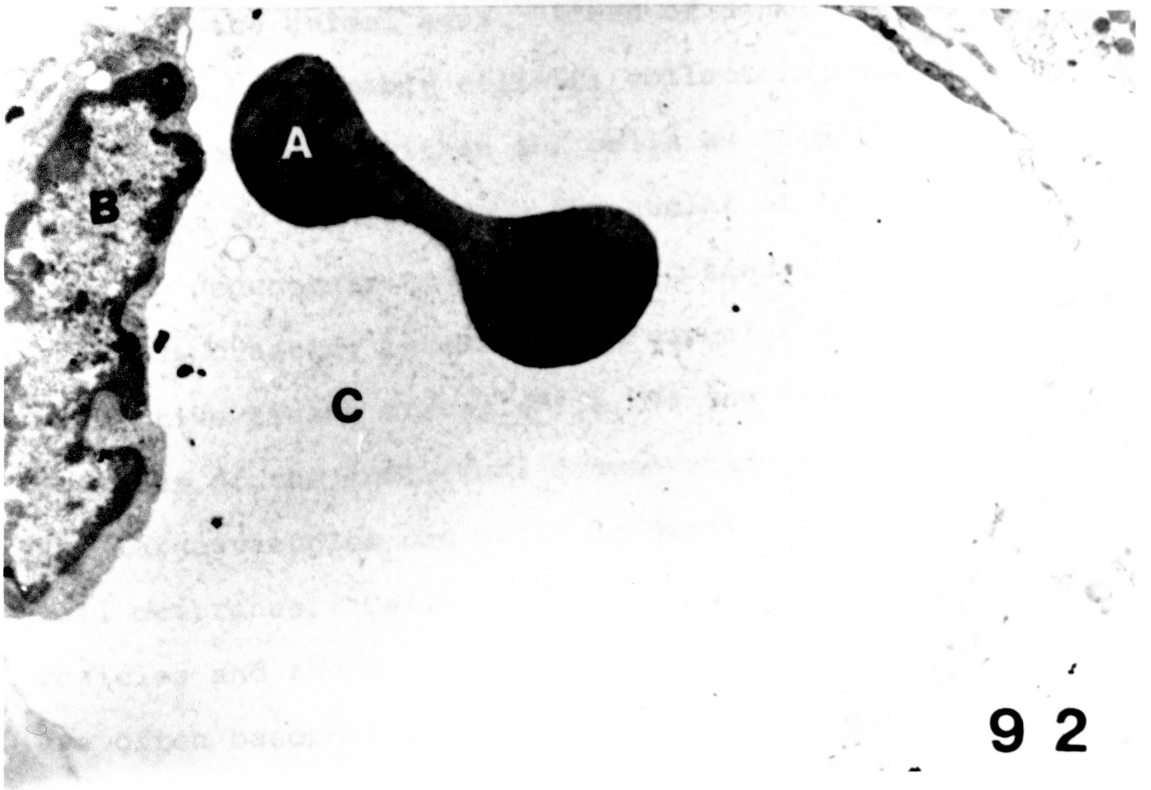
generous amount of cytoplasm which contains numerous granules. Two cell types are found among chromophils. The acidophils (or alpha cells) are prominent and take up dyes such as eosin well (Fig. 88) whereas the basophils (or beta cells) stain weakly with basic dyes such as hematoxylin.

Transmission electron microscopy (TEM) distinguishes cell types by size and form of secretory granules. The granules of the basophil are finer and more variable in size (140-200 nm) and density than the granules of the acidophil which are more uniform in size and density and are more osmiophilic (Fig. 91). Basophils include gonadotrophs (FSH- and LH-producing cells) and thyrotrophs (thyroxin-producing cells). Gonadotrophs can be readily distinguished by TEM. The thyrotrophs are angular or polygonal whereas the gonadotrophs are rounded cells with larger granules than the thyrotrophs (Sanborn, 1972). Only the gonadotroph will be considered in great detail. Gonadotrophs of a sexually mature animal are revealed by electron microscopy to be cells with greatly rounded contours, usually in close association with vascular spaces, and containing numerous rounded granules (Fig. 91). The lightly staining nucleus is eccentrically located within the cell and contains prominent nucleoli within

Figures 91-92. Transmission electron photomicrographs of the pars distalis of sexually mature female hamster reveals greater detail. (Fig. 91) The gonadotrophic cell (A) is readily distinguished by its rounded contours and numerous rounded granules of variable size. The nucleus (B) stains lightly and contains finely scattered chromatin and one or more darker nucleoli. The mitochondria (C) are seen to contain shelf-like cristi. Electron dense bodies (arrow) are presumed to be lysosomes. The larger granules of an acidophil (D) are seen in the lower right hand corner. X 20,250. (Fig. 92) The endothelium of the capillaries in the pars distalis is of the fenestrated type. An erythrocyte (A) and a white blood cell (B) are seen in the lumen (C) of the capillary. X 9225.



91



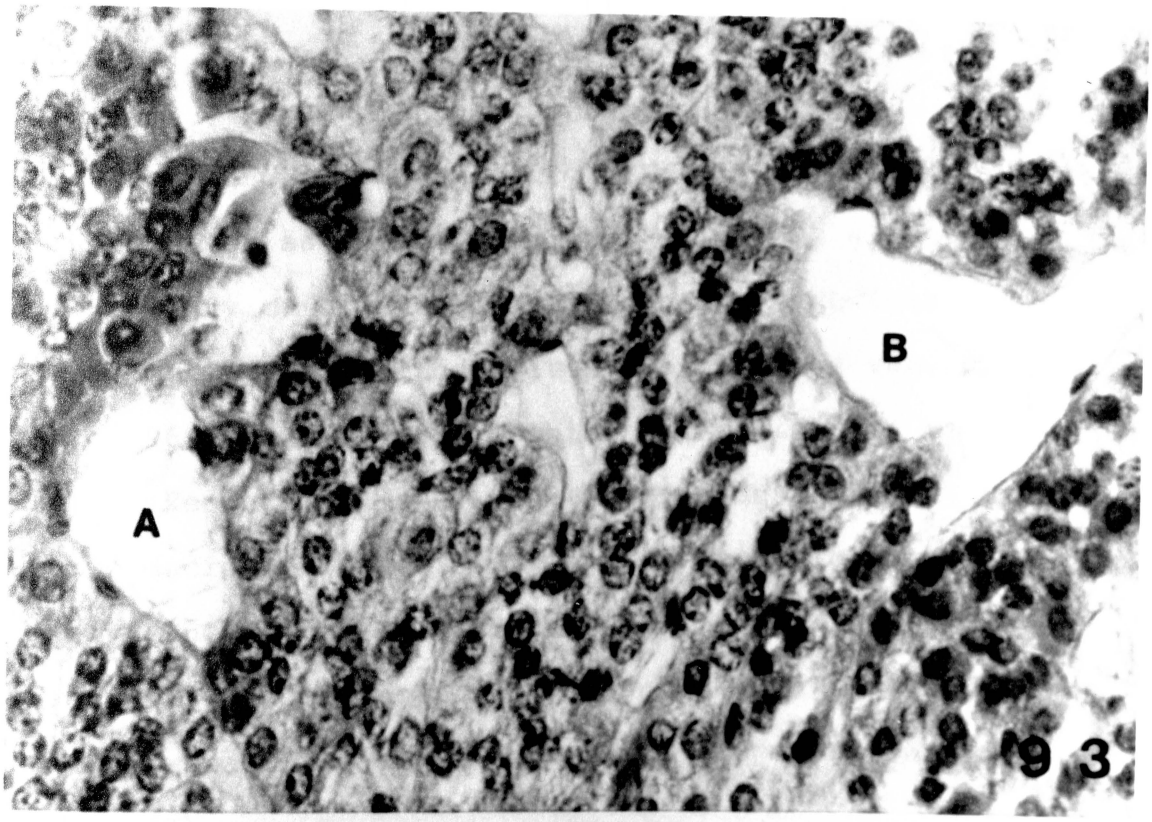
92

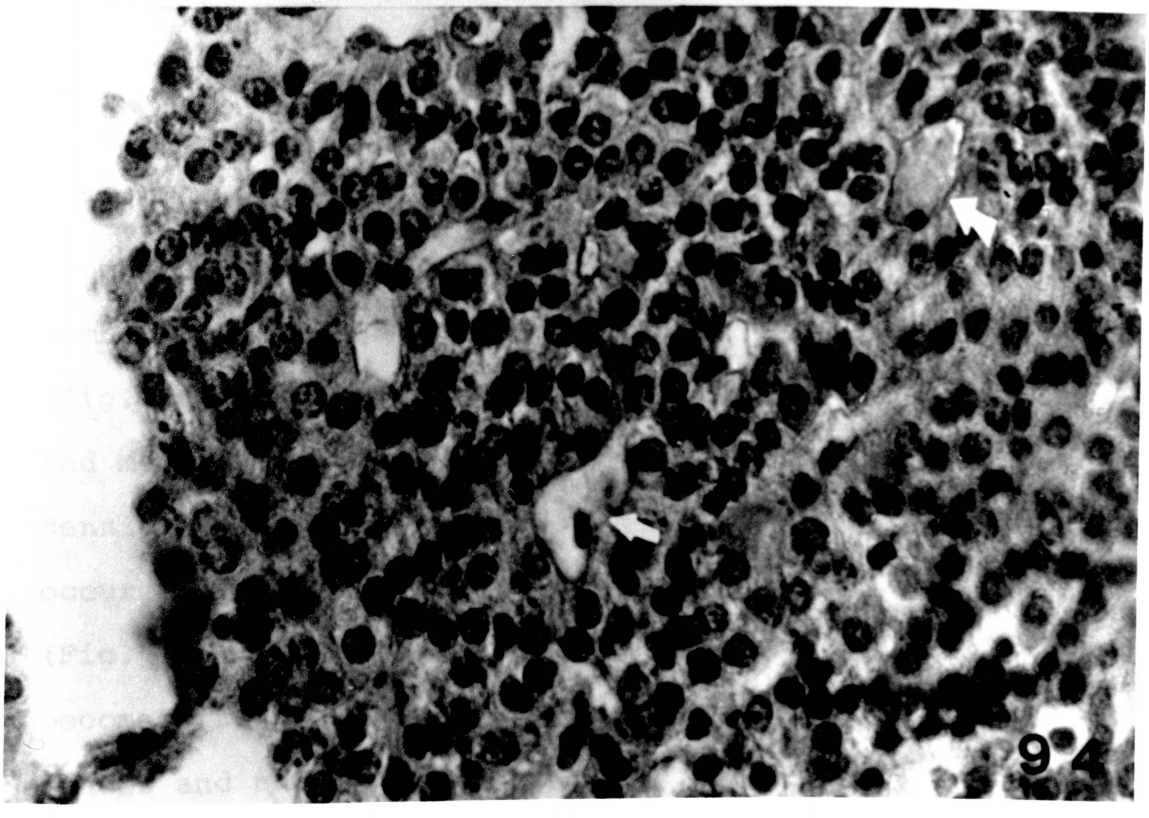
the finely scattered chromatin. The granules are of variable density and up to 200 mμ in diameter. The mitochondria are elongated and cristae are shelf-like. Electron dense bodies larger than the secretory granules are often seen and presumed to be lysosomes. The endothelium of the capillaries in the pars distalis is of the fenestrated type (Fig. 92).

