Changes with age in the canine female genitalia: histomorphological study

George Goulding Stott

Iowa State University

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Iowa State University, Ph.D., 1970
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CHANGES WITH AGE IN THE CANINE FEMALE GENITALIA:
HISTOMORPHOLOGICAL STUDY

by

George Goulding Stott, D.V.M.

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa
1970
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INTRODUCTION

Aging is a multidefinitional term. One definition was that used by Sonneborn, (Strehler, 1960) in which aging was described as a degree of departure from the "normal" with respect to morphology, histology, cytology, physiology, biochemistry, etc. Getty (1966) felt that the aging process was "normal", and that to know the "normal" picture over the life span of years was the important objective, for without an understanding of the normal the determination of the departure from it was frustrated. The important facet of the present study was to describe the microscopic changes that occurred in the canine ovary and various portions of the tubular genital tract from shortly after birth, through the period of functional maturity and on into old age. It was hoped that the information gained from such a study would aid the pathologist in determining the extent of disease conditions more accurately in various aged animals, and that it might also be used as a baseline for further histochemical, biochemical, biomedical and electron microscopic studies, in furthering the understanding of the aging phenomenon. In addition to this the findings hopefully would be useful as a baseline for experimental studies in the various disciplines.

The importance of the reproductive systems and aging was put in focus by Talbert and Hamilton (1965). They found that one means of slowing down aging in rats and to increase their life span by about 20 percent was by gonadectomy at birth. This was interesting although somewhat impractical, but it did emphasize that the reproductive mechanisms in both the male and female contributed significantly to the aging process.
LITERATURE REVIEW

Ovary

Since Galen reported erroneously about 1800 years ago that the ovary was a filter for women's semen (Ringrose, 1963) much knowledge has been accumulated about this organ. The pattern of the estrous cycle, the age of sexual maturity, the time of year and length of the cycle were all factors which determined the number of young that could be born in the life span of one female. At one end of the scale was the Alaskan fur seal in which, according to Pearson and Enders (1951), each ovary underwent a yearly cycle alternating with the other ovary. The corpus luteum persisted for 21 months following ovulation and normally the females gave birth to their first pup at 3 to 7 years of age. At the other end of the scale was the rabbit in which the ovary produced mature follicles every 7 to 10 days until conception resulted (Eckstein and Zuckerman, 1956). The existence of anovular follicles in both seals and rabbits also affected the conception and thereby the number of young born. Anovular follicles have also been described in the ovaries of aged mice and sterile dingo's by Tamura (1927). According to Ringrose (1963) a gradual replacement of endocrine and ova producing tissue by fibrous tissue as well as a decreased nutrient supply to this tissue as a result of vessel wall thickening were aging factors that limited ovarian function. In summing up ovarian aging Leathem (1958) stated that as the available ova were consumed in the earlier years of life, the aged ovary reverted to a residue of connective tissue that showed metabolic alterations.
Embryology and development

Germ cells and fetal development of the ovary

Over the years, various investigators (Allen, 1904, Duke, 1941, Franchi, 1962 and Arey, 1965) agreed in general on 4 phases of ovarian development namely, 1) the migrations of germ cells from the lateral splanchnopleure and later from the area at the caudal end of the developing gut or stalk of the yolk sac to the genital ridges, (in the entoderm of the yolk sac near the allantoic invagination in the human, Witschi, 1948), 2) indifferent gonad stage, 3) division of the gonad into a cortex and medulla and 4) sex differentiation which consisted of development of the cortex and involution of the medulla. According to Allen (1904) the medullary and Pflüger cords were derived from peritoneal invaginations and contained oogonia and follicle cells. Swezy and Evans (1929) concurred and indicated that from those germinal epithelial invaginations ovogenesis continued throughout adult life in the dog, cat, guinea pig and man.

As to the ovarian development in the dog, Latimer (1955) reported that fetal ovarian weight plotted against body weight represented a single line. He stated that although the ovaries from larger fetuses increased in weight more rapidly than those from smaller fetuses the weights of the fetal ovaries varied exceedingly.

Postnatal development

Raps (1946) observed that the germinal epithelium underwent cyclic activity in which the cells varied from squamous to cuboidal in shape. He described the tunica albuginea as an incomplete, irregular layer of connective tissue in the 2 day old specimen, as a continuous connective tissue basement membrane in the 4 day old specimen, and as a single celled struc-
ture in which septae joined radiating cords from the medulla in the 6 day old specimen. By 17 days, Raps (1946) found that the tunica albuginea was several cells thick and the septae were continuous with the trabeculae-like formations of the medulla. It was 6 plus cells thick in the 2.5 month old specimens and was a thin continuous layer beneath the squamous germinal epithelium in the 5.5 month old specimens.

Cells in the outer cortex, beneath the tunica albuginea, were described by Raps as masses of follicle precursor cells in the 2 day postnatal ovary. These cells contained voluminous granular cytoplasm and large spherical to oval granular nuclei. Differentiation of the precursor cells and the appearance of follicular cells around them was reported in the 6 day specimen. According to Raps the number and appearance of the primary follicles was similar in the 2 and 8 week old specimens. Primary follicles were found at birth in the pig ovary (Hadek and Getty, 1959, and Bel, 1966) and at 13 days of age in the rabbit (Duke, 1941). By 13 weeks of age cords of primordial ova, each with its complement of granulosa cells were observed to extend from the periphery of the cortex to the medulla. The first follicle cells surrounded by stratified granulosa cells were observed in the 15 week specimens as well as some atretic follicles (Raps, 1946). Hyalinization of the zona pellucida and theca folliculi were considered by Tanaka (1962) to be the most characteristic features of small to medium sized atretic follicles. He reported that when the large growing follicles underwent atresia proliferation of the theca interna and replacement by connective tissue were observed as well as hyalinization.

A definite cortico-medullary junction present in the ovaries of young
dogs was lost by 13 days of age as cortical cellular activity moved into the medulla (Raps, 1946). The medulla of the 4 day ovary as described by Raps consisted of numerous large blood vessels. In the medulla of 6 day specimens he found parenchymatous tissue cords. These showed mitotic activity in the 13 day postnatal specimens but were inactive in the 10 week ovary. Raps observed follicular growth in the vascular medullary tissues in ovaries from 17 week old animals and also in older specimens.

In the porcine ovary, the medulla was found to be practically free of follicles during the first 112 days of life and the cortex contained 2 distinct zones of developing follicles (Casida, 1935). In the outer zone only primary follicles were found up to 73 days of age. Further information by Hadek and Getty (1959) revealed that in the young porcine, the ovaries were trilobed up to 3 months of age. The first fully developed follicular cavity was observed in porcine ovaries 2.5 to 3 months of age by Hadek and Getty (1959) and Bal (1966).

As an interesting comparison with other species, it was found by Arai (1920) that the first ovulation in the rat was at about 65 days of age, and in man at 65 months. It was also found that cessation of the breeding period in the rat was 18 to 22 months (45 years in man). In the older animals as the total ovarian weight increased the total number of ova decreased. Arai concluded that new ova were formed postnatailly from the germinal epithelium and were then covered by adjacent epithelial cells. More recent investigations showed that the follicular cells which surrounded the ova of the mouse stemmed from stroma cells (Peters and Pederson, 1967).
Physiology

Cyclic changes

In 1945, Barton described the changes that occurred in the canine ovary through the estral cycle. It was found that one of these changes was in the germinal epithelium, in the production of epithelial cords. During metestrum only a few primary follicles were found and localized invaginations of epithelial cords were seen. Barton felt that these cords produced new follicles or else degenerated. During anestrus the isolation and atrophy of the existing cords resulted in a new wave of germinal epithelial activity and pinching off of more cords from the invaginating germinal epithelium. Because primary follicles developed deep in the cortex Barton felt that there was no morphological connection between the primary follicles, epithelial cord remnants and invaginating germinal epithelium of the same cycle. The largest numbers of primary and anovular follicles were found during anestrous. These structures were also observed but not as frequently in late proestrum, estrum, one-month metestrum, parturition and two and four months postpartum. Barton (1945) explained that the pronounced activity of anestrus, a single period of receptivity with ovulation at estrous showed that the dog was monestrous but the periodic formation of large numbers of epithelial cords, anovular and primary follicles suggested abortive estrous cycles and polyestrous rhythm.

Allen (1923) and Allen and Creadick (1937) reported that waves of mitotic activity in the germinal epithelium of the mouse ovary resulted in the formation of new ova at each normal estral period. While Peters and Levy (1966) found that mouse ova failed to label with tritiated thymidine and felt that this was evidence that new oocytes were not formed in the
adult mouse during estrous.

**Ovulation**

Before ovulation took place at least one of the primary follicles had to migrate toward the medulla, then back to the cortex while developing into a mature follicle. The maturing follicle migrated through the cortical stroma by eccentric growth of the theca interna to form a theca cone which in turn provided a path of least resistance for the expanding follicle to follow (Strassmann, 1961). The theca cone was found in ovaries in pro-estrous and estrous in man, as well as in the mammalian orders Carnivora, Ungulata and Rodentia. Strassmann stated that the theca cone demonstrated a tropism toward the ovarian surface like a sprout of a plant, and grew divergently toward the nearest ovarian surface in most mammals while in the horse the theca cone grew convergently toward the ovulation fossa. Within the follicle itself other changes were observed in the fox ovary. Near ovulation the granulosa of the follicle was only a few cells deep. In the fox Pearson and Enders (1943) found a folding of the granulosa which gave the appearance of tufts or villi. These increased in size and complexity as the follicle matured and the corpus luteum formed. They mentioned that the unique enlargement of blood vessels under the basement membrane may have actually pushed the granulosa into these folds. This vascular arrangement was described in the sow also (Corner, 1919). Corner (1921) found in the porcine, that follicle maturation consisted of growth of the theca interna by hypertrophy of its cells and partial dissolution of the cumulus oophorous by separation of its cells so that the ova was nearly freed from the follicular wall. The cells forming the granulosa layer which were 6 to 9 cells deep were round to poly-
hedral, contained round nuclei and a few vacuoles (Corner, 1919). According to Corner (1919) the theca interna of the follicle at ovulation was about half the thickness of the granulosa cells and contained 3 to 5 layers of large epithelioid cells. He also described the presence of large fatty cells in the theca interna, and a narrow zone of spindle cells without fatty inclusions of vacuoles, between the granulosa and theca interna. Corner (1919) observed that just before ovulation enlarged vascular loops penetrated the granulosa in the area of the cumulus oophorus, in porcine follicles but not in those of the rat or mouse. Immediately prior to ovulation in the rabbit Asdell (1962) observed that the blood supply at the surface of the follicle increased except at a small avascular region near the center portion of the follicle wall protruding from the ovary. The avascular area expanded to form a cone, some of the blood vessels broke so that a small lake of blood formed at the follicular apex, and the point of the cone broke permitting the ovum, its corona radiata plus other granulosa cells and viscous follicular fluid to ooze out resulting in a completed ovulation (Asdell, 1962).

Corpora lutea

After extrusion of the follicular fluid and the contraction of smooth muscle cells in the follicular wall of the porcine ovary, Corner (1921) found that the diameter of the structure was cut nearly in half. He indicated that in this species most of the membrana granulosa remained intact in the follicle, hypertrophied, became laden with lipoid substances and thus became lutein cells. Increased vascularization of these cells from the theca and migration of hyperplastic theca interna cells into the granulosa cells was also described by Corner (1921). Only a slight amount
of hemorrhage from the follicle (Corner, 1919) occurred at ovulation. Harrison (1962) found that invasion of the granulosa by theca interna cells between the first and third day after ovulation was common to all species thus far studied. The migrating thecal cells became spindle shaped and were thought by Corner (1919) to be endothelial type cells. Pearson and Enders (1943) described a similar phenomenon of corpus luteum formation in the fox but felt that the exaggerated infolding of the follicular wall played a greater role in the filling of the cavity than hypertrophy of the thecal cells. They stated that the folding of the granulosa in the fox was not as prominent as it was in the dog. Migrating theca interna cells of the dog ovary laid down collagen tissue in between the granulosa cells (Harrison, 1962) and other theca interna cells remained at the periphery of the corpus luteum without developing into theca lutein cells. Harrison found that vacuoles appeared in the luteal cells by the sixteenth day but were prominent by the fiftieth day indicating degeneration of the corpus luteum. In the mouse ovary Peters and Levy (1966) found, by means of autoradiographs taken after flash labelling with tritiated thymidine, that after rupture a follicle received, along with the quickly forming and invading blood vessels, cells which were originally part of the follicle. The corpus luteum was displaced from the periphery of the ovary by the next cycle and further so by the next so that it lay close to the central blood vessels and became shrunken. By the third cycle Peters and Levy (1966) found only a small island of central stroma existed and by the fourth it had usually disappeared without a trace in the mouse ovary.
The aging ovary

The first physiological breakdown in the human ovary was an irregularity in the structure and function of the corpus luteum, resulting in decreased progesterone and a shortened luteal phase (Kretzschmar and Stoddard, 1964). After this, ovulation failed and consequently no corpora lutea were formed and no progesterone was formed. This resulted, according to Kretzschmar and Stoddard, in continued estrogen stimulation and a uniphasic ovarian cycle. Ringrose (1963) felt that ovarian vessel walls became thickened, rigid, sclerosed and hyalinized in the post-menopausal state of the human which resulted in a 57 percent decrease in blood flow if the lumen was reduced by one-fifth. The decrease in blood flow hindered progressive ovarian function. Another phenomenon associated with the aging human ovary was the formation of ovarian cysts (Leathem, 1958). It was reported by Paulsen, et al. (1958) that the post-menopausal human ovary still produced some estrogens. The decrease in function with age appeared to be the most significant aspect, however, as verified by Labhsetwar (1967) who reported a decreased ovarian compensatory hypertrophy in unilaterally ovariectomized rats. It was not known whether the decrease was due to a decline in gonadotrophin available or a decrease in sensitivity of the aged ovary. Even with both ovaries present as Laishaura (1963) pointed out there was a decrease in ovarian weight in ovaries from women over 30 years of age.