VIII. Morphological and ultrastructural observation of the pars distalis of senescent female hamsters

Light microscopy of the cells of the pars distalis in senescent hamsters reveals several gross changes which occur as the animal ages. Areas of darkly-staining substance, presumably colloid, collect intracellularly and form vesicles within the cells as it pushes the cytoplasm to the outside. The nuclei of these cells tend to degenerate and the cells coalesce into large vesicles creating intercellular vacuoles within the connective tissue stroma (Figs. 93 and 94). The faint outlines of the individual degenerating cells within the large vacuoles can still be distinguished by their cell membranes. Cells in the immediate area of these vesicles and those cells just beginning to degenerate are often basophils, indicating the probability that

Figures 93-94. High magnification photomicrographs of sections of the pars distalis of the hypophysis from senescent hamsters are stained with H and E. (Fig. 93) An accumulation of substance which appears to be hyaline results when cells containing colloid coalesce into a single vesicle (A) with the cytoplasm pushed to the outer walls. This is shown here in a Day 1 senescent animal. Intercellular vacuoles (B) and dialated or cystic sinusoids are visible. X 2331. (Fig 94) A section from a Day 2 female shows several vesicles (arrows) of which contain colloid. X 2331.

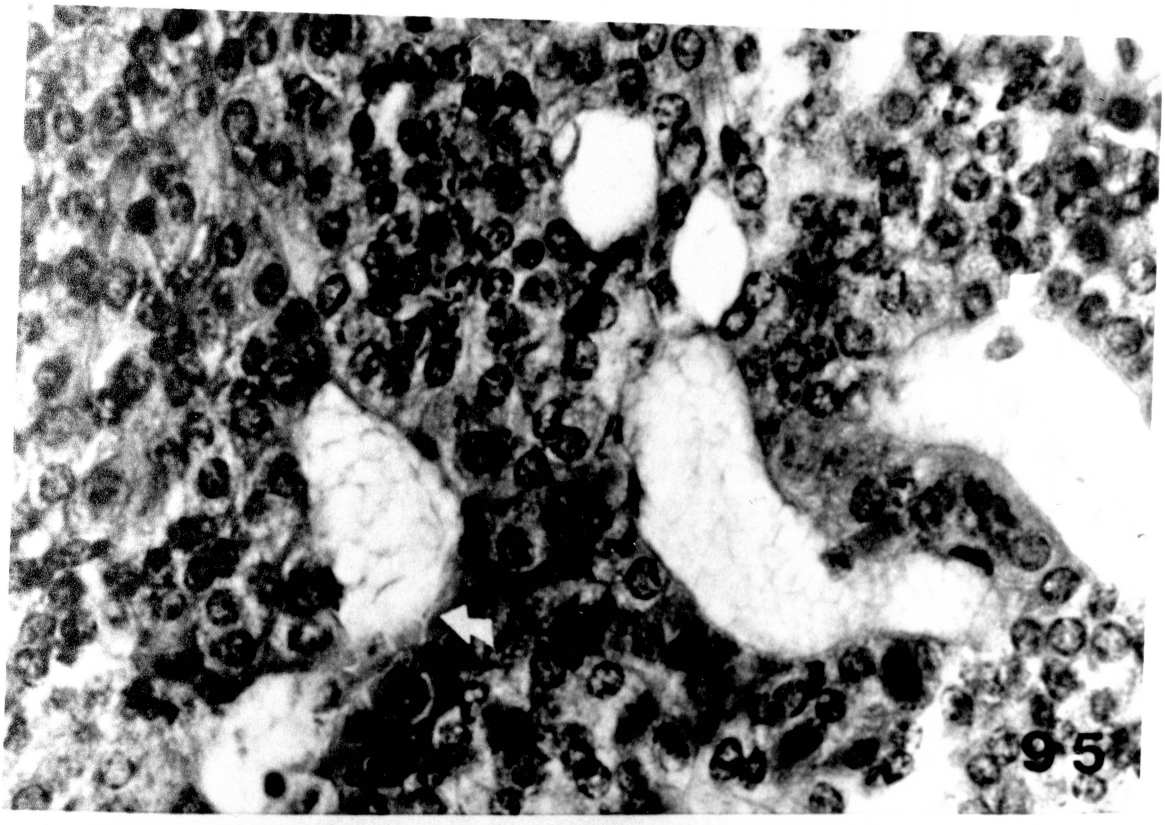

A
B
9 3

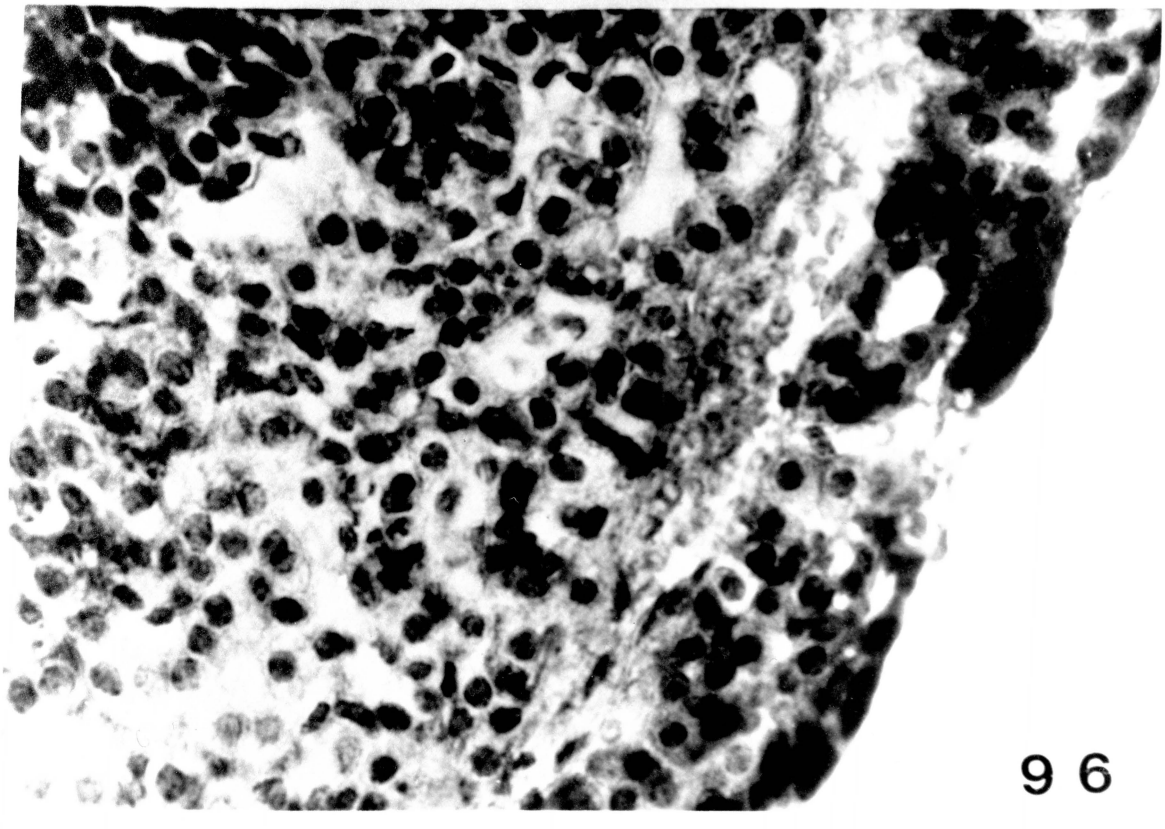

9 4

vesicles are usually the end result of the aging process of basophils. The regularity of cell arrangement deteriorates as vesicles enlarge (Fig. 95) and a large variability of size of basophils becomes obvious. Small cells often arrange themselves around these vesicles (Fig. 96), become hypertrophic, and stain in a manner much like chromophobes. The entire adenohypophysis shows decreased vascularity and an accumulation of pigment is not rare (Figs. 96 and 99). These hypertrophic basophils become inactive and hyperplastic (Fig. 98). Larger areas of disruption possibly represent adenoma (Fig. 99).

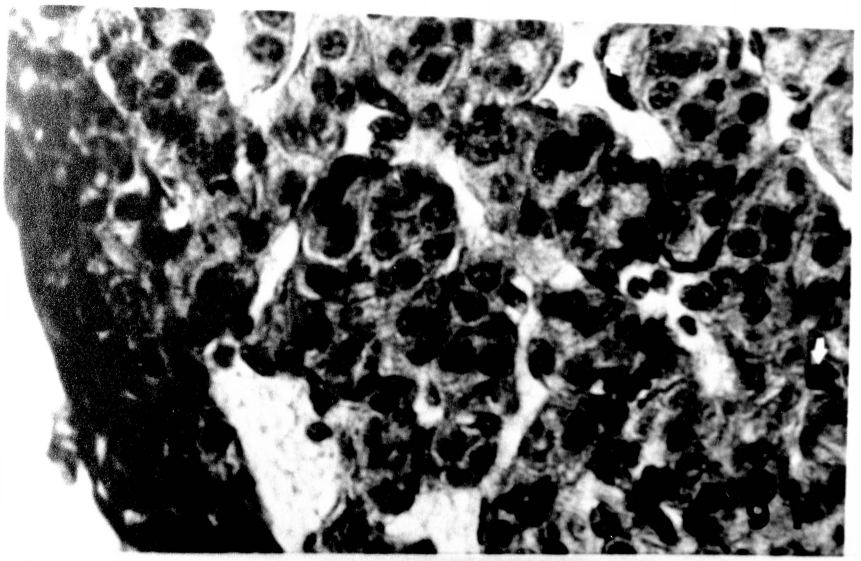
TEM reveals numerous intracellular changes which occur in gonadotrophs of senescent female hamsters. The gonadotrophs lying next to the vascular spaces are hypertrophied, the nucleus bizarre in shape, the nuclear membrane becomes irregular, and the cortex becomes dense (Fig. 100). Lipid vacuoles accumulate in the cytoplasm and membrane-surrounded inclusions of variable electron density become apparent (Fig. 100). Pigment accumulations occur and are often localized within cytoplasmic inclusions (Fig. 101). The scanty endoplasmic reticulum (Fig. 100) becomes fragmented and narrow with marked cisternlike spaces and numerous attached and free ribosomes (Fig. 101).

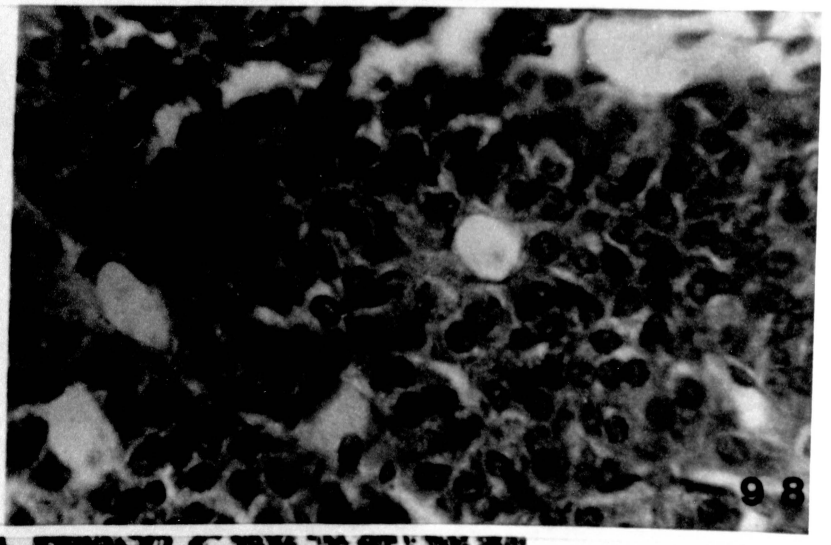
Figures 95-96. High magnification photomicrographs of H and E stained sections of the pars distalis of the hypophysis from senescent hamsters are shown. (Fig. 95) Vesicles in basophils of aged hamsters, some containing colloid (arrows), can be seen in the par distalis in this section from a Day 3 female. A distinct decrease in the regularity of cell arrangement is obvious. X 2331. (Fig. 96) Small cells are arranged as a vesicle in this section from a Day 4 animal. The decreased regularity of cell arrangement and variability in size is seen. X 2331.


95


96

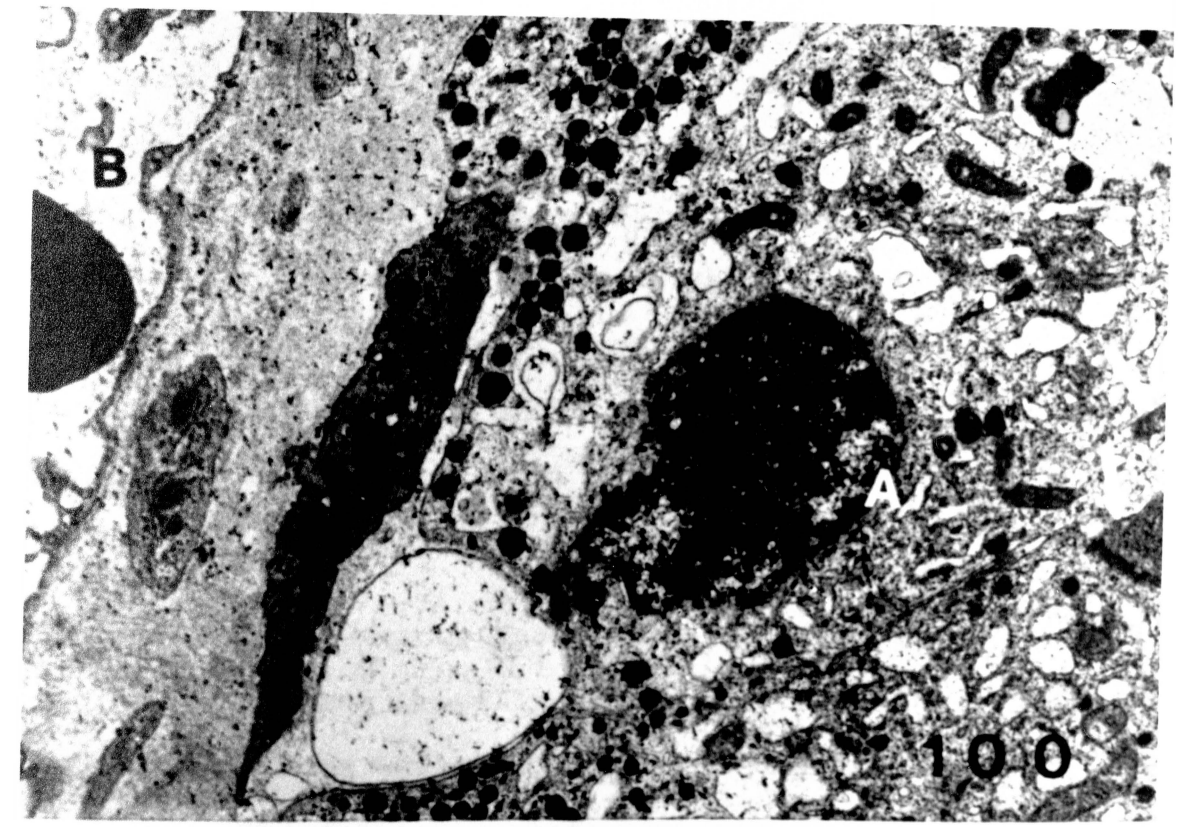
Figures 97-99. Photomicrographs of sections taken from senescent female hamsters are stained with H and E. (Fig. 97) A high magnification photomicrograph reveals decreased vascularity and accumulation of pigment (arrows). X 1665. (Fig. 98) High magnification photomicrograph reveals small nests of hypertrophic cells arranged as vesicles. These stain in a manner similar to that of chromophobes as they become inactive and hyperplastic. X 1665. (Fig. 99) Low magnification shows area of disruption of tissue that represents adenoma. X 416.

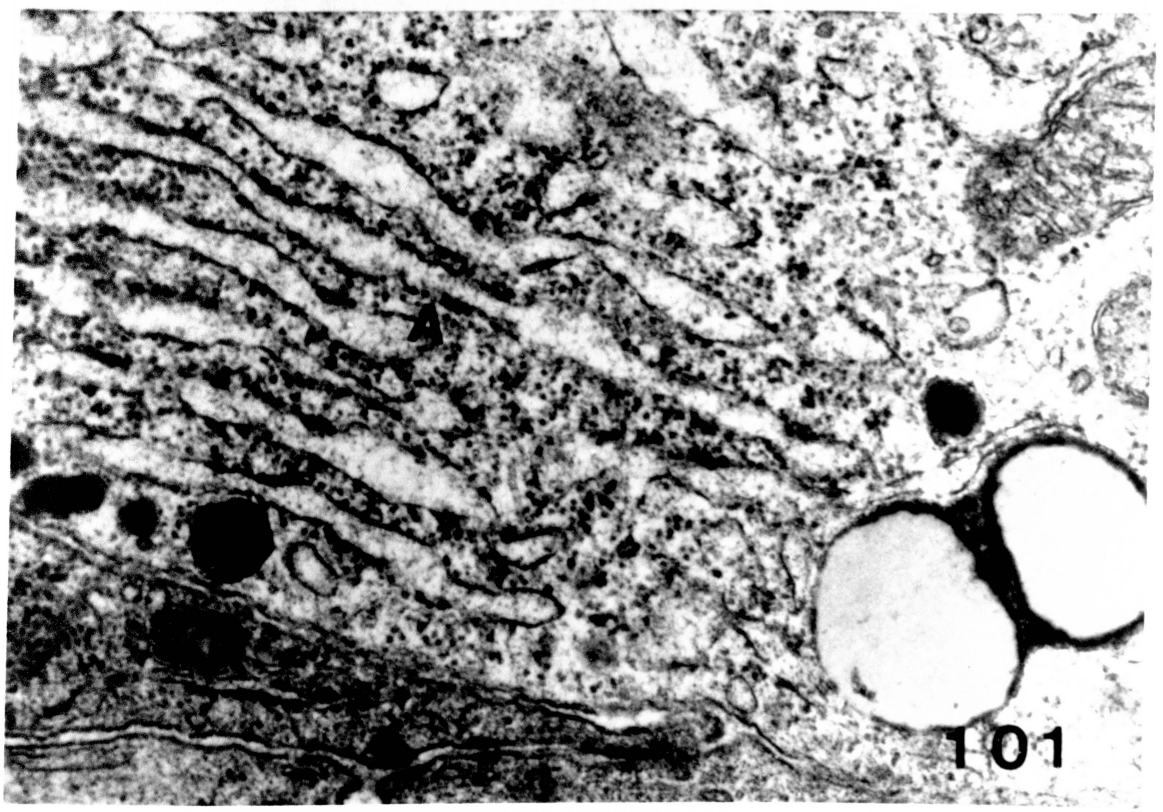



98


99

Figures 100-101. Transmission electron photomicrographs detail the pars distalis of senescent female hamsters. (Fig. 100) A hypertrophied gonadotroph (A) lying adjacent to vascular space (B) displays a decreased number of secretory granules. The bizarre-shaped nucleus is irregular in shape, and possesses a cortex of greater density. Numerous inclusions of variable electron density accumulate in the cytoplasm. Areas of pigment accumulation (arrows) are visible. X 13,500. (Fig. 101) The endoplasmic reticulum (A) of gonadotrophs becomes fragmented and narrow. Cisternlike spaces are visible. Numerous ribosomes, both attached and free, are seen. X 45,000.