Histology

In embryonic and early postnatal life, the mammalian ovary was composed of a definite cortex and medulla. The literature regarding the cortex included the germinal epithelium, tunica albuginea, interstitial cells,
primary, growing and Graafian follicles, atretic follicles and corpora lutea and was discussed in that order. Then the medullary structures, including vascularization and innervation were then reviewed.

**Cortex**

**Germinal epithelium**  
Jonckheere (1930) reported that proliferations of the germinal epithelium were continuous from a short time before birth to old age. In the 6 month old canine ovary Raps (1946) found that the germinal epithelium was a well defined layer of cuboidal cells. From the deep surface of the epithelium small masses of cells protruded at intervals into the tunica albuginea. Proliferation of the human germinal epithelium was felt to be under the influence of estrogens according to Ludwig (1965) who stated that it was possible that neo-oogenesis during the reproductive span of a woman seemed possible. Peters and Pederson (1967) felt that it did not take place in the mouse ovary. However, as far as the germinal epithelium was concerned histologically, it was not a static structure according to Valdes-Dapena (1967) who indicated that in the human at birth it was a single continuous layer of prominent columnar and cuboidal cells. By puberty the germinal epithelial cells had become flattened over the greater part of the ovary, with areas of plump cuboidal cells present in all ages studied. In the mouse the inactive germinal epithelium consisted of a single layer of flattened cells with scanty cytoplasm and bulging nuclei (Duke, 1941). Duke described stratified areas at activity sites and invagination of cortical cells at other points. According to Brambell (1956) the germinal epithelium, a peritoneal investment, was composed of low columnar or cuboidal cells and had a well developed basement membrane. Harrison (1962) also described a basement
membrane. Bloom and Fawcett (1962) agreed with this description for the ovary of the infant except the last phrase and stated that in the adult ovary the cells became lower and portions of the epithelium were further flattened under tension. They felt that a basement membrane was absent. The peritoneal investment of the mesovarium according to Leeson and Leeson (1966) ceased abruptly at the hilum and was replaced by a layer of cuboidal epithelium which covered the free surface of the ovary. They also stated that there was no basement membrane beneath the ovarian germinal epithelium.

Franchi (1962) and Harrison (1962) explained that the cells were frequently cuboidal and were capable of rapid mitotic activity to accommodate extensive changes in ovarian size during normal cycling. Even phagocytic activity of the germinal cells was observed (Latta and Pederson, 1944).

These cells in the adult human ovary (Simkins, 1932) became stretched and in some cases were changed into a connective tissue layer of hyperplastic fibers.

**Tunica albuginea** Both Brambell (1956) and Harrison (1962) stated that the structure underlying the germinal epithelium was composed of connective tissue of varying thickness. Smooth muscle fibers as well as fibrous connective tissue (Brambell, 1956) were included and the thickness varied with the species. In the 6 month canine ovary it was described as a thin continuous layer which Raps (1946) called a cellular basement membrane. Copenhaver and Johnson (1958), Bloom and Fawcett (1962) and Leeson and Leeson (1966) all referred to the tunica albuginea as a dense connective tissue structure in the human ovary.

**Interstitial cells and subsurface epithelial cell structures**

In the cortical stroma reticular fibers, connective tissues and smooth
muscle-like cells, interstitial cells and other epithelial structures were found (Bloom and Fawcett, 1962). According to Copenhaver and Johnson (1958), elastic tissue was found only in the vessels. The origin and function of the interstitial cells was controversial (Harrison, 1962). Histologically, they were usually described as relatively large epitheloid cells with clear vesicular nuclei (Kingsbury, 1914). Harrison (1962) referred to them as large polyhedral cells with polychromatic nuclei and cytoplasm filled with lipid droplets. It was found by Tsukaguchi and Okamoto (1928) that they were abundant in young dogs but relatively few were observed in the adult. Yellowish brown lipoid droplets found in these cells according to Dean and Fawcett (1952) were identical to the so-called brown degeneration in the adrenals of certain strains of mice. Kingsbury (1914) stated that in the cat ovary, they were modified stromal cells of connective tissue origin and hypertrophied thecal cells which contained granular contents of a lipoid nature. Tsukaguchi and Okamoto (1928) also referred to them as functionally active stromal cells. They were found thickly massed in the rabbit ovary, abundant in the cat ovary, few in number in the human ovary and very sparse in the ovaries of pig, sheep and cows (Kingsbury, 1914).

They changed in size and number (Brambell, 1956 and Mossman, et al., 1964) depending on the cyclic stage of the ovary, such as a hypertrophy and hyperplasia during the latter part of pregnancy. Interstitial cells were found in the atretic follicle as well as in the Graafian follicle (Kingsbury, 1939). It was reported by Guraya and Greenwald (1964), that the interstitial tissues of the canine ovary originated from theca, adjacent stromal cells of normal and atretic follicles, as well as from invaginations of
normal epithelium.

Subsurface epithelial structures (O'Shea, 1966) in the form of solid and hollow groups or cords of epithelial cells were found in the outer cortex of the dog ovary. Histochemically these cells showed evidence of mucin production and were differentiated from interstitial cells. O'Shea stated that these subepithelial structures are also found in the ovaries of seals, sea-lions and elephants.

**Primary follicles** Most follicles seen in an ovary were primary follicles (Leeson and Leeson, 1966) which were usually located near the periphery of the cortex. According to these investigators a primary follicle consisted of an immature ovum surrounded by a single layer of flattened follicular cells. In the canine according to Harrop (1960) these cells later became cuboidal to columnar.

The large round ovum (Bloom and Fawcett, 1962) had an eccentric nucleus which contained a loose network of chromatin and a large nucleolus. Okamoto (1928) indicated that the source of follicle cells which surrounded the ovum was the sexual cord cells during first two weeks of life then germinal epithelial invaginations, medullary and stroma cells. Follicle cells he felt were of epitheloid origin. Leeson and Leeson (1966) agreed and stated that the cells were believed to be derived from the germinal epithelium. It was reported by Peters and Pederson (1967) that the follicle cells of the mouse ovary originated from stromal cells.

Hadek (1965) stated that as long as the oocyte was surrounded by a layer of flat follicular cells it was a primordial follicle and that the follicle was regarded as a primary follicle when the cells changed into the cuboidal granulosa type. After the second layer of cuboidal cells
developed he called the structure a multilaminar primary follicle.

Growing follicle  As the animal reached sexual maturity, or after puberty in man, a progressive follicular growth took place (Leeson and Leeson, 1966) which was characterized by proliferation of follicular cells and development of a connective tissue capsule from the nearby stroma. Harrison (1962) found that as the follicles migrated deeper into the cortical tissues, they increased in size. According to Bloom and Fawcett (1962) as the ovum enlarged, its nuclei also increased in size and the mitochondria became more evenly distributed in the cytoplasm, later yolk granules appeared. Surrounding the ovum was a vitelline membrane (Arey, 1965; Hadek, 1965). As the epitheloid cells surrounding the primordial follicle, become cuboidal and proliferated to form layers, they were termed epithelial cells (Brambell, 1956), granulosa cells (Harrison, 1962) or follicular cells (Copenhaver and Johnson, 1958; Bloom and Fawcett, 1962 and Leeson and Leeson, 1966). At about this stage in the developing follicle, the follicular cells secrete what, Hadek (1965), Bloom and Fawcett (1962) and Leeson and Leeson (1966) referred to as a deep staining, refractile membrane, the zona pellucida. In the dog the time formation of this structure varied considerably (Harrop, 1960). Hadek informed us that this tough, elastic membrane which surrounded the vitelline membrane of the ovum had a mucopolysaccharide base. The follicular cells surrounding the zona pellucida continued to divide by mitosis. This activity was more rapid on one side of the ovum so that the follicle became oval in shape and the ovum was pushed to one side (Leeson and Leeson, 1966). Most investigators agreed that about this point small, irregular spaces appeared between the follicular cells and became filled with follicular fluid. Soon
those spaces coalesced and the antrum were formed. The several uniform layers of follicular cells, (Brambell, 1956; Copenhaver and Johnson, 1958) which surrounded the antrum were now separated from the surrounding ovarian stroma by a basement membrane which was sometimes called a glassy membrane (Leeson and Leeson, 1966). Harrison (1962) stated that at first the antrum was surrounded by a single layer of follicular cells which later divided into several layers. Accompanying the formation of the membrana granulosa, the outer layer of granulosa cells, was the appearance of the theca folliculi outside of the basement membrane. As the antrum enlarged the ovum was pushed to one side and remained attached to the wall of the antrum by a hillock of granulosa cells commonly termed the cumulus oophorus or discus proligerus. The layer of cells immediately surrounding the ovum were called the corona radiata. Appearing among the granulosa cells of the mammalian ovary, especially those of the prairie dog, cat and man (Brambell, 1956) were the bodies of Call and Exner. They consisted of a small darkly staining coagulum filled cavity surrounded by follicular cells and contributed to the formation of follicular fluid (Brambell, 1956) which filled the newly formed antrum.

Outside of the basement membrane, the theca folliculi, as most investigators agreed differentiated into a theca interna which was composed of a loose vascular connective tissue filled with capillaries. Surrounding this was a highly collagenous theca externa which blended into the stroma.

**Graafian follicle** If the follicle continued to develop in preparation for ovulation it grew until it occupied the thickness of the ovarian cortex, bulged from the free surface of the ovary (Bloom and Fawcett, 1962) and indented the medulla (Leeson and Leeson, 1966). The latter
authors indicated that when the ova had reached full size it was surrounded by a thick zona pellucida and a prominent corona radiata. Dawson (1952) found that finely reticulated, uniformly dispersed argyrophilic substances were typical of developing and mature ova. Liquor folliculi filled the antrum and the cells of the cumulus oophorus loosened prior to ovulation. Pearson and Enders (1943) found in the mature follicle of the fox, that the granulosa layer was only a few cells thick. They observed that the nuclei of the basal layer were apical in position and that a prominent folding of the granulosa took place especially at estrous. Brambell (1956) observed that this infolding took place prior to ovulation in the dog and fox. Yamashita (1959) made the same observation in the sow. An internal limiting membrane, lining the antrum of the mature follicle was mentioned by Brambell (1956) for the dog, human, ferret and bank-vole. He felt that it arose from penetrating cells of the theca interna which spread over the inner surface of the flattened granulosa cells. Loutfi (1965) found that the internal limiting membrane was argyrophilic, composed of a condensation of reticular fibers and was a basement membrane which provided the antrum with the characteristics of an epithelium. He stated that it was of mesodermal origin, was not penetrated by blood vessels and lacked connective tissue. In the rabbit the granulosa cells were not folded as described above, but were thought to be a true pseudostratified epithelium in which each cell base reached the basement membrane (Lipner and Cross, 1968).

The concentrically arranged cells of the theca interna were enlarged polygonal shaped cells, with vacuolated cytoplasm and vesicular nuclei. In the dog there was very little hypertrophy or very little cytoplasmic
differentiation of the theca interna cells which made it difficult to differentiate them from the surrounding stromal cells (Guraya, 1968). Copenhaver and Johnson (1958) indicated that the theca interna doubled in thickness during follicular maturation. Harrison (1962) observed that the cells of the interna enmeshed in a reticular and fibrous network along with a plexus of engorged capillaries and lymphatics (Brambell, 1956). In agreement with Brambell was Yamashita (1960a) who stated that the meshwork of the theca interna was composed of argyrophil fibers. Although the degree of development of the theca interna varied with different species of placental mammals, Harrison found that it was always maximal just prior to ovulation. Yamashita (1960a) reported that small masses of argyrophil fibers protruded from the inner side of the theca interna into the granulosa, giving it a plica-like configuration. Reticular fibers were found only in the thecal coverings of the Graafian follicle and in the ovarian stroma in the guinea pig ovary (Loutfi, 1965). Loutfi reported that the membrana granulosa contained no fibers, but that the argyrophil fibers of the theca interna blended with those of the theca externa and of the ovarian stroma. The thecal layers were of mesodermal origin (Loutfi, 1965). Brambell (1956) recorded that the theca externa had a rich vascular supply, numerous lymphatics and was composed chiefly of fibrous connective tissue and smooth muscle. In 1947, however, Claesson had reported that there was no smooth muscle in the theca externa of the cow, swine, rabbit or guinea pig. Bloom and Fawcett (1962) and Leeson and Leeson (1966) stated that the theca externa was composed of closely packed collagen fibers and fusiform cells.

**Corpus luteum** Textbook descriptions of the metamorphosis of
the post-ovulatory follicle indicated that there was minor hemorrhage into the follicle, then collapse, folding of the walls, and a hypertrophy of the granulosa cells into lutein cells. Lutein cells were said to be large, pale-staining cells with large vesicular nuclei. An extensive investigation in the corpus luteum of the sow was done by Corner (1919). Pearson and Enders (1943) found in the fox that the folds of the granulosa layer in the Graafian follicle played a leading part in filling the antrum after ovulation. The new lutein cells accumulated cytoplasmic inclusions, including granules, fats and lutein pigments (Brambell, 1956). Blanchette (1966) using the electron microscope, found through systematic examination of the rabbit corpus luteum sufficient cellular distinctions to recognize the first, second and third stages of pregnancy.