B
AJ
100

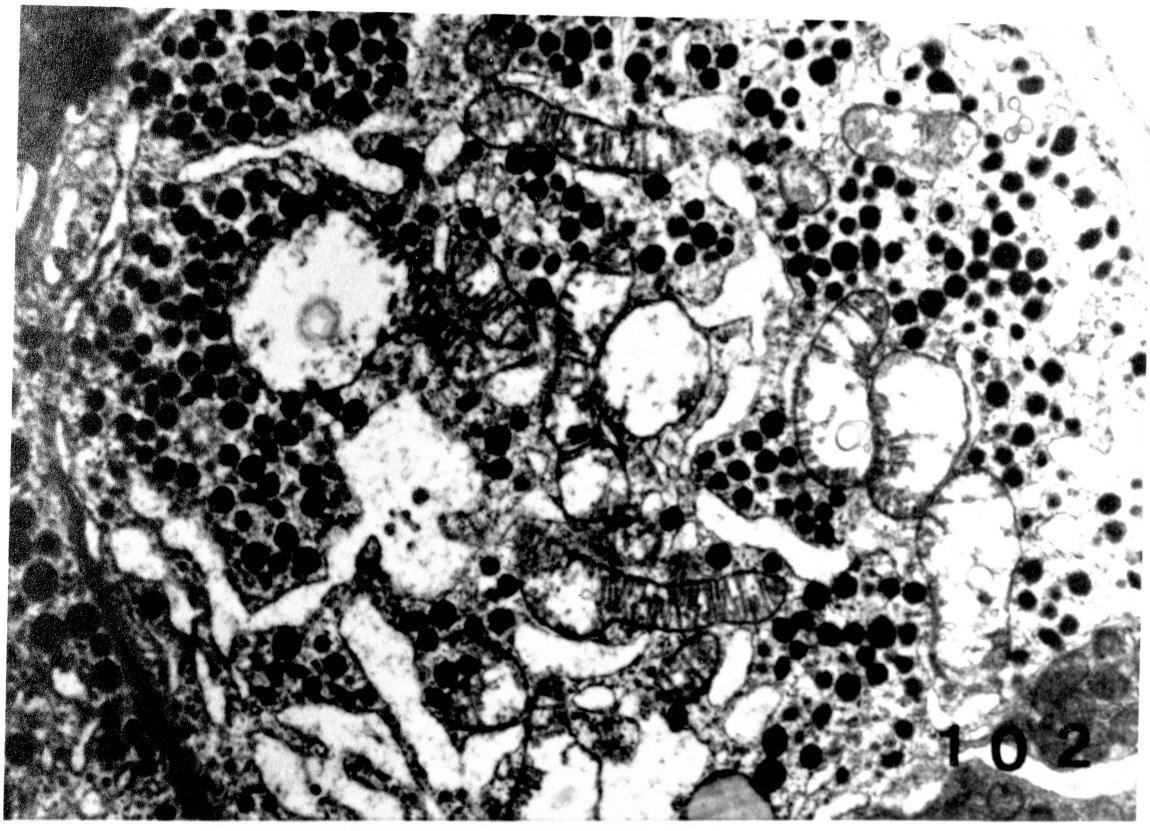

A
101

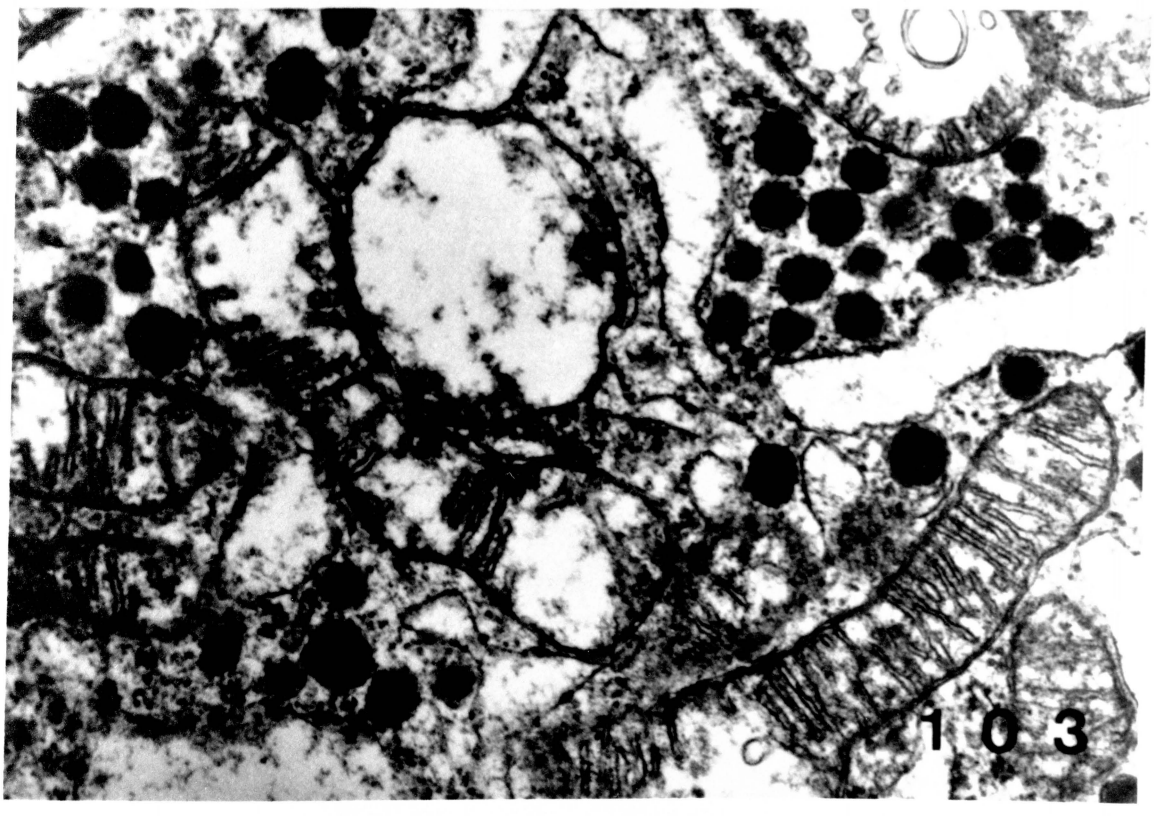
The mitochondria enlarge to several times their normal diameter (Fig. 102). The double-membraned septa often remain as mere stubs as vacuolation occurs. The vesicles fill with cytoplasm and appear to enlarge by coalescence of smaller ones to produce large vesicular areas within the cell (Figs. 102 and 103). The capillaries and sinusoids possess enlarged lumen (Fig. 104) and extrusion of granules is observed through the endothelia (Fig. 105).

IX. Behavioral estrous and litters obtained

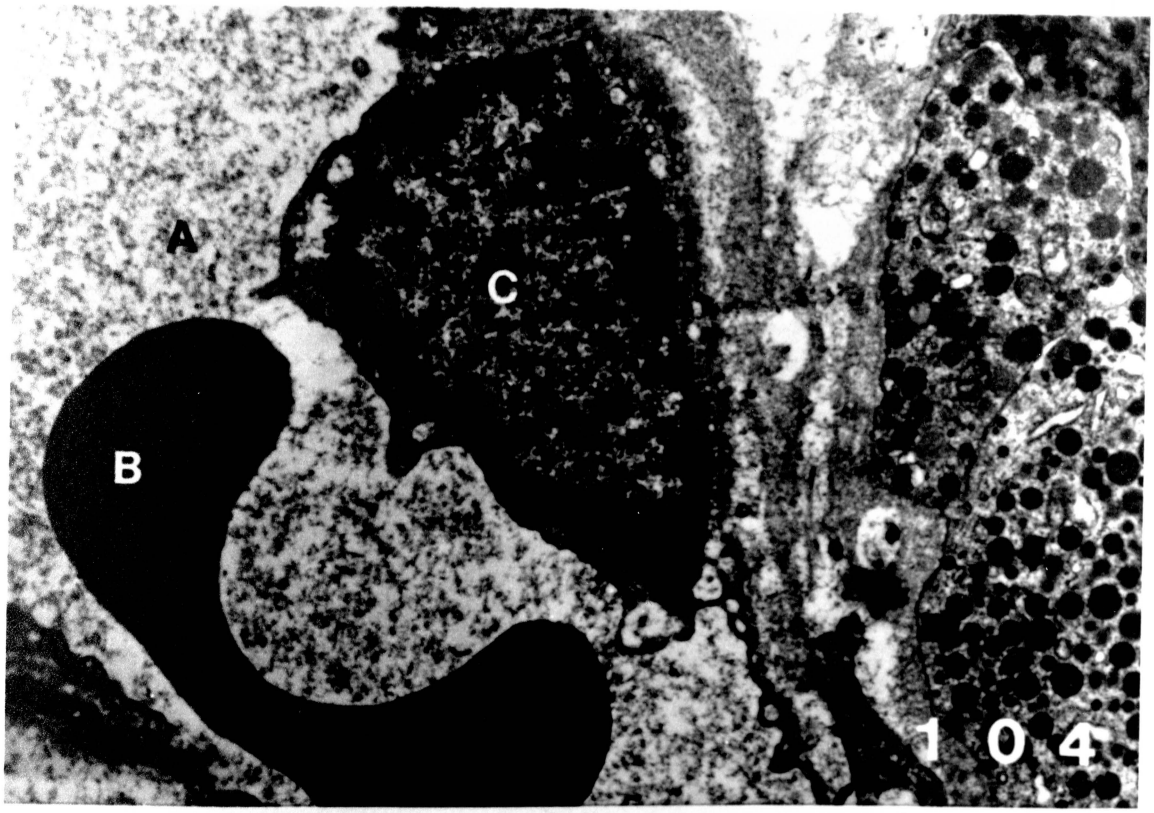
Twenty young sexually mature female hamsters and twenty senescent females were mated without benefit of vaginal readings. The males were left with the females for one week. Seventeen (85%) of the young females and two (10%) of the senescent females littered with average litter sizes of 9.1 and 3 respectively.