Pearson and Enders (1943) found that in the canine there were two main types of corpora lutea, those in which the convoluting granulosa entirely filled the antrum and those that gained assistance from the granulosa and theca cells lying between the folds. In either case it was 2 or 3 days after ovulation before the antrum was filled. In the first type of corpus lutea formation the cellular growth was so rapid that the luteal cells were unable to consolidate, resulting in a loose, diffuse corpus luteum, especially noticeable the first day after ovulation. Pearson and Enders indicated that only part of the cells luteinized at first while the remaining cells retained their follicular characteristics and became luteinized later. This type of corpus luteum was referred to as "lace-work" luteal tissue in the bitch, by Evans and Cole (1931). Corpus luteum formation of the second type, with the aid of the granulosa and thecal cells, was rapid, but slow enough to result in a consolidated well organized
structure. Arterioles, venules and lymphatics (Brambell, 1956) grew out along the thecal core of each granulosa cell and formed anastomosing capillary beds in the membrana granulosa.

In the cat, the wall of the ruptured follicle was deeply folded, even to involve the theca interna (Harrison, 1948), and no hemorrhage occurred into the collapsed follicle. Harrison (1962) found that migration of the theca interna and endothelial cells into the granulosa occurred 48 hours after ovulation and penetrated the wall of the corpus luteum by the end of the third day. Also at this time the theca interna cells lost their rounded appearance, became fibroblastic, and migrated between the granulosa cells laying down collagen. Peripheral theca interna cells did not change in appearance. At 11 days the vacuoles disappeared from the lutein cells and at 27 days involution of the corpus luteum of the cat was pronounced. By the fiftieth day the luteal cells became highly vacuolated and were considered to be degenerating.

In the lactating feline the corpora lutea were bright pink and showed increased vascularization. Histologically, the smaller vacuoles disappeared and some "giant" follicles persisted (Dawson, 1946).

In the bitch the life span of the corpus luteum was 6 to 8 months from mating according to Dawson (1946) and as it involuted the luteal tissue was reduced by progressive cytolysis usually not accompanied by leucocytic infiltration (Harrison, 1948). Harrison found that brilliant acidophilic granules which were present in late pregnancy and the first few weeks post-partum were not present in the other corpora lutea. In mice the luteal cells of newly formed corpora lutea were hematoxyphilic while those of old corpora lutea were eosinophilic (Loeb, 1948; Fekete, 1953 and Mody, 1963).
Mody found that in degenerating corpora lutea the cell cords had become loose, the cells had enlarged and the cytoplasm had become vacuolated. Then the thecal layers broke away and the degenerated lipochrome filled lutein cells were scattered in the ovarian stroma. Occasionally, according to Mody an intact entirely hyalinized corpora lutea was found in older mice. Vascularization was decreased due to a decreased lumen size in the vessels or partial occlusion of the lumen by adventitial cells or detached endothelial cells.

Foley, et al. (1964) found that the size and weight of corpora lutea of the bovine during the estrous cycle was not correlated with age or live weight. They observed an extreme variability in size of corpora lutea. In the sow Yamashita (1962) was able to correlate the stage of development and regression with a history of parturitions. He presumed that the pregnant corpora lutea "vascular bodies" of sows were organized completely and disappeared within 3 years. In an earlier publication Yamashita (1960b) classified the corpora lutea of the pregnant sow into 9 types and of the non-pregnant sow into 6 types, but had not correlated them. He gave the term "obsolete" corpora lutea to those that had ceased to function. Van Lennep and Madden (1965) called the functionless corpora lutea, "involuted" and indicated that the process in the human consisted of disintegration and phagocytization of the luteal cells by macrophages. They reported after electron microscope studies that fibro-hyaline material of the future corpus albicans was an amorphous substance with some protofilaments and collagen fibrils which were deposited between the shrinking lutein cells.

**Atretic follicle** Follicles at any stage of development often
ceased to grow and began the process of atresia. Primary follicular degeneration was accompanied by chromatolysis and fragmentation of the nucleus first and then of the follicular cells (Copenhaver and Johnson, 1958). Ingram (1962) found that the atretic primordial oocytes were differentiated from the normal ones by the crinkled appearance of the nuclear membrane. It was stated by Bloom and Fawcett (1962) that the follicular cells tended to engulf the debris of the shrunken degenerated ovum before they degenerated. The above investigators agreed that after degeneration of the primary follicle, no tract remained. Thus it was termed an obliteration type of atresia by Kovacs (1933) with regard to the canine ovary. Even in the growing follicle fatty disintegrative changes, accumulation of fatty granules in the cytoplasm, chromatolysis of the nuclei and ultimate liquifacation began in the ovum and then spread to the follicular cells. Kovacs described atresia of the growing follicle as a cystic type. According to Dawson (1952) large size and many aggregations of argyrophilic substance in the ova was associated with follicular atresia in the juvenile periods of development of the rat. Copenhaver and Johnson (1958); Bloom and Fawcett (1962) and Leeson and Leeson (1966) agreed that the zona pellucida became swollen, folded and then persisted in that state for sometime. Kingsbury (1939) observed in the cat ovary that the first demonstrable changes in a Graafian follicle destined to undergo atresia appear in the liquor folliculi as it changed from a coarse mesh appearance to a fine mesh. He found that the cumulus oophorus became atypical and the ova flattened. The follicular fluid thickened into a colloidal mass and then collapse of the wall resulted in antrum obliteration. A few years later Strasemann (1961) indicated also with regard to the cat that as the
follcicles underwent degeneration the cumulus oophorus flattened and the ovum sank to the membrana granulosa. At the same time the granulosa commenced to grow and fill the entire follicular cavity. Strassman also stated that physiologic degeneration of the Graafian follicle began in the nucleus of the ovum and proceeded centrifugally to the periphery. Pathologic degeneration of the follicle began in the outer layers and proceeded centripetally toward the ovum. Kingsbury (1939) observed that as the follicular epithelium of the cat ovary disappeared and the ova degenerated the theca thickened due to cellular hypertrophy and the acquisition of a rich lipoid material. This increase in size was accompanied by a radial arrangement of the thecal cells (Leeson and Leeson, 1966) and vascular penetration of the basement membrane and follicular cells. Guraya and Greenwald (1964) found that in the dog ovary follicular atresia was not accompanied by hypertrophy of the theca so that the place of degenerating granulosa cells was occupied by a cavity which persisted for a time. Next, according to Kingsbury (1939) the thecal cells lost their lipid content, reverted to the stromal cell type and were then called interstitial cells. They were broken up and separated into islands of cells by invading fibrous tissue (Bloom and Fawcett, 1962).

Miscellaneous follicle types In many ovaries there were follicles not fitting into the categories described above, such as polynuclear, polyovular and anovular follicles. According to Hartman (1926) polynuclear ova had been reported in the ovaries of the human, fetus through adult, monkey, calf, dog, cat, rabbit, pig, cow, mouse, guinea pig, armadillo, opossum, snake, frog, Teleost, bird, ascarid and butterfly. The same author stated that polyovular follicles had been found in the
ovaries of humans, dogs, cats, bats, pig, sheep, rabbits, mice, guinea pigs, armadillo, kangaroo, Dacyurus and opossum. These multiple structures were generally found only sporadically in the ovaries of most species except in the case of the dog (Hartman, 1926) in which polyovular follicles appeared to be common. Tanaka (1962) also studying the dog found polyovular follicles underwent atresia at some stage of development (Hartman, 1926). Anovular follicles according to Brambell (1956) have been described in the ovaries of bats, rabbits, rats, lemur, opposum, armadillo, monkey, dog, dingo, and mouse. Brambell stated that these structures were small and never developed an antrum.

**Medulla**

The mammalian ovary had a relatively small spongy medulla which adjoined the hilus and occupied the center of the ovary (Brambell, 1956). Brambell stated that it contained numerous large lymphatic sinuses and larger blood vessels among the connective tissue and strands of epithelial elements. Leeson and Leeson (1966) indicated that fibroelastic tissue was found in the medulla. Chromaffin-like cells were found in the hilus region of the human ovary. Harrison (1962) called them "hilus cells" and stated that well developed clusters were often found in relation to a prominent rete ovarii. In the camel Shehata (1964) reported that medullary tubes continuous with the mesonephric tubules of the mesovarium and related to the rete ovarii were very prominent even in adult animals. He indicated a possible secretory function which may have caused persistence of both mesonephric structures. Medullary tubes were scanty in the buffalo, absent in the cow, sheep and goat and only present in one porcine specimen (Shehata, 1964). Also in the hilus area were strands of smooth muscle
fibers which extended inward from the mesovarium (Copenhaver and Johnson, 1958 and Bloom and Fawcett, 1962).

**Vascularization**

The ovarian artery arose from the aorta and often made one or two spirals or coils between the aorta and ovary (Harri­son, 1962), and continued to branch and spiral after entering the medullary substance of the ovary. Bloom and Fawcett (1962) and Leeson and Leeson (1966) referred to ovarian arteries as helicine arteries and the former authors indicated that they showed longitudinal ridges in their intima as did the vessels of the corpora cavernosus penis. Kladetzky and Rosen­bauer (1965) described similar helicine arteries in the ovary of a lioness. These arteries formed a plexus in the cortico-medullary boundary area (Copenliaver and Johnson, 1958) and supplied blood to the parenchyma of the medulla and cortex and to the follicular theca (Christensen, 1964). In arteries frequently extended in a longitudinal direction, such as in the lung, longitudinally oriented muscle cells were found in the split internal elastic membrane (Bader, 1963). Christensen indicated that in the dog the development and regression of the vascular pattern followed the cyclic changes of the ovary. According to Duke (1945) the amount and distribution of elastic tissue also varied with the cyclic state. Changes were also seen in collagen and Wissler (1967, 1968) indicated that the arterial cell, a smooth muscle cell might be of a multifunctional nature and be the source of elastin, collagen, smooth muscle fibers and basement membrane.

Due to the helicine nature of the ovarian arteries high blood pressure of the aorta was quickly and efficiently reduced before it reached the delicate minute vessels of the follicles and parenchyma (Reynolds, 1950).
The degree of tortuosity according to Reynolds (1947) depended upon gonadotrophic stimulation. Delson, et al. (1948) agreed with the above statement, then added that in the presence of corpus hemorrhagicus cysts and during the period of follicular enlargement there was distortion of the spiral artery. In addition to the effect of spiralling on the physiology of the ovary, Horváth, et al. (1964) reported that in the cow, changes in periodic hyperemia and the blood supply in the resting stage caused vascular congestion and edema because of overstrain of capillary capacity. He explained that normalization of circulation resulted from functional closure of the arterial valves, arteriovenous anastomoses and venous sinus system.

Regarding the vascularization of follicles Brambell (1956) and Harrison (1962) agreed that the primordial and primary follicles had no special blood supply but simply lay among the vessels of the stroma. Anderson (1926) found that several small vessels approached each of the larger follicles through the stroma. As the follicle reached the antrum stage it acquired a vascular wreath which doubled by the time the theca interna was fully established (Brambell, 1956). Harrison (1962) explained that the inner plexus lay just outside of the membrana propria and communicated with the outer plexus or wreath of the theca externa. He stated that no vessels entered the granulosa and that a non-vascular area the, macula pellucida, developed on the protruding part of the follicle near rupture. Both Brambell and Harrison indicated that the venules of the plexuses anastomosed freely but that the arterioles only occasionally anastomosed. Near ovulation according to Brambell (1956) arterioles grew into the granulosa layer from the inner wreath. After ovulation and collapse of the follicular wall arterioles, venules and lymphatics grew along the thecal cores.
of folds toward the middle of the corpus luteum. Capillaries from these, grow into the granulosa, formed simple loops and then blood was returned via 6 or more venules. Lymphatics followed the arteries in their formation, were slower to develop but ultimately formed two systems, superficial and central. Bloom and Fawcett (1968) indicated that valves were found only in lymphatics outside the hilus. As the corpus luteum degenerated, the lymphatic systems disappeared first (Brambell, 1956) and later the blood vessels. Basset (1943) stated that the arteries and veins of the rat ovary remained as a knot of vessels located by shrunken degenerated corpora lutea. According to Yamashita (1960b) they underwent hyaline swelling of the intermediate coat and gradually disappeared. Histologically, it was difficult to distinguish between the veins and lymph vessels within the ovary because their walls were nearly identical in structure (Burr and Davis, 1951). Burr and Davis indicated that, in the rabbit the endothelial cells were more numerous in the veins than in lymphatics.

Innervation  Nerves, mostly unmyelinated, entered the ovary at the hilus along with the arteries (Copenhaver and Johnson, 1958 and Bloom and Fawcett, 1968). Bloom and Fawcett indicated that a few thin myelinated nerves were present and that the presence of sympathetic nerve cells in the ovary had not been confirmed. In 1920, Kurtz reported that in the dog ovary prepared by the pyridine-silver method, an abundant supply of sympathetic nerves to the smooth muscle cells of the arteries were found, but no sympathetic ganglion cells. Innervation of the canine ovary was said to be richer than that of the human ovary and in the dog ovary the nerve cells never ran into the follicular layer (Yamaguchi, 1957). Yamaguchi stated that the theca layers of functional and atretic follicles
contained many vegetative fibers. According to Karmsev (1958) terminations of nerve fibers were found in the blood-vessel walls cortical parenchyma, primordial follicles, Graafian follicles and in the corpus luteum of cat, dog, guinea pig and rat ovaries. Three kinds of sensory endings were found in the stroma of dog and cat ovaries, 1) arborized (most frequently found), 2) ball type and 3) the encapsulated ending (Tcheng, 1957). Tcheng and Tcheng (1957) further stated that the ball-like encapsulated receptors, infrequently found in the canine ovary were not found in the human and cat ovary. They found that the nerves of primary follicles terminated in the follicular epithelium. In larger follicles the theca was richly innervated. In the human Graafian follicle nerve fibers penetrated into the membrana granulosa (Tcheng and Tcheng) and in the corpora lutea some of the nerve fibers were near the blood vessels and others were distributed among the luteal cells. These authors stated that ovarian follicles and corpora lutea were directly innervated.