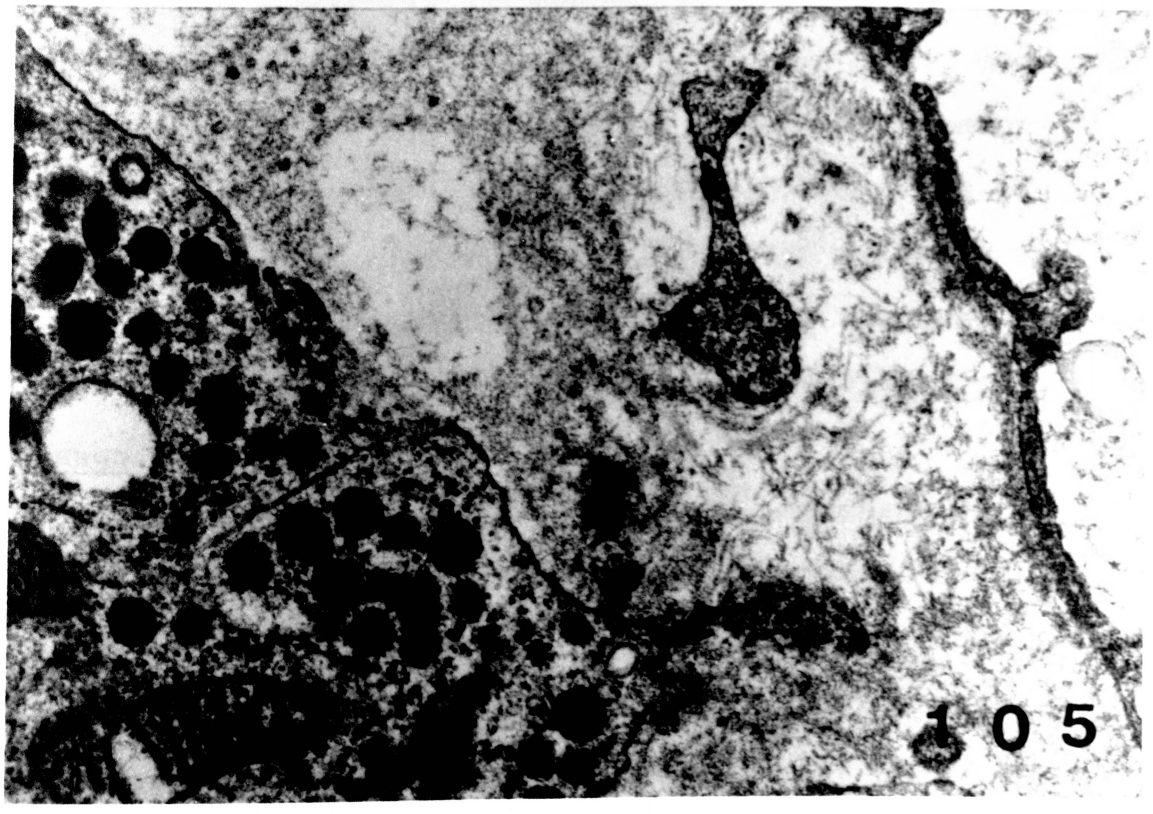
Figures 102-103. Transmission electron photomicrographs detail the pars distalis of senescent female hamsters. (Fig. 102) The mitochondria within this gonadotroph have enlarged several times their normal diameter. Degeneration results in vacuolation, and as the vesicles fill with cytoplasm, the spaces enlarge by coalescence. X 15,750. (Fig. 103) A higher magnification of mitochondria reveals degeneration within the double membraned organelle. The septa remain as stubs of the original shelf-like structures. X 40,500.


102


103

Figures 104-105. Transmission electron photomicrographs detail the pars distalis from senescent female hamsters. (Fig. 104) A capillary or sinusoid of a gonadotroph possesses an enlarged luman (A). A erythrocyte (B) and a white blood cell (C) are visible. X 13,500. (Fig. 105) A greatly magnified section of the endothelium reveals extrusion of granules (arrow) occurring. X 36,000.


A
C
B
104


105

DISCUSSION AND CONCLUSIONS

The present study reports morphological and ultrastructural observations on the hypothalamic-hypophyseal-ovarian axis of sexually-mature female hamsters and relates similar observations to those seen in aged animals. Vaginal phenomena were observed and correlated with cycle days and morphological observations of the ovary. These findings have been catalogued. The possible mechanisms of ovulation in the sexually mature animal and of disruption of ovulation in the senescent animal are discussed. The principal technical problems encountered in the use of light microscopy - the attainment of a high degree of resolution and the observation of external surface changes, were overcome by the utilization of transmission electron microscopy and scanning electron microscopy respectively. EM proved to be an excellent and valuable biological tool and provided a means of determining intracellular changes.

The correlation of vaginal phenomena with morphological change.

The attempt to correlate vaginal phenomena with cycle day and observed morphology yielded much useful data. The study indicated that vaginal phenomena is a reliable method for the determination of cycle day. Normal changes expected as recorded in the literature, do occur, and when correlated

with light microscopy, scanning electron microscopy, and behavioral estrous, add much validity to the use of vaginal phenomena as a research tool for the reproductive biologist. Young sexually-mature females cycled regularly through each day of the 4-day cycle. Young animals displaying Day 1 phenomena by 10:00 a.m. were exposed to the male on that evening only. Of these animals, 95% littered with the average litter size being 11.4. Females whose vaginal phenomena were ignored were placed with the male for one week. Of these animals 85% littered and the average litter size was 9.1. This indicates that the vaginal phenomena is a useful research tool and suggests its application to the breeding of hamsters. The female is notorious for her aggressive behavior toward the male when she is not in heat. Many males are maimed and killed during the periods when they are left for days with females. Not only does the percent of females littering increase, but the litter size is somewhat greater when the two animals are placed together at the appropriate time.

Due to the intricate hormonal feedback mechanisms which collate the operation of this four-day cycle and result in ovulation, one must take a close look at the three tissues involved and consider their relationship to each other in order to understand the reproductive

statis of the animal. The three-point axis determines the endocrine environment of the animal and it must be considered during any attempt to explain morphological changes in these tissues as they occur with age. Although no hormonal studies were attempted during this investigation, the literature abundantly supplies the necessary information. A failure at any point would initiate a chain reaction. If FSH-RF fails to reach the adenohypophysis, FSH would be withheld from the ovary, follicular maturation could not occur, and lower estrogen supplies in the blood would result in loss of the estrogen-mediated LH response. The unstimulated granulosal cells could not produce progresterone which is required by the hypothalamus before a new cycle is initiated by FSH-RF. Thus the female is dependent upon coordinated operation of the three tissues in the maintenance of a "normal" cycle and anything which disrupts the feedback mechanisms at any point - either in a primary cycle or a secondary cycle is likely to disrupt the entire system. The results of this study indicate that aging interferes with this mechanism.

Mechanism of ovulation in "normal" young sexually-mature females.

The normal hamster ovary is a globose, lobate organ with relatively few fissures suspended from the dorsal body wall by the mesovarian. An abundance of secondary and vesicular follicles are apparent in the ovary of a sexually-mature hamster. At proestrous, a rapid follicular growth occurs, follicular liquid and granulosa cells increase in amount, and the vascularity of the organ is enhanced. Granulosa cells swell and follicular liquid-filled spaces form before ovulation occurs. The present study has described observations on the hypothalamic-hypophyseal-ovarian morphology and ultrastructure and related them to the process of aging. The study has revealed that there is a progressive degeneration of the epithelial covering of the follicle on Day 1. Increasing pressure, produced by the edema, ruptures the weakened areas in the epithelium. The ovum is released on Day 2 and the evolution of the corpus luteum is seen on Day 2 ovaries.

These morphological changes must be considered in the light of the endocrine environment as it has been reported in the literature. A complete and critical review of the literature concerning the mechanism of

ovulation is not the purpose of this paper. In attempting to interpret observed changes and relate them to follicle rupture, it is however, of benefit to consider the cyclic nature of the vaginal phenomena in the light of biochemical and hormonal influences as reported recently by other investigators and to consider these influences as effectors of the observed morphological changes seen in the hamster during the present investigation. The tentative scheme represents a working hypothesis that includes many results from the last two decades of experimentation and much conjecture on the part of the author.

As the previous corpora lutea begin to degenerate, an FSH rise, induced by FSH-RF, stimulates follicular growth (Fig. 9), but whether this involves follicles that will be ovulated two or six days later remains indefinite. Microvilli increase in size and number about this time and may correspond to the increase in LH receptors and LH stimulable cyclase which have also been observed at this time (Chang et al., 1977) -- especially in view of the fact that LH binding is dependent upon the number of receptors (Channing and Kammerman, 1974). The ovarian response to the LH rise (Alleva et al., 1976; Leavitt et al., 1973; Lukaszewska and Greenwald, 1970) may be

mediated by prostaglandins or estrogen or both. The functional relationship between LH and the prostaglandins remains obscure and the role of prostaglandins may be one of an obligatory mediator of adenylate cyclase (Rondell, 1974), or two entirely different mechanisms converging on the same pathway (Lamprecht et al., 1973; LeMaire et al., 1973). The estrogen rise which either preceeds the LH surge (Rondell, 1974) or coincides with it (Schwartz, 1974) influences the ability of the ovary to respond to LH by either turning on the release of LH or by regulating the expression of LH via the turning on of a memory center (Freeman et al., 1976; Rondell, 1974).

As LH increases and becomes greater than FSH, the ovarian multiphasic response is seen on Day 1 of the estrous cycle. The level of $_{c}$AMP increases and implicates $_{c}$AMP as a second messenger in negotiating a change (Rondell, 1974). Cholesterol esters are mobilized for the production of progesterone which peaks late in the afternoon of Day 1 (Leavitt et al., 1973; Leavitt and Blaha, 1970; Lukaszewska and Greenwald, 1970; Guraya and Greenwald, 1965; Deane, 1952). Negative feedback mechanisms abolish the stimulatory effect of LH by estrogen (Freemen et al., 1976). This progesterone peak has been shown to stimulate protein synthesis which would be necessary to produce the postulated

"ovulatory" enzyme which may possess a collagenolytic activity and cause the observed disruption of collagenous tissues such as the theca externa. Some reports state that the observed activity--although capable of disruption--is not one of a true collagenase (Espey, 1974). The decrease in proliferation of granulosal cells (Fig. 14) and loss of microvilli (Fig. 15) seen in Day 1 are attributed to LH influences and preceed the formation of a functional corpus luteum.

The increase in prostaglandin synthesis as ovulation approaches (Yang et al., 1973) has let to the conclusion that prostaglandins are involved in the process of rupture. This involvement could involve enzyme or steroid synthesis or both (Rondell, 1974). Inhibitors of prostaglandin synthesis block ovulation; thereby suggesting that prostaglandins in some way mediate the ovarian response to LH (Tsafriri et al., 1972; O'Grady et al., 1972), indirectly by inhibiting the stimulatory effect of LH on adenyl cyclase thus inhibiting $_{c}$AMP synthesis (LeMaire et al., 1973) or directly by their absence. Prostaglandins are capable of stimulating contractile processes. This may have functional significance to the process of ovulation but is probably not an absolute prerequisite (Espey, 1978). Prostaglandins have been noted to originate from sympathetic nerves of

stimulated dog spleen (Davies et al., 1967), and the LH-triggered local release of catecholamines in the ovary is potentially capable of stimulating prostaglandin synthesis or stimulating adrenergic nerves whose existence in the theca externa has been shown (Walles et al., 1975; Bahr et al., 1974). This nerve stimulation may be controlled by certain prostaglandins, especially E_2 (Stjarne, 1973).

The Day 4 estrogen rise induces synthesis of a variety of enzymes such as the proteolytic enzymes capable of the disruption of collagenous tissue (Fig. 16) which is seen later (Espey and Rondell, 1968). A nine-fold increase in the number of multivescular bodies in the tunica albuginea just prior to the onset of ovulation (Espey, 1974; Espey, 1971) and increase in estrogen-stimulated acid phosphotase activity has been reported, suggesting a direct relationship between lysosomes and the breakdown of the surface epithelia and the theca externa layers seen in Day 1 ovaries (Fig. 16). Production of prostaglandins capable of labelizing the lysosomal membranes (F_2) greatly increases about this time (Bjersing and Cajander, 1975; Weiner and Kaley, 1972) and may supercede the stabilizing effect of other prostaglandin groups such as E_1 (Ferguson et al., 1973), causing the release of lysosomal contents in the immediate area

resulting in the disruption of collagenous connective tissue (Espey, 1976; Espey, 1974), fibrinolytic activity, and breakdown of the follicular wall. At this time, estrogen levels have dropped, introducing fragility of capillaries and allowing rupture of blood vessels (Cherney et al., 1975; Rona, 1963). Prostaglandin E_1 and estrogen have permitted vascular permeability (Bjersing and Cajander, 1975) and fluid accumulation resulting in local edema and separation of connective tissue (Fig. 14).

Estrogen may induce the formation of mucopolysaccharides, and hemostasis becomes apparent in the area of locally secreted estrogen (Cherney et al., 1975; Rona, 1963) contributing to follicular wall breakdown. The thecal or granulosal cells that are mature, and have been estrogen-primed (Rao et al, 1978) undergo luteinization (Channing and Kammerman, 1974; Koering, 1969) concurrently with ovulation to evolve the corpora lutea (Fig. 17-20).

Disruption of ovarian cycle in senescent animals.

Several morphologic changes occur in the ovaries of senescent hamsters which could interfere with the passage of substances between the blood stream and the tissues. The blood vessels become thickened due to sclerotic walls and hyalinization of the membranes (Fig. 62); the epithelial structures within the ovary also proliferate.

They form many papilliform projections (Figs. 46-52) and become hyperplastic as growth is uncontrolled (Bourne, 1961). The tunica albuginea appears to be thickened and takes on a stratified appearance due to the proliferation of dense fibrous connective tissue between it and the germinal epithelium (Fig. 31). This could result in a loss of sensitivity to gonadotropins from the pars distalis. A distinct decrease in the number of Graaffian follicles and the absence of fresh corpora lutea is apparent. Thus ovulatory failure results in failure of luteinization which in turn results in failure of new follicles to grow. However, the total hormone environment of the senescent animal as recorded in the literature must be examined carefully to determine which event occurs first and initiates such a chain reaction. If an apparently-exhausted ovary from an old animal is transplanted into a younger female, ovulation does occur (Aschheim, 1976). Therefore, the cause of atresia in the large preantrial and vesicular follicles may be extra-ovarian. Ovarian failure in young animals may by hastened by an increased level of gonadotropin (Everitt and Burgess 1976). This seeming contradiction necessitates a careful look at the feedback mechanisms involved as the other members of this three-point axis are considered.

Morphologic variations in the pars distalis of senescent animals.

Since the pituitary is known to synchronize reproductive activity with instructions from the brain, any change in the cells of this tissue would be suspect in negotiating a change in ovarian function. A deficiency of hormones within the cells due to lack of production or a decreased output due to lack of stimulation could play a role in the decreased fertility observed in senescent animals. However, one must also consider the fact that observed morphological changes could be a consequence of senescence and not the causative agent. Many pituitary changes were observed in the present study but there is no evidence that the pituitary holds the primary responsibility for reproductive failure. Gross disorganization of cells and decreased vascularity were apparent by light microscopy. Differential cell counts were not attempted due to the lack of validity of such counts. This is an unreliable indicator because the appearance and staining properties of cells reflect variable states of hormone synthesis, storage and release (Tixier-Vidal and Farquhar, 1975). Because of the essentially simultaneous release of LH and FSH, attempts to determine cell type responsible for their individual

production have been relatively unsuccessful (Martin, 1976; Harris and Donovan, 1966; Thompson, 1960; Serber, 1958) and it has been speculated that the same cell is responsible for both (Ham, 1974).

The morphologic and ultrastructural changes observed in the pars distalis of senescent hamsters reflect signs of increased activity rather than lack of it. These include degranulation of cells (Fig. 100), peripheral location of remaining granules, and increased size and number of mitochondria (Fig. 102). Increased vacuolation of basophils becomes apparent (Fig. 102). These results indicate an increased state of activity and suggest a decrease in the efficiency of the negative feedback system to control LH and FSH by the hypothalamus. This is in agreement with the increased levels of gonadotropin observed in human females at menopause (Verzar, 1966).

Morphologic variations in the hypothalamus of senescent hamsters.

Cellular disruption and decreased order in the arrangement of cells were observed in the area of the arcuate nucleus of the hypothalamus. The tissue retracts from blood vessels in the area creating an extracellular vacuolization (Fig. 81). The fact that such shrinkage occurs such that contact with the vascular network is

interrupted suggests that difficulty may arise in processing and transporting releasing factor. The great diversity in size of cells suggests the possibility of hypertrophy of some of them indicating excessive function.

Mechanism of disruption of ovulation in senescent animals.

The generalized disruption of cells and their resulting disorder in all three of the tissues examined suggests that responsiveness of cells to their environment (hormonal or otherwise) diminishes with increasing age. The question before us is whether such failure is in the primary domains of the hypothalamus, the immediate regulatory system of the pars distalis or within the somatic functions they regulate in the ovary. Thus, we must consider each tissue in turn as a possible culpret.

The aging cause could be within the ovary resulting in the insensitivity of follicular cells to LH and FSH. If the follicle then failed to mature properly, estrogen levels would decrease and remain low in the blood; thereby causing an increase in FSH due to lack of positive feedback to the hypothalamus. The ovulatory surge of LH late in the follicular phase depends on increased levels of estrogen to mediate its action. This suggests that something innate in the young host may be capable of reversing the stratified condition of the epithelium

such that the follicle is allowed to rupture through its wall rather than atrophy. This is likely because the increased levels of estrogen mediate the LH response and prepare the theca and the granulosal epithelia for rupture by weakening collagen, separating connective tissue, causing the synthesis of enzymes, and causing the pressure from the ever-increasing edema.

The second possibility is that ovarian failure is a result of the FSH to be released from the pituitary. This means that either the FSH is not produced or that it is not released into the blood stream. The electron microscopy in this study indicated that the gonadotropin cells are highly active in synthesis and release of gonadotropin. This could be FSH, or LH, or both. If we extrapolate data from the post-menopausal woman, we can assume that high levels of FSH remain in the blood long after ovulation has ceased (Everitt, 1976). Therefore, we are forced to assume that the sensitivity of the gonadotrophs to RF has not decreased. This indicates failure of receptors to suppress trophic hormone release thus causing overstimulation of target tissues and their ultimate exhaustion. Thus the receptors in the hypothalamus or the pituitary or both (Fig. 106) change with age such that an elevated feedback threshold for estrogen

exists, or the receptors in the hypothalamus for FSH and LH are modified such that this threshold for positive feedback is elevated. The cellular disruption suggests that aging changes would have less long range consequences in tissues which are capable of repair. As long as the rate of repair exceeds the rate of degradative changes, the consequences of change produced by aging is of little concern. However, such is not the situation in neurons. Since these are nondividing cells, the primary candidate is likely to be in a nondynamic system such as the hypothalamus (Everitt and Burgess, 1976). The administration of L-DOPA reinitiates the estrous cycle in aged rats (Quadri, Kledzik and Meites, 1973) by raising the level of brain catecholamines. This suggests that brain catecholamine deficiencies result in disruption of estrous cycles and that such deficiencies can be repaired by sympathomimetric drugs.

Conclusions

The study indicates that vaginal phenomena may be correlated with morphological changes within the ovary, and behavioral estrous. Biochemical and hormonal variations as recorded in the literature may then be utilized in a speculative manner to consider the possible cause of such changes. When used in a conscientious manner, the

vaginal phenomena of the hamster is an excellent research tool for the reproductive biologist.

The post-reproductive, aging female hamster may express the vaginal phenomena in the predictable manner or may be acyclic. Those animals expressing acyclic vaginal phenomena showed no signs of ovulation or corpora lutea formation within their ovaries. This indicates that vaginal phenomena is a result of the endocrine environment of the animal and may be independent of the actual follicle release.