**Age changes**

Histological changes observable in the aging ovary correlated with the physiological changes discussed earlier. Although the canine has been known to cycle up to 18 years of age (Taylor and Dorn, 1967) the regularity or irregularity of the cycles was not reported. One of the physiological changes of aging ovaries was estrogenic failure according to Kretzschmar and Stoddard (1964) which took place gradually, over a period of years. Thus histological changes were also expected to be gradual changes. Some of these as described by Bonifirraro and Subrizi (1966) were vascular changes, decreased follicular activity and
cystic formations. They cautioned, however, that not all ovaries from older specimens had these changes.

**Cortex**

**Germinal epithelium**

According to Andersen and Simpson (1961) an indication of decreased activity was the absence of invagination of the germinal epithelium into the cortex in an 8.5 year old Beagle in which large crypts were found on the ovarian surface. Mody (1963) reported that as the mouse ovary became shrunken with age the germinal epithelium became loose, folded and was multilayered in some areas. In some of these areas Mody found that anovular buds were formed which consisted of 6 or more cells arranged in a ring with more cytoplasm toward the lumen. These were found singly or in clumps beneath the germinal epithelium. In the human Tóth and Gimes (1964) found that the surface became corrugated. Various papillomatous surface growths as well as cystic structures were described by Thung (1961) in the human ovary.

**Tunica albuginea**

This structure was the main source for fibrosis in the aged human ovary (Woll, et al. 1948). It was stated by Tóth and Gimes (1964) also regarding the aged human ovary that the tunica albuginea was thick and showed signs of hyaline degeneration at some points. They found a layer of elastic fibers, forming a network around the cortex immediately beneath the tunica albuginea and indicated that with advancing years this ring decomposed. It should be remembered that Harkness (1964) stated that there appeared to be little if any elastic tissue in the ovary except in the blood vessels. The tunica albuginea of the porcine ovary became an easily distinguishable structure at 3 months of age, developed gradually and did not increase in thickness.
beyond 30 microns in pigs up to 33 months of age (Hadek and Getty, 1959).

**Cortical stroma**  
The cortical stroma of the aging human ovary was classified as hyperplastic, intermediate or atrophic by Bigelow (1958). Hyperplastic stroma was characterized by dense areas of stromal cells frequently arranged in a whorled pattern without follicles or their derivatives. The intermediate group included those with cortical stroma 0.1 to 0.2 cm. in diameter. If the cortical stroma was less than 0.1 cm. it was considered to be atrophic. Bigelow also described cortical stromal fibrosis in the aging ovary of two types, superficial or in conjunction with cortical stromal hyperplasia. In the 2 year old hamster Rolle and Charipper (1949) described an increase in amount of fibrous connective tissue but did not classify it as did Bigelow. Woll, et al. (1948) found in the human, that during senility, stromal cell nuclei shrank greatly, fibrous tissue penetrated the cortical stroma from the tunica albuginea and medulla and reticular fibrils became hyalinized. They concluded that ovarian stroma had the competence to differentiate into cells of the granulosa, theca interna, corpus luteum, and corpus albicans and that in senility this ability sometimes led to stromal hyperplasia, thecomatosis, cortical granuloma formation, granulosa and thecal cell tumors. Fienberg (1958; indicated that in cases of thecomatosis, endometrial hyperplasia was a coexisting condition due to excess ovarian hormone production. In studying 47 cases of stromal abnormalities Fienberg categorized them as 1) interna thecosis, 2) combined interna and stromal thecosis and 3) stromal thecosis. A progressive thinning and wrinkling of the cortex was the next step in the aging ovary (Leathem, 1958).

Yamauchi (1963) indicated that with the disappearance of oocytes from
the ovaries of aged cows (17, 21 and 28 years of age) the ovary became a solid stromal mass. In some strains of mice Thung et al. (1956) reported that anestrous of old age was relative to amyloid infiltration and cystic degeneration of the ovaries.

**Interstitial cells** Ringrose (1963) explained that the increase in fibrous tissue slowly replaced the hormone and ova producing tissue thereby contributing to functional failure. "Wheel cells" or "deficiency cells" (Wolfe, 1943) which formed as a result of nuclear changes in the interstitial cells were observed in 12 month old rats and occurred in increasing numbers in the ovaries from old specimens. Paulsen, et al. (1958) maintained that the post-menapausal ovary still produced some estrogenic substances.

**Follicles** According to Bloom (1954) ovarian cysts, the most common of which were follicular cysts increased in frequency with age in both the dog and cat. Gilmore (1965) indicated that they were the most common non-neoplastic lesion found in the ovaries of these genera, that they were usually multiple, thin walled and occurred in both ovaries. Sometimes they were associated with endometrial hyperplasia. Especially in dogs over 6 years of age adenomas, granulosa or granulosa-theca cell tumors were often found. The latter were thought to produce estrogens. According to Gilmore over 50 percent of the dogs with ovarian tumors had cystic endometrial hyperplasia.

In 28 out of 48 post-menopausal ovaries studied Ringrose (1963) found no primordial follicles. In very old mice small follicles surrounded by one to 3 cell layers but no Graafian follicles were found (Mody, 1965). Mody observed that in all groups studied the number of follicles diminished
with advancing age and small follicular cysts were occasionally observed in old ovaries. A similar pattern was recorded by Rolle and Charipper (1949) for the hamster. No normally growing nor primordial follicles were found in the ovaries of aged cows (Yamauchi, 1963). Tamura (1927) found anovular follicles in the sterile dingo and aged mouse. In the rat an increase of atretic follicles was observed both early and late in life but not in between. (Wolfe, 1943).

**Corpora lutea**

Mody (1963) found pigment-containing cells in adult and old ovaries of mice which generally increased in amount with age. He observed that fibrous scars were occasionally seen in the ovaries of old pseudopregnant and postbreeding mice which perhaps represented a mode of involution characteristic of persistent corpora lutea. Regarding the human ovary, Joel and Foraker (1959) found that resorption of the corpora albicans and corpora fibrosa decreased with age. A fairly constant number of corpora lutea were found in the rat ovary during reproductive life and then a rapid decrease was noted beyond 19 months of age (Wolfe, 1943). Erickson (1966) found a similar decrease in the aging bovine.

**Medulla**

**General**

Since the chief function of the ovarian medulla was to support the vessels and nerves on their way to the more functionally active cortex it was understandable that it received but little attention in the literature. Neither the nerves nor lymphatics were mentioned with regard to the aging ovary, blood vessels on the other hand received much attention.

**Vascularization**

Ringrose (1963) gave reasons for vascular
changes that took place in various types of vessels. He stated that the short renal artery with its wide lumen was constructed for longevity and the long tortuous ovarian vessels were constructed for movability, thereby sacrificing longevity. It was reported by Murri (1957) in studying the ovaries of women between 37 and 75 years of age that the vascular components showed pronounced sclerotic alterations. Kladezky and Rosenbauer (1965) found some vascular changes which they called hyaline changes in the ovary from a 3 year old lioness. Sauramo (1954) explained that in the ovaries from older women the perifollicular ovarian vessels often underwent hyaline-elastoid degeneration of the media and thickening of the intima. He found that in women over 30 years of age the middle-sized arteries were marked by senile sclerosis. Occasionally arteriosclerosis of the hilar vessels appeared first as an ovulatory sclerosis and gradually spread to the large vessels. More recently Tóth and Gimes (1964) reported, regarding aging cortical vessels, that there were numerous hyalinized vessels with thick walls around the corpus fibrosa and in some instances the tunica media was replaced by a homogenous substance. According to Balo (1963) even in the young animal muscular arteries contained small amounts of collagen and pre-collagen fibers which increased in amount with age. Tóth and Gimes found that the lumen of some vessels was constricted as a result of intimal proliferation, thickening and proliferation (elastohypertrophy) of elastic fibers. In addition to changes within the vessels Amirov (1958) reported that after 30 years of age in the human the number of branches of the ovarian artery decreased so that where a maximum of 50 to 60 had been found at age 30, only 20 to 30 branches were seen in old age.
In studies performed on porcine ovarian tissues, intimal thickenings of the blood vessels were observed by 4 years of age according to Bal (1966). He found a gradual increase in thickening that resulted in a near occlusion of the vascular lumen by 8 years of age. The smaller arteries of old guinea pig ovaries (over 6 years of age) were entrapped in hyaline nodules according to Batali, et al. (1961) whereas the larger arteries were thickened by concentric rings of fusiform cells and had narrowed lumens. One possible result of decreased lumen size and consequent decreased blood volume reaching the ovary was a disturbed ovary-pituitary relationship which Bernstorff (1957) indicated caused an increased gonadotrophin secretion.

Oviduct

According to Leeson and Leeson (1966) the oviduct was a tube between the ovary to the uterus. In the dog (Christensen, 1964) each oviduct was located between the peritoneal layers of the mesosalpinx and connected the peritoneal cavity with the uterine cavity. Christensen stated that the canine oviduct was 4 to 7 cm. long, 1 to 3 mm. in diameter, and consisted of an infundibulum at its cranial end, near the opening of the ovarian bursa, and a caudal tubal portion. From within outward it was composed of: 1) a partially ciliated columnar epithelium that lay on, 2) the mucosa, which was slightly folded at the uterine end of the oviduct and greatly folded near the ovarian end, 3) a muscular layer of primarily circular bundles of fibers with a variable number of longitudinal and oblique fibers and 4) a tunica serosa which was composed of the peritoneum that made up the mesosalpinx.
Embryology and development

In the human embryo, the Mullerian duct, referred to as paramesonephric duct, by Velardo (1958) and Arey (1965) was first observed lateral to the cranial end of the mesonephric duct, at 37 days (Velardo) or 42 days (Arey). Velardo stated that it was probably derived from an invagination of the coelomic mesothelium. Gruenwald (1941) suggested that it may have developed from a splitting of the mesonephric duct. In a recent text, Arey (1965) wrote that the Mullerian duct appeared as a furrow in the thickened epithelium at the cranial end of each urogenital ridge. According to Burns (1955) the cranial portion of the oviduct, the ostium, in certain fishes and amphibians arose from persistent pronephric tubules, and then the oviduct developed by the caudal growth of a cord of cells from this primordium. He indicated that the pronephros even in mammals was probably related to ostial formation. Development of the Mullerian duct, of which the cranial portion became the oviduct, was dependent upon the presence of the nephric duct (Burns, 1955). At any rate the cranial most portion of the groove remained open while according to Arey (1965) the lips of the groove, a little more caudally closed to create a funnel-like tube. As the solid cord of cells grew caudal from this area cavitation began cranially and then progressed caudal (Velardo, 1958). All of this began in the 37 day or 10 mm. human embryo (Velardo). As growth of the Mullerian duct continued, the oviduct portion remained slender (Patten, 1958) in deference to the greater development of the more caudal portions. Arey stated that at 10 weeks in the human embryo, the cranial end of the oviduct had developed a fringe of fimbria. He indicated that as the mesonephric system degenerated the oviduct lay in a mesenteric
fold (the mesosalpinx), while the cranial portion (the ostium), retained a close relationship with the gonad (Velardo, 1958). Velardo stated that in the human at 10 weeks of intrauterine life the caudal ends of the Mullerian duct had fused and from the fused portion caudad developed the uterus and vagina. In the 15 cm, human female fetus the fallopian tubes or oviducts were patent canals throughout their entire length as indicated by Fleming (1926-1927) were lined with a single layer of epithelial cells and had two coats to their walls. Mature muscle fibers were not recognizable in these layers. Ultimately, the oviduct became differentiated into the fimbriated funnel-shaped infundibulum cranially, the intermediate enlarged ampulla and the narrow caudal portion, the isthmus, which joined to the uterus (Bloom and Fawcett, 1962). Voinot (as cited by Novak and Everett, 1928) found that in the human oviduct cilia were absent during intrauterine life and appear in slight number just before birth. Up to puberty, he found ciliated and non-ciliated cells. Very little more information, however, was available in the literature on the morphology of the reproductive tract near the end of fetal and during the early postnatal periods.