The histological and ultrastructural morphology of the neuroendocrine reproductive axis in the female hamster indicates extensive changes that accompany the aging process. These changes do not result in total cessation of function and may even enhance the function such as is the case with gonadotrophs; therefore, the aging of the reproductive system in the female is probably the result of a modification of function rather than merely the cessation of function. Due to the fact that all three tissues show cellular disruption, the fault must lie within the "weakest link" and one is forced to consider the failures in a chronological manner. The results of this study indicate that the pars distalis is not only functioning, but is doing so at an increased rate. No

signs of ovulation were found in any of the ovaries of senescent hamsters; therefore, hormonal imbalance can be assumed. Whether the fault is in the hypothalamus or the ovary is uncertain. The cells in both fail to respond to their hormone environment; probably due to a structural alteration in receptors or perhaps a decrease in number of receptors.

In the literature, definitions of aging usually include the stipulation that an aging change must be irreversible, and would therefore necessitate a reappraisal of any research results if one is content with such a definitioan. Changes occurring in tissues such as the ovary, that can be reversed when transplanted into a young animal and result in a rejuvenation of function, cannot truely be considered in the light of such a definition as aging changes.

Changes within cells may be the result of alteration at the supracellular level or they may be the cause of such changes. Much more research is necessary to determine which is the fact, and ultimately it may be a delicate interplay between the two. Those in pursuit of a molecular mechanism of aging would do well to superimpose any results onto the supracellular picture to get the full benefit of the research as it applies to the metazoan.

This research has investigated the events that are precursors of ovarian regression and the investigator has attempted to interpret all results in the light of knowledge of the hormone mediated influences reported in the literature. It is hoped that the knowledge gained in searching for the mediators of female reproductive senescence might ultimately contribute to the search to gain an understanding of the aging process in general.

LITERATURE CITED

Adams, C. E. 1910. Ageing and reproduction in the female mammal with particular reference to the rabbit. J. Reprod. Fert. Suppl. 12:1-16.

Alleva, J. J., F. R. Alleva, and M. W. Lipien. 1976. Use of vaginal concretions of calcium carbonate to detect ovulation in hamsters. Laboratory Animal Science. 26(1):57-62.

Alleva, J. J., F. R. Alleva, B. E. Fry, Jr., and E. D. Eanes. 1971a. Calcium carbonate concretions: Cyclic occurrence in the hamster vagina. Science. 174:600-603.

Alleva, J. J., M. W. Waleski, and F. R. Alleva. 1971b. A biological clock controlling the estrous cycle of the hamster. Endocrin. 88:1368-1379.

Alleva, J. J., M. V. Waleski, F. R. Alleva, and E. J. Umberger. 1968. Synchronizing effect of photoperiodicity on ovulation in hamsters. Endocrin. 82:1227-1235.

Amoss, M., R. Burgus, R. Blackwell, W. Vale, R. Fellows, and R. Guillemin. 1971. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem. Biophys. Res. Comm. 44:205-210.

Andrew, W. 1971. The anatomy of aging in man and mammals. Grune and Stratton, New York and London. pp. 186-205.

Ascheim, P. 1976. Aging in the hypothalamic-hypophyseal ovarian axis in the rat. In A. Everitt and J. Burgess, eds., Hypothalamus, pituitary, and aging. Charles Thomas, Springfield, Illinois. pp. 376-418.

Baba, Y, H. Matsuo, and A. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem. Biophys. Res. Comm. 44:459-463.

Bahr, J., L. Kas, and A. V. Nalbandov. 1974. The role of catecholamines and nerves in ovulation. Biol. of Reprod. 10:273-390.

Barnes, R. D., B. L. Lasley, and A. G. Hendricks. 1978. Midcycle ovarian histology of the bonnet monkey, Macaca radiata. Biol. of Reprod. 18:537-553.

Belt, E. 1952. Leonardo da Vinchi's studies of the aging process. Geriatrics. 7:205-210.

Birren, J. E., ed. 1960. Handbook of aging and the individual. The University of Chicago Press, Chicago, Illinois. pp. 5-16.

Bjersing, L. and S. Cajander. 1975. Ovulation and the role of the ovarian surface epithelium. Experientia. 31:605-608.

Bond, C. R. 1945. The golden hamster (Cricetus auratus): Care, breeding, and growth. Physiol. Zool. 18:52-59.

Bourne, G. H., ed. 1961. Structural aspects of ageing. Hafner Publishing Company, Inc., New York. pp. 113-301.

_____. 1968. Aging changes in the endocrines. In Leo Gitman, ed. Endocrines and aging: A symposium presented before the Gerontological Society seventeenth annual meeting, Mineapolis, Minnesota. Charles Thomas, Springfield, Illinois. pp. 66-75.

Burgus, R. and R. Guillemin. 1970. Hypothalamic releasing factors. A. Rev. Biochem. 39:499-526.

Campbell, H. J., G. Feuer, and G. W. Harris. 1964. The effect of intrapituitary infusion of median eminence and other brain extracts on anterior pituitary gonadotrophic secretion. J. Physiol. 170:474-486.

Chang, S. C. S., W. Anderson, J. C. Lewis, R. J. Ryan, and Y. H. Kang. 1977. The porcine ovarian follicle. II. Electron microscopic study of surface features of granulosa cells at different stages of development. Biol. of Reprod. 16(3):349-357.

Channing, C. and S. Kammerman. 1974. Binding of gonadotropins to ovarian cells. Biol. of Reprod. 10:179-198.

Cherkin, A., C. E. Finch, N. Kharasch, F. L. Scott, T. Makinodan, and B. Strehler, eds. 1979. Physiology and cell biology of aging. Vol. 8. Raven Press, New York. pp. 3-107.

Cherney, D. D., J. A. Liberato, and P. Motta. 1975. The development of rabbit ovarian follicles following copulation. Fertility and Sterility. 26(3):257-270.

Cooper, E. R. A. 1925. The history of the more important human endocrine organs at various ages. Oxford University Press, London. pp. 5-32.

Davies, B. N., E. W. Horton, and P. G. Withrington. 1967. The occurrence of prostaglandin E_2 in splenic venous blood following nerve stimulation. J. Physiol. (London). 188:38-39P.

Deane, H. W. 1952. Histochemical observations on the ovary and oviduct of the albino rat during the estrous cycle. Amer. J. Anat. 91:363-413.

DeGraff, R. 1974. De mulierum organis generation inservientibus tractatus novus, 1672. Annotated translation in J. Reprod. Fert. Supplement. 17:77.

Encyclopaedia Britannica, Inc. 1972a. William Benton, Publisher. Vol. 18:219. Chicago, London, Toronto, Geneva, Sydney, Tokyo, Manila.

Encyclopaedia Britannica, Inc. 1972b. William Benton, Publisher. Vol. 13:971. Chicago, London, Toronto, Geneva, Sydney, Tokyo, Manila.

Encyclopaedia Britannica, Inc. 1972c. William Benton, Publisher. Vol. 1:870. Chicago, London, Toronto, Geneva, Sydney, Tokyo, Manila.

Espey, L. L. 1971. Decomposition of connective tissue in rabbit ovarian follicles by multivesicular structures of thecal fibroblasts. Endocrin. 88: 437-444.

_____. 1974. Ovarian proteolytic enzymes and ovulation. Biol. of Reprod. 10:216-235.

_____. 1976. The distribution of collagenous connective tissue in rat ovarian follicles. Biol. of Reprod. 14:502-506.

_____. 1978. Ovarian contractility and its relationship to ovulation: A review. Biol. of Reprod. 19:540-551.

Espey, L. L. and Rondell, P. 1968. Collagenolytic activity in the rabbit and sow graafian follicle during ovulation. Am. J. Physiol. 214(1):326-329.

Everitt, A. 1976. The female climacteric. In A. Everitt and J. Burgess, eds. Hypothalamus, pituitary and aging. Charles Thomas, Springfield, Illinois. pp. 419-430.

_____. 1973. The hypothalamic-pituitary control of aging and age-related pathology. Exp. Gerontol. 8:265-277.

Everitt, A. and J. A. Burgess, eds. 1976. Hypothalamus, pituitary and aging. Charles Thomas, Springfield, Illinois. pp. 1-42.

Ferguson, W. W., A. W. Edmonds, J. R. Starling, and S. L. Wangenstein. 1973. Protective effect of prostaglandin E_1 (PGE_1) on lysosomal enzyme release in serotonin-induced gastric ulceration. Ann. Surg. 177:648-654.

Finch, C. E. and L. Hayflick, eds. 1977. Handbook of the biology of aging. Van Nostrand Reinhold Company, New York. pp. 318-357.

Freeman, M. C., K. C. Dupke, and C. M. Croteau. 1976. Extinction of the estrogen-induced daily signal for LH release in the rat: A role for the proestrous surge of progesterone. Endocrin. 99(1):223-229.

Fugo, N. W. and R. L. Butcher. 1971. Effects of prolonged estrous cycles on reproduction in aged rats. Fertil. Steril. 22:98-101.

Geiger, R., W. König, H. Wissmann, K. Geisen, and F. Enzmann. 1971. Synthesis and characterization of a decapeptide having LH-RH/FSH-RH activity. Biochem. Biophys. Res. Comm. 45:767-773.

Green, J. D. 1951. The comparative anatomy of the hypophysis with special reference to its blood supply and innervation. Am. J. Anat. 88:225-312.

_____. 1952. Comparative aspects of the hypophysis. In G. E. W. Wolstenholme and M. P. O'Connor, eds. Ciba Foundation colloquium on endocrinology. Anterior pituitary secretion and hormonal influences in water metabolism. Vol. IV. Churchill, London. pp. 72-86.

Green, J. D. and G. W. Harris. 1947. The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol. 5:136-145.

Griffin, J. J. 1950. Aristotle's observations on gerontology. Geriatrics. 5:222-226.

Guraya, S. S. and G. S. Greenwald. 1965. A histochemical study of the hamster ovary. Amer. J. Anat. 116: 257-267.

Guyton, A. C. 1971. Basic human physiology: Normal function and mechanism of disease. W. B. Saunders Company, Philadelphia, London, and Tronto. pp. 607-616 and 684.