Physiology

In order to gain a better appreciation of the importance of the oviduct as a structure, some of the functions were reviewed. Eckstein and Zuckerman (1956) indicate for the canine species that during anestrus the mucosal folds near the ovarian end were well developed while those near the uterotubal junction were inconspicuous. They found that at this time the epithelium consisted of a single layer of small, non-ciliated columnar cells with large nuclei and cytoplasm that gave no evidence of secretion.
During proestrous according to Eckstein and Zuckerman (1956) the tube increases in volume, the mucosal folds become attenuated and the glandular epithelium developed into ciliated and non-ciliated cells which contained fuchsinophilic granules. At the time of estrous, the oviducts increased greatly in motility and the fimbria actually appeared to massage the ovary (Nalbandov, 1958), Eckstein and Zuckerman, (1956). These investigators indicated that the above described secretory appearance was maintained. When ovulation occurred the ova were expelled into the peritoneal cavity and were then engulfed by the oviduct (Guyton, 1961). The massaging action of the fimbria aided in this. Guyton indicated that the active cilia lining the abdominal ostia created a slow current into the oviduct, which along with possible feeble contractions aided in carrying the ova into the oviduct where fertilization could take place. Even morphology of the secretory cells in the ampulla varied during the days when ova passed down the mouse oviduct (Reinius, 1966). Secretory granules and visicles became more numerous. According to Schilling (1962) in the ovine and bovine the muscle system was multidirectional so that rapid closure of the ovarian bursa was possible. Mastroianni (1962) indicated in agreement with Guyton that tubal contractions may then have secondarily aided ciliary action for ova transport. In the luteal phase in the canine, Eckstein and Zuckerman (1956) found that none of the oviducal cells were ciliated. Throughout lactation changes in structure and height of the epithelium occurred in the sow (Palmer, 1965).

Histology

According to Harrop (1960) the canine oviduct consisted of a funnel shaped infundibulum with fimbria, a long ampulla and a short narrow isthmus
near the uterine horn. Velardo (1958) described a cross section of the human oviduct as a musculo-membranous structure which consisted of a mucous membrane and a muscle layer covered by a serosal layer. The musculature as described by Nalbandov (1958) consisted of an inner circular and an outer longitudinal layer. The epithelium, lamina propria, musculature, vascularization, innervation and serosa were reviewed and when appropriate, cyclic changes due to physiologic state were described.

**Epithelium**

Lining the canine oviduct was a single layer of columnar epithelium (Harrop, 1960). In reference to the mammalian species in general Nalbandov indicated that this epithelium contained ciliated and non-ciliated cells, some of which changed to goblet cells. Zupp (1924) found in the bovine that the epithelial cells, and their cilia were longest at the ovarian end. In man according to Clyman (1966) ciliated epithelial cells made up most of the infundibulum and isthmus while in the ampullae secretory cells were most abundant. Fredricsson (1959) found that these 2 cell types were cytogenetically and morphologically separate entities and that they regenerated independently.

**Proestral** In the canine according to Harrop (1960) the epithelial cells increased in height, developed a secretory cytoplasm and became ciliated during proestrous. Eckstein and Zuckerman (1956) had agreed with this but stated further than fuchsinophilic granules were a prominent component of the cytoplasm at this phase. In the bovine cilia were distinct, especially at the ovarian end and a small amount of secretion was on the surface (Zupp, 1924) while very little pseudostratification was observed (Weeth and Herman, 1952). A similar picture of
proliferation but with definite pseudostratification was observed by Abdalla (1968) in the ovine. In swine, Snyder (1923) had also observed a gradual increase in epithelial height in preparation for estrous.

**Estral**  No change from that observed during proestrous was seen in this phase in the canine (Harrop, 1960). It was felt by Eckstein and Zuckerman that the cells at estrous contained fine fuchsinophilic granules and by Andersen and Wooten (1959) that there was an increase in secretory cells. Zupp (1924) found during estrous in the bovine, that the epithelium stained lighter and contained distinctly granular nuclei. There were no granular bodies on the surface and no secretion at this phase. The epithelial cells reached their maximum height (34 microns) at estrous according to Weeth and Herman (1952) were goblet shaped with round, vesicular nuclei, vacuolated cytoplasm and had bush-like tufts of cilia. Snyder (1923) found that the epithelium reached its maximum height (25 microns) at estrous also in the pig. Casida and McKenzie (1932) agreed but found the porcine epithelium to measure 22 to 56 microns in height, with cilia 8 to 14 microns in length.

**Post-estral**  After ovulation and during what would be termed metestrum in the canine, signs of secretory activity disappeared and during the luteal phase proper, none of the cells were ciliated (Eckstein and Zuckerman, 1956 and Harrop, 1960). In the ovine this was considered the secretory phase and Abdalla (1968) described a tall epithelium with cytoplasmic projections protruded from the free border of some of the non-ciliated cells. Later the epithelium became irregular. Snyder (1923) reported that in swine the length of projections from the non-ciliated cells varied inversely with the height of the epithelium. Thus,
as the epithelium decreased in height the second week after ovulation (at implantation time) cytoplasmic processes jutted out from the apical end of the non-ciliated cells far beyond the adjacent cilia. A similar phenomenon was reported by Casida and McKenzie (1932) in the ovine about 3 days after estrous at which time the cytoplasmic projections were at their greatest height 6 to 11 microns, compared to their average height of 3 to 8 microns at other stages. In both the porcine and ovine species, deep staining nuclei were observed during estrous. Casida and McKenzie (1932) indicated that as the cytoplasmic projections increased in length there was a tendency for them to become pedunculated. This was perhaps a similar phenomenon to that described by Weeth and Herman (1952) for the bovine as they reported extrusion of nuclei 2 to 4 days post-estrous. These authors also observed pseudostratification of the epithelium in early diestrous. At 15 days post-estrous or during the diestral period the columnar epithelium of the bovine oviduct was at its minimum height of 18 microns (Weeth and Herman). Weeth and Herman reported that cilia which were never well developed or evenly distributed in the bovine were very sparse in this late diestral period. Snyder (1923) observed that in the porcine oviduct, cilia were present at all stages of the cycle without variation in number. In the human Novak and Everett (1928) found that peg cells "stiftchenzellen" were present in large numbers during pre-menstrual and menstrual periods corresponding to the post-estral period of animals. They felt that peg cells were simply secretory cells after secretion. Only at the level of electron microscopy were differences noted between the secretory cells of the infundibulum than those of the ampulla according to Reinius (1966)
who studied mouse tissues. He found that the latter had more and longer microvilli.

Gestational Snyder (1925) found in the porcine that if conception was followed by normal implantation periodic changes were inhibited. He found that the epithelium remained in a condition characteristic of the second week post-estrus when the cells were lower and the cytoplasmic projections became prominent. A similar phenomenon was observed by Veeth and Herman (1952) who reported that the epithelium was about 20 microns during estrus and was only slightly pseudostratified in appearance. They found nuclear extrusions and goblet-like cells which they indicated appeared to be related. During gestation cilia in the bovine oviduct were sparse, poorly distributed and often non-existent (Veeth and Herman). During the first month of pregnancy in the ovine, Abdalla (1968) found cytoplasmic projections or large droplets of secretion on some cells and also found other tall columnar ciliated cells. In the second half pregnancy the cytoplasmic projections decreased in number and only a few cells were ciliated. Finally at the 140 day of gestation Abdalla observed that cytoplasmic projections and protrusion of nuclei had reappeared. In the human during pregnancy according to Novak and Everett (1928) the ciliated cells became extremely low but still retained their cilia. In swine, Snyder (1925) found no change in the distribution or activity of cilia during pregnancy. Veeth and Herman reported that acidophilic cytoplasmic projections were more numerous during pregnancy than in the non-gravid oviduct and that epithelial glycogen, located at the bases of the plicae was observed throughout gestation. No cytoplasmic projections were present in the human during the gravid phase of the apical border of the non-ciliated
cells was gently rounded and gave the free border a wavy outline (Novak and Everett, 1928).

**Postparturient and lactational** According to Palmer (1965) changes in structure and height of the porcine oviducal epithelium occurred throughout lactation and in the early post weaning period. He found that one to 3 days after farrowing the epithelium appeared pseudostratified columnar and was 30 to 35 microns in height. At 7 days postparturient the epithelium decreased to 15 to 20 microns, was simple columnar, and contained numerous cytoplasmic processes projecting from the apical border. This observation agreed with Snyder’s (1923) of the postparturient sow oviduct even to the finding of nuclei in the cytoplasmic processes that Palmer stated were extruded into the lumen of the oviduct. A decrease in cellular extrusion was reported during the later stages of lactation, 52 and 62 days postparturient, (Palmer, 1965). Three and 4 days after weaning Palmer found that the epithelium increased up to 35 to 40 microns in height and regained the earlier described pseudostratified appearance. No information was found in the literature regarding this phase for other animals.

Small epithelial lined pockets were found in the folds of the bovine oviduct near the periphery according to Weeth and Herman (1952) who stated that throughout most of their length they were surrounded by connective tissue stroma. They did not indicate whether cyclic changes occurred or did not occur in these pockets.

**Membrana propria**

Leeson and Leeson (1966) stated that there was no definite basement membrane underlying the epithelium of the oviduct, while Arey (1968) in-
icated that it was present but was inconspicuous with the light microscope. In 20 human specimens studied by Lamb, et al. (1960); 7 had no evidence of a basement membrane; in 10 a discontinuous and variably thick membrane was noted and in 3 a thick continuous membrane was present. Reticular fiber stains on 7 of these showed the condensation of fibers to be slight and discontinuous in 5 and of moderate thickness and continuous in the other 2, one of which was from a functional tract and the other from a senile tract. Pauerstein and Woodruff (1967) found that although it was inconstant in the human, it was sufficiently visible to enable the localization of what they called "indifferent cells." According to Abdalla (1968) it was found in the ovine oviduct.

**Lamina propria**

The whole mucosal lining of the oviduct was thrown into characteristic folds, low and simple next to the uterus and thin, high and intricate next to the ovarian end (Arey, 1968). The lamina propria comprised most of the tissue of the rugae. In the dog, these folds were a permanent feature of the oviduct after puberty (Andersen and Wooten, 1959). Copenhaver and Johnson (1958) stated that it was composed of richly cellular connective tissue, compact in the isthmus and more loosely arranged in the high folds of the ampulla. This was in agreement with Leeson and Leeson (1966) who added that it contained a few scattered smooth muscle cells. Bloom and Fawcett (1962) described the oviducal lamina propria as consisting of a network of thin fibers, numerous fusiform or angular cells, some wandering cells and mast cells. According to Harrop (1960) this layer in the dog consisted of connective tissue cells, collagen fibers, muscle fibers and blood vessels. In the bovine
Weeth and Herman (1952) found that the connective tissue of the plica and that surrounding the tube was predominately reticular fibers of medium thickness with some collagen fibers in the wider folds. Oviducts from 94 cattle, sheep and swine were studied by Nellor (1965) who indicated that the connective tissues of the lamina propria contained large numbers of migrating cells. He indicated that they contained ample neutrophilic cytoplasm and had a characteristic spoke-wheel nuclear chromatin pattern. Nellor indicated that the nuclei became round after they penetrated the basement membrane then wedge-shaped after penetrating between the epithelial cells. After being expelled into the lumen these migrating cells assumed a compact nuclear form with limited cytoplasm, similar to a tissue lymphocyte. According to Nellor, whole cell extrusions were present in cattle and swine early in normal estrous and 8 to 10 days post ovulation, while in sheep they occurred from the sixth to the fifteenth day of the normal estral cycle. They also occurred in cattle and swine oviducts from the twentieth to the fortieth day of pregnancy. Snyder (1923) indicated that the stroma of the oviduct in the pregnant sow was closely packed and that at the ninth day of pregnancy, at implantation, an infiltration of eosinophilic leucocytes was evident. In the bovine (Weeth and Herman, 1952) the oviducal lamina propria was of moderate density (except in early pregnancy) and collagen fibers were more prominent than in the non-gravid oviduct. Nellor described two modifications of the leucocyte-like patterns as found during normal cycles. In swine, Snyder (1923) reported that the stroma of the lamina propria appeared swollen and edematous when the epithelium was high during the first and third weeks after ovulation which compared
favorably with the first migration of lymphoblast-like cells described by Nellor (1965). Snyder (1923) reported that it was composed of closely packed strands during the second week when the epithelium was low. During estrous in the bovine, Weeth and Herman (1952) found that reticular fibers were most prevalent in the stroma. Snyder described wandering cells of the eosinophilic and plasma cell types which he stated appeared in numbers that fluctuated sharply but irregularly without any relation to the cycle, in contrast to the cyclic pattern presented by Nellor.

Casida and McKenzie (1932) reported an increase in the stromal elements of the ovine oviduct similar to that described by Snyder for swine shortly after estrous, and stated that the individual connective tissue cells appeared swollen and vacuolated, a condition which he referred to as hydroptic degeneration.

It was stated by Arey (1968) and Bloom and Fawcett, (1962) that there was no muscularis mucosa in the oviduct and therefore the lamina propria extended without change down to the muscular coat. No glands and no submucosa were present in the oviduct (Copenhaver and Johnson, 1958 and Arey, 1968).

Muscularis

This structure in the canine consisted of some oblique muscle bundles, a circular layer with elastic fibers extending into the lamina propria, a vascular layer and a thin longitudinal muscle layer (Harrop, 1960). The textbooks (Copenhaver and Johnson, 1958; Bloom and Fawcett, 1962; and Leeson and Leeson, 1966) described this structure as being composed of an inner circular and an incomplete outer longitudinal layer of smooth
muscle. Bloom and Fawcett (1962) explained that the inner layer may also be spiralled but that there was no distinct limit between the two and that they were both embedded in an abundant loose connective tissue with elastic networks that extended far into the serous layer and into the broad ligament. According to Copenhaver and Johnson (1958) the outer longitudinal layer was complete only in the isthmus and at the uterine attachment. Both muscle coats increased in thickness from cranial to caudal (Leeson and Leeson, 1966).

In contrast to these textbook descriptions, Horstmann (1952) described 3 discrete layers of musculature in the human oviduct: 1) a subperitoneal layer which was directed longitudinally; 2) a middle vasomotor layer, whose fibers paralleled the vessels encircling the duct and 3) an inner, autochthonous layer, arranged in spirals which originated from different directions and intersected at regular intervals. Kiper (1950) and Toni and Maccaferri (1951a, 1951b) described 3 layers and indicated that they were intimately connected anatomically and functionally. It was of physiological interest to note that at the cranial end of the oviduct Stange (1952) observed a tubal attracting muscle the muscle attractens tubae which originated at the tubal end of the fimbria, from the vascular layer and terminated at the cranial pole of the ovary. Stange postulated that the muscular layer in the para-ovarium lifted the ovary while the muscle attractens tubae brought the fimbriated end of the tube down on the ovary.

**Serosa**

The human oviduct according to Leeson and Leeson (1966) was invested with a fold of reflected peritoneum. It consisted of a loose connective
tissue, the deep portions of which contained the longitudinal bundles of
the muscularis and the surface of which was covered with a mesothelial
sheet.

**Vascularization and innervation**

**Blood vessels**  According to Bloom and Fawcett (1962) the
mucous membrane and its folds as well as the serous coat contained abun-
dant blood and lymph vessels. The mucosa was supplied by spiral arterioles
and had a rich capillary plexus as described by Gillet and Pietri (1967).
Copenhaver and Johnson (1958) indicated that the blood vessels ran in
the stroma along the bases of the oviducal rugae, and that they sent
off branches which gave rise to a dense capillary network in the stroma.
Venous drainage as described by Velardo (1958) followed a similar pattern
to the arteries and established a large plexus in the oviducal muscles.
The abdominal ostia contained in its mucosa a ring of large blood vessels
especially veins that extended into the fimbria (Bloom and Fawcett, 1962).
This structure around which the muscle fibers composed a network, formed
erectile tissue that Bloom and Fawcett indicated became turgid and aided
in ovum pickup at ovulation. This was described as a "rotliche Masse",
for the dog by Andersen and Wooten (1959). During gestation in the
bovine (Weeth and Herman, 1952) the stroma of the mucosal folds appeared
avascular, especially after the first trimester.

**Lymph vessels**  Anderson (1927) described in detail the lymph-
atics of the fallopian tube of the sow. The results of this investigation
showed that in the ampulla, the lymphatics lay in three separate strata of
tissue; two capillary networks in the subserous and sub-epithelial con-
nective tissue respectively and the larger collecting vessels in the inter-
muscular connective tissue layer. In the outer third of the oviduct, where the longitudinal muscle layer was sparse the lymphatics were actually in the subserous connective tissue but in cross-section the most conspicuous was the mucosal plexus. The lymph systems of the infundibulum and isthmus were not completely worked out but appeared to be similar to the ampulla, although the lymphatics of the isthmal muscles were of larger lumen size. A sudden increase in complexity was observed at the tubo-uterine junction although the fundamental pattern remained the same.

Lymphatics of the human oviduct were described by Copenhaver and Johnson (1958) as large lacunae in the stroma of the mucosal folds which emptied into narrow channels, passed through the muscularis and formed a rich subserous net.

According to Anderson (1927) during estrous the two sets of vessels in the ampulla and isthmus were twice as wide as at mid-estrous whereas the greatest size of the lymph vessels of the tubo-uterine junction was observed 12 to 14 days after ovulation. Lee (1928) observed that the lymph vessels were small and few in this region in the dog. It was then stated that the vessels of the latter junction had the same cycle as the uterus and not of the oviduct (Anderson).

Innervation In the human oviduct, large nerve bundles were found with the vessels in the serous layer and in the periphery of the longitudinal muscle, while the circular muscle contained a dense plexus of thin nerve bundles which supplied the muscle fibers and penetrated the mucous membrane (Bloom and Fawcett, 1962).

Tubo-uterine junction

Investigations on the tubo-uterine junction in carnivora revealed that
in the cat and dog they were morphologically similar (Anderson, 1928). In the dog Lee (1928) found that the oviduct entered the uterus obliquely, and not at the apex of the uterine horn. He reported that protrusion marked the entrance of the tube and that at the tubal ostium the ciliated epithelium was tall columnar, resembling that of the oviduct proper, rather than that of the uterus. Uterine glands were found near the opening of the oviduct. The stromal cells were loosely arranged at the extremities of the mucosal projections and densely packed at the base where fibrous connective tissue was found. Lee indicated that the circular muscle layer was well developed and was continuous with the corresponding layer of the oviduct proper. In a recent report Hook and Hafez (1968) indicated that no ciliated cells were found at the junction in the dog or rat, whereas they were very abundant at estrous in the rabbit. The muscularis of the dog and pig lacked a definite inner longitudinal layer whereas this layer was described in man, rat and cow (Hook and Hafez). Hook and Hafez indicated that neither the circular muscle of the oviduct nor of the uterus extended deeply into the uterine mound in the dog but that some sphincteric action of the base of the oviduct was not precluded. Lee (1928) reported that in the rabbit and pig numerous macroscopically visible polyp-like projections extended into the uterine cavity. Similar folds, but of lesser prominence were found in the mouse, rat, guinea pig and cat while in the dog and lioness the least development was found. In all of the animals studied Lee also observed that the circular smooth muscle layer was well developed and suggested a sphincteric action which together with the mucosal folds might effectively shut off the tube. Anderson (1928) stated that no sphincter was observed at
the junction in the cow.

The polyp-like structures described by Lee (1928) for the porcine junction were referred to as villi by Anderson (1928) who stated that they were present in specimens from all stages of growth and pregnancy, although they varied in size, number and distance extended from the tubal opening. Using cross-sections instead of longitudinal ones as studied by Lee (1928) and Anderson (1928), Rigby and Glover (1965) reported that in the sow the lumen of the oviduct decreased markedly in the first centimeter from the uterine ostium, remained narrow for about 6 cm, then widened out into the ampulla. According to Rigby and Glover characteristic diverticulae found in the first few millimeters of the tube appeared as branches of the lumen, were directed rostrally and were of possible functional significance.

Age changes

Kretzschmar and Stoddard (1964) reported that the fallopian tubes of the aging human female underwent general atrophy. They found that the walls lost their folds, became thin and constricted while the mucous membrane also became thin, flattened and lost its cilia.

Epithelium

In the human oviduct, for several years after cessation of the menstrual cycle there was but limited evidence of atrophic changes in the epithelium (Novak and Everett, 1928). These investigators observed that the cells remained high, both types persisted, cilia were abundant and no evidence of secretory change was seen in the non-ciliated cells. In some specimens from subjects over 60 years of age Novak and Everett found that the epithelium was of cuboidal type or even flattened. These changes were
found to vary in different parts of the oviduct so that in parts the epithelium was tall and in others it was similar to simple squamous type with no trace of cilia.

**Lamina propria**

Novak and Everett (1928) reported that the tubal rugae from some human subjects over 60 years of age became rounded and had a fibrous appearance. Agreement with this was made by Pinero and Foraker (1963) who stated that there was evidence of increasing amounts of plical fibrous tissue after the menopause and that collagen fibers were more pronounced in older specimens. Sani (1950) found that there was an increase of elastic elements in the human oviduct from the fetal period to the 35 to 40 year age period followed by regressive changes of elastic fibers that paralleled an increase in collagen as connective tissue beginning at the 45 to 50 year age group. Among the connective tissues elements of the lamina propria of aged cows Yamauchi (1964) found many pigment cells and various kinds of leucocytes such as lymphocytes, eosinophils, monocytes and plasma cells.

**Muscularis**

According to Toni and Maccaferri (1951b) the diameter of the oviducal musculature decreased progressively from the uterine to the abdominal ostium in the same subject and with age at any given level. They found that at the isthmal level, the mean transverse diameter of the muscle cells at 20 years was 6.2 microns and at 50 years was 3.4 microns. It was observed that while the muscularis increased from 20 to 50 years of age, atrophy and regression were observed beyond 50 years of age (Toni and Maccaferri, 1951a). Thus, the number of muscle cells per unit area in
general increased from 50 to 80 years (Toni and Maccaferri, 1951b). These investigators also observed that the amount of intercellular substance increased with age. Epithelial ingrowths that extended deep into the muscularis and formed cystic structures were described in the porcine species by Bal (1968). He also indicated that in these 6 and 8 year old specimens there was an increased fibrosis of the muscularis.

**Vascularization**

In the porcine oviducal vascularization of 6 to 8 year old specimens the arteries were severely affected with intimal thickening and intimal plaques were observed in large veins (Bal, 1968). According to Bunce (1964) the intima was normally absent in arteries from young healthy dogs but was found in more vessels in older dogs (6 years of age). In 1965 however, he stated that by his definition an intima was not found in distended arteries from dogs less than 7 years of age. Pinero and Foraker (1963) reported that in the human oviduct no generalization as to vascular changes with age appeared possible.

**Uterus**

Christensen (1964) defined the canine uterus as a hollow muscular organ which served as a habitation for developing young, gave attachment to the fertilized ovum and served as the route by which the sperm reached the oviduct. He stated that it consisted of a neck (cervix), body, and two horns or cornu. (Only the body and horns were discussed in this portion of the review.) Structurally, the uterus of the dog consisted of three tunica: the mucosa, muscularis and serosa. According to Christensen, the tunics mucosa (endometrium) was the thickest of the three layers and was lined with a layer of temporarily ciliated low columnar cells, under
which were the long branched tubular glands of the lamina propria. He described the muscularis as being composed of a thick circular inner layer and a thin longitudinal outer layer of non-striated muscle, which encompassed a vascular layer which contained blood vessels, nerves and circular and oblique muscle fibers, all of which was covered by the serosa.

**Embryology and development**

The origin and development of the cranial portion of the Mullerian ducts into the fallopian tubes was previously discussed. As the portion of these ducts immediately caudal to the oviducts excavated and developed, the paired Mullerian ducts fused caudally and eventually formed the horns and body respectively of the bicornuate uterus of carnivores (Patten, 1958). In the 98 mm. ovine fetus the epithelium of the whole uterus was typically columnar and often consisted of two layers of cells (Bulmar, 1964). In the human fetal uterus the glands invaginated early in the third trimester but remained small until puberty (Arey, 1965). Arey observed that the muscular wall was foreshadowed by the end of the first trimester, by mesenchyme condensing about the epithelial lining.

**Physiology**

There were a number of accounts of cyclic functions in the dog (Evans and Cole, 1931, Mulligan, 1942, Eckstein and Zuckerman, 1956). Eckstein and Zuckerman provided descriptions for all of the major mammalian orders among which was the Carnivora, including the cat, lion, spotted hyena, ferret, weasel, mink, fox and domestic dog.

Some of the smaller breeds of dogs were said to have 3 or 4 cycles each year compared to an average of 2 (Mulligan, 1942) for most breeds.
Using Heape's classification as a base, Evans and Cole (1951) divided the canine estrous cycle into: 1) anestrum, the 2 month period from the end of regressive changes of one estrum to the incidence of the proestrum; 2) proestrum, the 9 day period from the first show of blood at the vulva until male acceptance; 3) estrous, the 9 day period of male acceptance and 4) metestrum, the 90 day period of corpora luteal regression.

During anestrous in the canine as described by Eckstein and Zuckerman (1956), the uterus was small and flattened with a shallow mucosa and poorly developed muscular wall. Rapid growth during proestrous included mucosal thickening due to increased vascularity, with some focal areas of slight hemorrhage per diapedesis, and edema associated with glandular proliferation. Laguchev (1965) found a statistically significant increase in mitotic activity during proestrous in the mouse. During estrous the vascular congestion was less, hemorrhage stopped and the uterine glands decreased their activity. A second increase in mitotic activity was observed by Laguchev during the first day after estrous. Following estrous either pregnancy or pseudopregnancy resulted. Eckstein and Zuckerman stated that in the latter there was a continued proliferation of the uterine glands and surface epithelium and a folding of the mucosa. Later two zones were distinguishable in the endometrium, an inner compact zone with superficial crypts and stroma and a deeper zone which consisted of a network of dilated uterine glands set in a scanty lamina propria. During the true luteal phase the compact zone transformed into a mass of epithelial villi, and the spongy zone glands decreased in number. Regression of the endometrium began in the second half of pseudopregnancy and by the end of the period returned to the anestrous state.
If pregnancy resulted the early development of the endometrium was the same as in pseudopregnancy and appeared the same between the implantation sites (Eckstein and Zuckerman, 1956).

**Histology**

As indicated previously, the appearance of the uterus varied with the stage of the estral cycle. Changes that occurred in the morphology of the epithelium, lamina propria and muscularis including vascularization and innervation were given for each phase of the estrous cycle namely: proestrual, estral, metestral (post or diestral) and anestral, gestational, postparturient and lactational as far as information was available.

**Epithelium**

**Proestrual**

During proestrual most of the glandular epithelial cells were tall except those undergoing mitosis in which the nuclei were small and basally located (Eckstein and Zuckerman, 1956). Andersen and Wooten (1959) on the other hand stated that mitotic figures were found in the "low" columnar epithelium of proestrual. McKenzie (1926) indicated that in the sow, leucocytes were numerous at this phase. In the bovine during proestrual Cole (1930) and Weeth and Herman (1952) observed in the intercaruncular areas that pseudostratification was pronounced and the surface epithelium was tall, as it was during estrous. Cole indicated that the nuclei were oval or spherical and basally located.