Hafez, E. S. E., ed. 1976. Perspectives in human reproduction. Vol. 2. Aging and reproductive physiology. Ann Arbor Science, Ann Arbor, Michigan. pp. 1-38.

Ham, A. W. 1974. Histology. 7th ed. J. B. Lippincott Company, Philadelphia and Toronto. pp. 782-846.

Harmon, S. M. and G. B. Talbert. 1970. The effect of maternal age on ovulation, corpora lutea of pregnancy, and implantation failure in mice. J. Reprod. Fert. 23:33-39.

Harris, G. W. 1955. Neural control of the pituitary gland. Arnold, London. pp. 20-102.

Harris, G. W. and B. T. Donovan, eds. 1966. The pituitary gland. Vol. 1. University of California Press, Berkeley and Los Angeles. pp. 147-233.

Hayat, M. A. 1975. Principles and techniques of scanning electron microscopy. Vol. 3. Biological applications. Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne. pp. 1-80.

_____. 1978. Introduction to biological scanning electron microscopy. University Park Press, Baltimore, Maryland. pp. 1-125.

Humason, G. L. 1972. Animal tissue techniques. W. H. Freeman, San Francisco. pp. 148-169.

Ingram, D. L., A. M. Manol, and S. Zuckerman. 1958. The influence of age on litter size. J. Endocrinol. 17:280-285.

Jeffcoat, S. L. and J. S. M. Hutchinson. 1978. The endocrine hypothalamus. Academic Press, New York. pp. 271-332.

Kent, G. C., Jr. 1968. Physiology of reproduction. In R. A. Hoffman and P. F. Robinson, eds. The golden hamster. Iowa State University Press. pp. 119-138.

Kent, G. C., Jr. and J. P. Mixner. 1945. Use of vaginal smears in mating the golden hamster. Proc. Soc. Exptl. Biol. Med. 59:251-253.

Kerdelhue', B., S. Catin, and M. Jutis. 1975. Short and long term effects of anti-LH-RH serum administration on gonadotropic regulation of the female rat. In M. Motto, P. G. Crosignani, and L. Martini, eds. Hypothalamic hormones. Academic Press, London, New York, and San Francisco. p. 29-107.

Koering, M. J. 1969. Cyclic changes in ovarian morphology. during the menstrual cycle in Macaca mulatta. Am. J. Anat. 126:73-101.

Kohn, R. R. 1978. Principles of mammalian aging. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 1-99.

Korenchevsky, V. 1961. Physiological and pathological aging. In G. H. Bourne, ed. Hafner Publishing Company, Inc., New York. pp. 5-6 and 347-364.

Lamprecht, S. A., U. Zor, A. Tsafriri, and H. P. Lindner. 1973. Action of prostaglandin E_2 and of luteinizing hormone on ovarian adenylate cyclase, protein kinase and ornithine decarboxylase activity during postnatal development and maturity in the rat. J. Endocrinol. 57:217-233.

Leavitt, W. W., C. R. Basom, J. N. Bagwell, and G. C. Blaha. 1973. Structure and function of the hamster corpus luteum during the estrous cycle. Am. J. Anat. 136(2):235-249.

Leavitt, W. W. and G. C. Blaha. 1970. Circulating progesterone levels in the golden hamster during the estrous cycle, pregnancy, and lactation. Biol. of Repro. 3:353-361.

LeMaire, W. J., N. S. Yang, H. H. Behrman, and J. M. Marsh. 1973. Preovulatory changes in the concentration of prostaglandins in rabbit graafian follicles. Prostaglandins. 3:367-376.

Lukaszewska, J. H. and G. S. Greenwald. Jan. 1970. Progesterone levels in the cyclic and pregnant hamster. Endocrin. 86:1-9.

Martin, C. R. 1976. Textbook of endocrine physiology. The Williams and Wilkins Company, Baltimore, Maryland. pp. 346-389.

Matsuo, H., Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Comm. 43:1334-1339.

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

Metchnikoff, E. 1907. The prolongation of life-optimistic studies. London, Heinemann. pp. 1-30.

O'Grady, J., P. Caldwell , V. Burton, F. J. Auletta, and L. Speroff. 1972. The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation pregnancy, and pseudopregnancy in the rabbit. Prostaglandins. 1:97-106.

Orsini, M. W. Sept. 1961. The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, lactation, and the anestrous hamster, Mesocricetus auratus. Proceedings of the Animal Care Panel. 11(4):193-206.

Ovadia, J., J. W. McArthur, L. Kopito, and H. Ulfelder. 1971. The cervical mucus secretion of the bonnet monkey (M. radiata): Anatomical basis and physiological regulation. Biol. of Reprod. 5:127-145.

Ordy, J. M. and K. R. Brizzee, eds. 1975. Neurobiology of aging. Plenum Press, New York and London. pp. 1-22.

Pearl, R. 1921. The biology of death. VII. Natural death, public health, and the population problem. Sci. Mon. 193-212.

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

Rao, M., A. R. Midgley, Jr., and J. S. Richards. May 1978. Hormonal regulation of ovarian cellular proliferation. Cell 14:71-78.

Rona, G. 1963. The role of vascular mucopolysaccharides in the hemostatic action of estrogens. Am. J. Obstet. Cynecol. 87(4):434.

Rondell, P. 1974. Role of steroid synthesis in the process of ovulation. Biol. of Reprod. 10:199-215.

Sanborn, E. B. 1972. Light and electron microscopy of cells and tissues. Academic Press, New York. pp. 165-171.

Schally, A. V., A. Arimura, C. Y. Bowers, A. J. Kastin, S. Sawana, and T. W. Redding. 1968. Hypothalamic neurohormones regulating anterior pituitary function. Rec. Prog. Hor. Res. 24:497-588.

Schally, A. V., Y. Baba, A. Arimura, T. W. Redding, and W. F. White. 1971a. Evidence for peptide nature of LH and FSH-releasing hormones. Biochem. Biophys. Res. Comm. 42:50-56.

Schally, A. V. and C. Y. Bowers. 1964. In vitro and in vivo simulation of the release of luteinizing hormone. Endocrin. 75:312-320.

Schally, A. V., R. M. G. Nair, T. W. Redding, and A. Arimura. 1971b. Isolation of the luteinizing hormone and follicle-stimulating hormone-releasing hormone from porcine hypothalami. J. Biol. Chem. 246:7230-7236.

Schally, A. V., A. Arimura, and A. J. Kastin. 1973. Hypothalamic regulatory hormones. Science. 179: 341-350.

Schuchner, E. B. and J. C. Stockert. 1974. Ultra-structural evolution of the corona radiata follicular cells. Cytologia. 39:257-264.

Schwartz, N. B. 1974. The role of FSH and LH and of their antibodies on follicle growth and on ovulation. Biol. of Reprod. 10:236-272.

Serber, B. J. 1958. A cytological study of the anterior pituitary gland of the normal, gonadectomized, and thyroid deficient hamster (Mesocricetus auratus). Anat. Rec. 131:173-192.

Shock, N. W. 1957. A classified bibliography of geron-tology and geriatrics. Stanford University Press, pp. 1-485.

Sievertsson, H., J. K. Chang, C. Bogentoft, B. L. Currie, K. Folkers, and C. Y. Bowers. 1971. Synthesis of the luteinizing releasing hormone of the hypothalamus and its hormonal activity. Biochem. Biophys. Res. Comm. 44:1566-1571.

Simmonds, M. 1914. "uber embolische prozesse in der hypophysis" Virchows Arch Pathol. Anat. , 217:226.

Stjarne, L. 1973. Inhibitory effect of prostaglandin E_2 on noradrenine secretion from sympathetic nerves as a function of external calcium. Prostaglandins. 3:105-109.

Strehler, B. L. 1977. Time, cells and aging. 2nd ed. Academic Press, New York. pp. 1-57.

Strong, L. C. 1952. Observations on gerontology in the 17th century. J. Gerontol. 7:618-619.

Talbert, G. B. 1968. Effect of maternal age on reproductive capacity. Am. J. Obst. Gynecol. 102:451.

Thompson, R. J. 1960. Cytology of the hypophysis in the adrenalectomized golden hamster (Mesocriectus auratus) Am. J. Anat. 106:55-71.

Thorneycroft, I. H. and A. L. Soderwall. 1969. The nature of the litter size loss in senescent hamsters. Anat. Rec. 165:343-348.

Tixier-Vidal, A. and M. G. Farquhar. 1975. The anterior pituitary, Academic Press, New York, San Francisco, and London. pp. 3-20.

Tsafriri, A., Lindner, H. R., U. Zor, and S. A. Lamprecht. 1972. Physiological role of prostaglandins in the induction of ovulation. Prostaglandins. 2:1-10.

Turner, C. D. and J. T. Bagnara. 1971. General endocrinology. 5th ed. W. B. Saunders, Company, Philadelphia, London, Toronto. pp. 490-543.

Verzar, F. 1966. Anterior pituitary function in age. In G. W. Harris and B. T. Donovan, eds. The pituitary gland. Butterworths, London. Vol. 2. p. 444.

Vickery, B. H. and J. P. Bennett. 1968. The cervix and its secretion in mammals. Physiol. Rev. 48:135-154.

Walles, B., L. Edvinsson, B. Falck, C. A. Owman, N. O. Sjolberg, and K. G. Svensson. 1975. Evidence for a neuromuscular mechanism involved in the contractility of the ovarian follicular wall: Fluorescence and electron microscopy and effects of tyramine on follicle strips. Biol. of Reprod. 12:239-248.

Weakley, B. S. 1972. A beginner's handbook in biological electron microscopy. Churchill Livingstone, Edinburgh and London. pp. 44-64.

Weiner, R. and G. Kaley. 1972. Lysosomal fragility induced by prostaglandin $F_{2\alpha}$. Nature New Biol. 236:46-47.

Yang, N. S. T., J. M. Marsh, and W. J. LeMaire. 1973. Prostaglandin changes induced by ovulatory stimuli in rabbit graafian follicles: The effect of indomethacin. Prostaglandins. 4:395-404.

Young, W. C. 1961. Sex and internal secretion. Vol. 1 Williams and Wilkins, Baltimore, Maryland. pp. 161-239.

Zamboni, L. 1974. Fine morphology of the follicle wall and follicle cell-oöcyte association. Biol. of Reprod. 10:125-149.