**Estral**

During estral in the canine, the epithelium of the uterine crypts and the surface exhibited proliferation (Mulligan, 1942), and became higher (Harrop, 1960). In the cat (Dawson and Kosters, 1944) the surface epithelium was tall columnar, 20 to 29 microns in height and
pseudostratified in appearance and according to Fabian and Preuss (1966) it was tallest during this phase of the cycle. The pseudostratified appearance of the epithelium at estrous was also observed in the sow (McKenzie, 1926 and Green, 1950), and cow (Cole, 1930). McKenzie reported that cilia were found in the epithelial cells of the crypts and glands of the sow's uterus but not on the free surface. He found that the epithelium increased in height all through estrous. Corner (1921) recorded that the uterine epithelial cells of the sow were 25 to 35 microns in height at estrous and that chromatolysis of the nuclei and vacuole formation was observed in some of the cells. In the ovine uterus at estrous the cells were 21 to 31 microns high according to Casida and McKenzie (1932) and had very large nuclei. The nuclei were spherical, tended to be basally located in the bovine and were arranged in 2 or 3 rows at estrous (Cole, 1930).

Metestral Eckstein and Zuckerman (1956) referred to the postestrual phase of the bitch as pseudopregnancy if conception did not take place and indicated that proliferation of the epithelium continued. In the sow (McKenzie, 1926) the pseudostratified condition continued until about a week after estrous. Corner (1921) explained also with reference to the sow, that the individual cells grew larger and piled up to form an epithelial layer 35 to 50 microns thick, but that no degeneration was evident, and that no neutrophils were seen in the epithelium. He stated that mitotic divisions were numerous throughout the first week after estrous. A clear zone was observed by McKenzie in the sow the day after estrous, between the basal end of the epithelial cells and the basement membrane which he stated was probably evidence of degeneration. In the
bovine the epithelium reached its lowest level (10 microns) 2 to 4 days postestrum and its highest level (40 microns) 15 to 19 days after estrous (Larson, et al. 1965). Weeth and Herman (1952) found that during the stage of decreased cell height, the bovine epithelial cell nuclei were middle to apical in location.

**Anestral** Mulligan (1942) stated that in late metestrous (seventieth day) which would be near early anestrous in the dog, the epithelium was composed of short, simple columnar, clear fat containing cells with apical nuclei. According to Eckstein and Zuckerman (1956) it was low columnar or cuboidal and the nuclei were basally located during anestrous proper.

**Gestational** In the canine according to Mulligan, if pregnancy took place the surface epithelium became greatly hyperplastic, was shed and disaggregation took place. Corner (1921) found in the sow that the same histological changes were found during the first 15 days of pregnancy as during the 15 days following estrous without copulation. He stated that by the seventh day the epithelium had passed into the high columnar stage, with a hilly arrangement of the surface and cessation of mitotic divisions. At 11 days the epithelium was low columnar, without hillocks and by 14 days the low columnar cells were covered with frayed or rounded protuberances. Later on, at 18 to 20 days the epithelial cells were lower and the roughness of the epithelial surface was more pronounced. As pregnancy progressed the epithelial cells of the sows uterus became medium or tall columnar and persisted throughout pregnancy (Corner, 1921). Palmer (1965) reported that at 110 days of gestation in the sow the uterine epithelium was greatly folded and the
epithelial cells were cuboidal in shape. In the pregnant bovine (Weeth and Herman, 1952) the epithelium varied from completely absent to 50 microns in height and showed karyorrhexis and pseudostratification during the first half of pregnancy. Later it becomes more uniform with predominately ovoid, basal nuclei although small amounts of lipid were seen in the surface epithelium throughout pregnancy, (Weeth and Herman).

Postparturient and lactational After parturition took place in the dog epithelial repair from the non-placentized portion was accomplished in a similar fashion to that described during metestrous, except that it began to form a more complicated pattern (Mulligan, 1942). At 8 days postpartum in the cow (Weeth and Herman, 1952) the surface epithelium was growing in from the intercaruncular area at the periphery of the placentome and cut off much of the loose, degenerating mass. At one day postpartum in the sow (Palmer, 1965) the epithelium was similar to what it was in late pregnancy, except that the folding of the uterine lining was not as pronounced. The cells were low columnar to cuboidal and were 10 to 15 microns in height. By 7 days after farrowing the cells were only 5 to 6 microns high with deep staining cytoplasm. Palmer found that in certain areas numerous mitotic cells were present and by this time the folding was gone. By 14 days the epithelium was 15 to 20 microns high and was pseudostratified in appearance. Cell multiplication and degeneration had occurred at this time in the cells near the basement membrane. Continued epithelial growth was observed so that at 28 days postpartum it was 25 microns in height and at 3 days post weaning 30 to 35 microns in height (Palmer, 1965).
Membrana propria

The basement membrane under the epithelium of the porcine uterus was distinct throughout the estral cycle (McKenzie, 1926). Casida and McKenzie (1932) reported that the basement membrane was usually fairly well defined in the ovine uterus. In 13 human specimens studied Lamb, et al. (1960) found that a basement membrane was absent in 2, formed a thin discontinuous layer of condensed ground substance in 9 and was fairly thick in the remaining 2 which were from senile patients. One of the latter ones, however, showed an inconstant membrane that was absent around most of the superficial and some of the basal glands. Out of 8 specimens stained for reticular fibers, a thin layer of condensed fibers was present in all, but was discontinuous in six. Lamb, et al. reported that no consistent differences in the character of the basement membrane with regard to stage of cycle was found.

Glands of endometrium

Eckstein and Zuckerman (1956) and Harrop (1960) reported 2 kinds of glands in the canine endometrium: shallow crypt-like invaginations with narrow necks, and long glands which penetrated the whole thickness of the mucosa and became slightly coiled as they approached the myometrium. According to Trautmann and Fiebiger (1957) the short glands were absent in other domestic animals and man. Eckstein and Zuckerman stated that the glandular epithelium was tall columnar, and except during mitosis the nuclei were relatively small and basally located. Sometimes the epithelium was ciliated (Trautmann and Fiebiger). The latter authors indicated that these glands had a lamellar connective tissue sheath.
Proestral The glandular epithelium proliferated during
proestrus according to Eckstein and Zuckerman (1956). Mulligan (1942)
found that it became tall columnar type during this phase.

Estral Progressing through estrous the basal layer and tubules
became increased in thickness and complexity through proliferation of
the gland epithelium which increased in height, showed pseudostratification
and mitotic figures (Mulligan, 1942). Evans and Cole (1951) indicated
that most of this growth, especially of the crypts and deeper glands,
ocurred during the second half of estrous. In the sow the glandular
epithelium did not appear to change much in height with respect to the
estrous cycle (McKenzie, 1926). Corner (1921) found only a few mitotic
cells in the superficial glands and none in the deep glands of the sow,
but did find highly chromatic extra-nuclear granules 1 to 2 microns in
diameter in some of the gland cells. These he found only during estrous.
In the ewe, Casida and McKenzie (1932) reported that the glandular epi-
thelium did not show cyclic changes, but that there was an increase in
coiling and branching of the glands as estrous progressed. Overall
uterine glandular activity was reduced at estrous in the bovine (Weeth
and Herman, 1952). Cole (1930) reported that during estrous in the bovine
the nuclei of the glandular epithelial cells were often elongated, although
basally located, and that the increase in height of the cells resulted
in a decreased lumen size.

Metestral A progressing proliferation of uterine glandular
epithelium as described for estrous continued until the end of the second
week of metestrous in the dog and regression began about the third and
fourth week (Mulligan, 1942). At 22 days in the dog the crypts and glands
were smaller according to Marshall and Haiman (1917) and the epithelium lining them was cuboidal. The gland lumen sometimes contained a colloidal substance. During metestrous (diestrous) in the bovine according to Weeth and Herman (1952) the gland lumina were atrophic. Weeth and Herman found that the gland epithelial lipid was maximal at 4 days postestrous and none was present at 11 or 15 days postestrous. Cole (1930) reported marked hypertrophy in the bovine at 8 days postestrous which continued to the eleventh day and then regression began at 15 days postestrous.

**Anestral** A deficiency in glands was noted during anestrous in the bitch by Eckstein and Zuckerman (1956) who indicated that the low columnar or cuboidal gland cells had basal nuclei.

**Gestational** If the dog conceived the glands of the basal layer became dilated and their epithelium flattened according to Mulligan (1942) who stated that the tubules of the glands persisted as remnants. He found that the crypts widened into large recesses lined with flattened epithelium and enclosed a mucinous material. In the bovine at 25 to 28 days of pregnancy Foley and Reece (1953) observed that the glands were larger, especially in the middle and deeper zones of the mucosa. They indicated that large active glands were found in tissues from 3 animals slaughtered between 78 and 116 days of gestation. At 110 days of pregnancy in the sow the glands were greatly hypertrophied with epithelial cells 35 to 40 microns high that contained well defined granular cytoplasm (Palmer, 1965).

**Postparturient and lactational** At one day postpartum Palmer observed that the gland cells of the sow's uterus were 12 to 15 microns in height, were vacuolated and many of them contained shrunken pyknotic
nuclei. Palmer (1965) found that the deeper uterine glands maintained this appearance throughout lactation while some of the superficial tubule cells increased in height after 28 days postpartum. Three to 4 days after weaning Palmer observed increased glandular activity (as indicated by numerous mitotic figures), more deeply staining cellular cytoplasm and increased numbers of glands especially near the inner muscle layer.

**Lamina propria**

Arey (1968) classified this portion of the uterine mucosa as a framework of reticular fibers which condensed into a basement membrane and abundant stromal cells that occupied the spaces in the mesh. It had a mesenchymatous appearance in the dog (Harrop, 1960). Scattered macrophages which contained brown pigment granules were found in the endometrium of all dogs that had gone through at least one cycle. Mulligan (1942) indicated that they were not found in immature animals. They stained positive for iron, with Turnbull's blue. Mulligan stated that these macrophages were most noticeable in proestrus and during the postparturient period and least conspicuous in pregnancy, metestrus and anestrus. Cells resembling immature and mature lymphocytes were observed to invade the subepithelial uterine stroma and epithelium of gilts and cows in the luteal phase of the cycle (Nellor, 1963). Occasional morphologically normal lymphoid follicles were found in the endometrium of human specimens according to Payan, et al. (1964) and Sen and Fox (1967). According to Harkness (1964) collagen fibers were also a part of the lamina propria.

**Proestral** In the canine during proestrus Mulligan (1942) and Eckstein and Zuckerman (1956) found that there was marked interstitial edema and hyperemia of the stromal layer, with focal extravasation of
erythrocytes into the stroma especially near the uterine lumen. They indicated that hemosiderin laden macrophages were abundant at this time. No extravasation was observed in the bovine endometrium in proestrous according to Cole (1930) although the blood vessels were congested. Cole described a decrease in stromal cells in the intercotyledonary area of the bovine and an increase in large mononuclear cells probably the same as the macrophages described for the canine.

**Estral** The stromal layer of the canine uterus became less edematous at this time although it was increased in total bulk (Mulligan, 1942). Eckstein and Zuckerman (1956) reported that at least in early estrous in the canine there was a persistence of edema, a decrease in vascular congestion and cessation of hemorrhage with a disappearance of the existing extravasated cells. They indicated that the brown pigment containing macrophages were still seen during estrous. In the sow (Corner, 1921) and ewe (Casida and McKenzie, 1932) uterine edema persisted during estrous as Eckstein and Zuckerman indicated was the case in the dog. Weeth and Herman (1952) reported that stromal edema was not a marked characteristic of the non-gravid bovine uterus but that moderate loose-ness of the stroma was seen during proestrous and estrous and considerable hyperemia and extravasation were noted in the lamina propria that encircled the caruncle. In contrast Cole (1930) reported a very edematous stroma with few cells in the bovine lamina propria at estrous.

**Metestral** After estrous, or in the pseudopregnant bitch Eckstein and Zuckerman (1956) found an increase in the stroma near the epithelium and a scanty stroma near the muscularis. In the sow there was a decrease in stromal edema with a resulting condensation of connective
tissue after estrous, and an increase in eosinophils in groups around the vessels and glands of the superficial zone but not in the epithelium (Corner, 1921). Marked congestion of the blood vessels in the deeper stroma of the ovine endometrium with an increase in epithelium invading leukocytes was described by McKenzie (1936) for the postestral phase of the cycle. He also described pigment deposits in variable amounts below the epithelium of the ovine uterus similar to that described by Weeth and Herman (1952) for the bovine, although in the latter case this light yellow to dark brown pigment was concentrated around the caruncle and was at a maximum in the late postestral period.

**Anestral** The canine lamina propria during anestrous was composed of a connective tissue layer that was relatively poor in epithelial elements. (Mulligan, 1942). Harrop (1960) described it as a shallow structure in this phase.

**Gestational** Mulligan found that if pregnancy took place the connective tissue of the stromal layer of the canine was stretched, thinned, and the supporting stroma between the crypt recesses was compressed into narrow "mesenteric" partitions. As pregnancy progressed the association of the chorionic villi with the maternal capillary bed became more complex. A similar density of stroma was described for the pregnant bovine uterus by Weeth and Herman (1952) but in this case ischemia of the compact stromal layer and disappearance of pigment was observed early in the second trimester. They also reported a decrease in reticular fibers with an increase in collagen as pregnancy progressed and an acellular hyaline appearing lamina propria. It was reported by Fainstat (1964) that the collagen and elastic fibers appeared to be reversible
during pregnancy and thus were thought to be the same extracellular fibers. An increase in quantitative collagen of over 800 percent was reported for the human uterus at term by Morrione and Seifter (1962). Fainstat (1968) described these changes in the rat as the uterine stromal ccc phenomenon or 1) cell hypertrophy and hyperplasia, 2) collagen bundle splitting, fraying, disaggregation and disappearance and 3) capillary and other blood vessel proliferation and expansion.

Postparturient In the canine at parturition according to Mulligan (1942) the mesenteric partitions were ruptured and sloughed away with the placenta while larger masses of partially hyalinized fibrous connective tissue remained in situ at the previous attachment sites. Morrione and Seifter (1962) indicated that during involution in the human uterus there was histological evidence of edema and partial destruction of the reticular framework. The rate of collagen loss from the involuting uterus was greater than had been recorded from any other tissue in mammals (Harkness, 1964).

Myometrium

According to Rudolph and Ivy (1930) the musculature of the dog uterus consisted of three layers, a thick inner layer of circular fibers, a middle layer composed of vascular tissue, connective tissue, circular and oblique muscle fibers, and a thin external layer of longitudinal fibers. From within outward Weeth and Herman (1952) referred to them as stratum submucosum, stratum vasculare and stratum subserosum, in the bovine and stated that the inner layer contained no collagen. Bird and Willis (1965) found it very difficult to differentiate between the endometrial stroma and the inner circular muscle layer of the human uterus and stated that
the two tissues interdigitated intimately, resulting in an irregular and ill-defined junction.

**Cyclic changes** In anestrous the muscular wall of the canine uterus was poorly developed (Eckstein and Zuckerman, 1956) or inconspicuous (Mulligan, 1942). According to Weeth and Herman (1952) the muscle cells of the bovine uterus begin a renewed growth about two days proestrous, were larger at estrous and smaller about the end of diestrous. They indicated that cyclic changes occurred only in the outer and middle layers.

In the ferret at estrous the myometrium made up 35 percent of the total diameter of the uterine horn (Buchanan, 1966). Bird and Willis (1965) stated with regard to myogenesis in the human uterus that the uterine muscle was probably not static even in the non-pregnant uterus and suggested that there was possibly a cyclic production of new muscle at the metrio-myometrial junction with resorption of the old muscle in the myometrium. Production of connective tissue components by smooth muscle cells was also suggested by Friederici and De Cloux (1967) for the rat.

Bergman (1968) found that the rat uterus was electrically and mechanically quiet in the absence of estrogen stimulation.

**Gestational** Mulligan (1942) stated that the smooth muscle of the canine uterus became hypertrophied as pregnancy advanced. According to Strauss (1965) enlargement of the uterus during pregnancy depended mainly on hypertrophy of the muscle to a limit and that above that limit hyperplasia was required. He stated that after a number of pregnancies a uterine weight was reached at which only hypertrophy was necessary to take care of pregnancy. Bird and Willis (1965) suggested that myogenesis was possibly a normal function of the endometrial stroma and did not limit
this ability to a certain number of pregnancies. According to Schwalm and Dubrauszky (1966) there was a 43 percent increase in the amount of muscle tissue in the region of the body and isthmus of the human uterus during pregnancy. Due to the hypertrophy of muscle cells in the pregnant bovine uterus Weeth and Herman (1952) found that the tissue became extremely loose and appeared increasingly hypoplastic. The latter investigators stated that the loose connective tissue which separated the muscle bundles was predominantly short reticular fibers in early pregnancy with collagen fibers apparent during the latter half.

**Involutional** In an average size dog the horns of the uterus decreased in size from 30 to 50 cm. in length and 9 to 11 cm. diameter to 13 to 17 cm. long and 4 to 6 cm. in diameter during normal parturition. Normal involution then took over from this point (Rudolph and Ivy, 1930). Palmer (1965) found that the myometrium of the sow was very thick 1 and 3 days after parturition due to hypertrophy of the individual muscle fibers and the abundance of connective tissue that surrounded the muscle bundles and lay between the layers. The greatest weight loss was in the first 5 days according to Graves, et al. (1967). Palmer observed a decrease in thickness of the myometrium up to 21 days after parturition due to a decrease in size of muscle fibers and amount of interspersed connective tissue. This decrease was as reported by Graves, et al. also due to a decrease in cell numbers as well as size. From 21 days until the end of a 62 day lactation period the myometrium remained unchanged (Palmer). According to Graves, et al. (1965) removal of the pigs from the sow immediately after parturition resulted in a slower involution of the outer longitudinal layer, but no change was observed in the other
muscle layer. It should be also remembered, however, that injury to the uterus caused delays in normal involution as reported by Morrione and Zai Ru (1964).

**Serosa**

In the canine uterus, Trautmann and Fiebiger (1957) found that the serosa (perimetrium), the stratum vascular and the longitudinal layer of muscle were all continuous with the broad ligament which invested and suspended the uterus. Weeth and Herman (1952) observed that in the bovine it was composed of a single cuboidal, epithelial covering on a narrow connective tissue base. They pointed out that in this connective tissue next to the myometrium was a dense bed of small blood vessels. During the latter part of gestation the connective tissue was acellular and hyaline-like in appearance, while during estrous and for 8 days post-estrous it contained both argyrophilic and collagen fibers (Weeth and Herman). These investigators found that by 11 days postestrous the argyrophilic reticular fibers decreased and the collagen fibers increased, a trend that continued through gestation.

**Vascularization and innervation**

**Blood vessels** Trautmann and Fiebiger (1957) indicated that the blood vessels entered the uterus from the broad ligament and that they were numerous, thick-walled and followed a tortuous course in the stratum vascular while giving off branches to the other layers of the uterine wall. In early postnatal development in primates Fanger and Barker (1961) found that arterioles were first seen near the myometrial junction at 2 weeks of age and on up in the endometrium to near the surface by 3 weeks of age. The mucosal twigs formed periglandular and sub-
epithelial networks according to Trautmann and Fiebiger (1957) and the veins lacked valves. During postestrus and postpartum the elastic connective tissue of the blood vessels increased noticeably (Duke, 1945). In the endothelial cells of arterioles found in the rat myometrium, Röhlich and Olah (1967) found cross-striated fibrils which they considered to be contractile units and called them myoendothelial cells. Trautmann and Fiebiger found in animals that had gone through a pregnancy that the arteries and veins exhibited cushion-like intimal thickenings and in all layers of the wall there was a great increase of elastic tissue, which formed blumps in old subjects. These were similar to the irreversible vascular changes that occurred in the human (Bloom and Fawcett, 1962) in which the vessels of the placental site showed irregular thickenings of the intima with neoformation of smooth muscle.

In the placental mesometrial arteries of the guinea pig subendothelial cells proliferated during the early stages of pregnancy and the internal elastic membrane was fragmenting (Albert, 1967). The elastic fibers were absent by term and cushions composed of several cell layers of subepithelial cells were present. These, however, disappeared by 22 days after parturition and a new internal elastic membrane had formed. Albert did not indicate whether any alterations were observed in the tunica media during this time. Bloom and Fawcett (1962) found that it was largely substituted by coarse elastic networks. Albert and Bhussry (1967) found that the distribution of elastic tissue and the number of muscle layers in the arterial walls was a general indication of the numbers of pregnancies up to 3, beyond which the only indication was that the animal was a multipara. They stated, however, that there was an increase in
elastin in the arterial walls with aging.

**Lymphatics** In the human histology texts (Arey, 1968; Copenhaver and Johnson, 1958; and Leeson and Leeson, 1966) it was stated that lymph vessels were abundant and formed plexuses throughout the layers of the uterus except in the superficial zone of the mucosa. Velardo (1958) described 2 sets of lymphatics in the human uterus; a superficial one, beneath the serosa and a deep one, throughout the outer layers of the uterus. In animals, lymphatics were numerous and their trunks lay in the stratum vascular (Trautmann and Fiebiger, 1957).

**Innervation** According to Trautmann and Fiebiger nerves of the animal uterus ended partly on the muscle fibers and partly in the mucosa. Leeson and Leeson (1966) indicated that in the human, myelinated nerve fibers entered the mucosa and formed a plexus beneath the epithelium, while non-myelinated nerve fibers supplied blood vessels and muscle bundles. No nerve cells were found inside the myometrium of the human uterus (Velardo, 1958; and Bloom and Fawcett, 1962).

**Age Changes**

According to Leathem (1958) the human uterus commenced to age with the first cycle and each succeeding cycle was a complete maturation process. He indicated that because estrogen favored an increase in fibrous connective tissue, the uterine changes became more marked with each cycle. After the menopause in the human according to Leathem atrophy began resulting in degeneration of the musculature and glandular components. Old hamsters showed a markedly reduced ability to develop deciduomata when compared to young animals (Blaha, 1964). There was a decline in litter size in aged mice possibly due to an unfavorable intrauterine environment.
Endometrium

In old dogs, endometrial hyperplasia was increased in incidence so that according to Bloom (1954) many dogs over 6 years of age showed some degree of hyperplasia. Kretzschmar and Stoddard (1964) found that in anovulation in the human, the endometrium was frequently thick, showed proliferative changes and as available estrogen decreased the endometrium became thinner. It eventually consisted of a thin surface layer and a thin dense stroma which contained a few small scattered tubular glands.

Epithelium  In old age, the surface epithelium of the endometrium consisted of a single layer of low cuboidal cells arranged in a fairly straight or slightly undulating line (Speert, 1949). According to Speert, only rarely was a connection observed between the glands and the surface epithelium.

Glands  Human postmenopausal endometrium were classified as senile, atrophic, cystic atrophic, and proliferative by Noer (1961) and Mansour and Baradi (1965). Bigelow (1958) described the senile atrophic as inactive and stated that it was classified primarily on the absence of epithelial and stromal mitosis. He indicated that the glandular epithelium was composed of narrow cells lined up in a single row around the lumen and the nuclei of these cells were generally hyperchromatic, elongated and occupied most of the cell cytoplasm. The stroma sometimes showed fibrosis according to Bigelow who indicated as did Kretzschmar and Stoddard that thinning of the endometrium sometimes occurred in older patients and then went on to explain that the glands tended to lose their vertical orientation to the surface and approached at an angle or even became
parallel to it. The cystic atrophic endometrium was like the above with the addition of cystically dilated glands lined by flattened epithelium (Bigelow, 1958), which gave a Swiss cheese pattern (Speert, 1949). The proliferative type or as Bigelow described it, the senescent proliferative was similar to the inactive type with the addition of mitotic figures in the epithelium or more rarely in the stroma. Foley and Reece (1953) observed only small to medium-sized uterine glands in most non-pregnant bovine except older animals in which they observed a few large glands which they felt were the result of age changes. Cystic glands were also reported in older bovine by Yamauchi (1964) and Mochow and Olds (1963, 1966).

**Myometrium and connective tissue**

In older dogs and cats over 10 years of age (Gilmore, 1965) found that leiomyomas were the most frequent tumor found and that they formed single or multiple masses in the wall of the uterus, usually near the cervix. Relatively few adenomas or adenosarcomas were found.

As the uterus of the hamster aged (6 to 15 months) there was a progressive increase in connective tissue between the muscle layers (Rolle and Charipper, 1949). Rolle and Charipper observed an apparent decrease in the circular smooth muscle in the 18 and 21-month old groups while the longitudinal muscle remained the same. They found that this phenomenon was more pronounced in the 24 month old specimen with the longitudinal muscle still well preserved. In the 24 month old hamster, the outer stroma of the uterus was very coarse, while the inner stroma had more connective tissue, but the fibers were finer. According to Schwalm and Dubrauszky (1966) the menopausal uterus had less muscle than
the premenopausal uterus, Kretzschmar and Stoddard (1964) found that atrophy of the myometrium progressed gradually in the human following cessation of ovulation before any significant changes occurred in the vagina or vulva. Uterine wall size decrease was due to fluid loss (Kretzschmar and Stoddard) as well as transformation into fibrous tissue (Bettzieche and Ebert, 1968).

A study carried out by Wolfe, et al. (1942) on the rat uterus from 5 to 323 days of age produced the following results which were summarized.

**Uteri of 5 to 7 day old rats** Only reticular connective tissue was found in the endometrium which was composed of radially arranged fine reticular fibers, that extended from the basement membrane of the epithelium to the inner border of the inner circular muscle layer. The muscle layers were not well differentiated. Arranged about the muscle cells were fine and often indefinite fibrils.

**Uteri of 15 day old rats** Thickening and transformation of the reticulum into collagen was observed. A narrow zone of collagen bordered the circular smooth muscle.

**Uteri of 30 to 60 day rats** An increase in replacement of reticulum by collagen was noted and the narrow zone of collagen observed in the 10 day rats was expanded to comprise 50 to 80 percent of the thickness of the endometrium. The serosal layer stroma, the trabecular stroma in the longitudinal muscle and the intermuscular stroma were collagenous.

**Uteri of 90 day old rats** In this group the zone of collagen occupied 75 to 95 percent of the endometrial thickness. The myometrium contained a well developed meshwork of reticulum about the smooth muscle fibers although in a few cases a transformation of the reticulum into
collagen was observed.

**Uteri of 180 to 360 day old rats** The major change in this group was a marked thickening of individual collagen fibers from 6 microns in the 180 day rats to 10 or 12 microns in the 360 day old specimens. Arrangement of the collagen fibers in the younger group consisted of a meshwork while in the latter group they had a broad fiber and sheetlike arrangement. Collagen continued to be deposited around the blood vessels of the vascular layer and formed a layer as wide as the circular muscle layer. The serosal layer also increased in collagen content so that it was 10 to 12 microns in thickness in the 360 day old rats.

**Uteri of 450 to 823 day old rats** There were maximum amounts of collagen present in all portions of the uterus. The only persisting reticulum of the endometrium was found in and just below the basement membrane. Collagen fibers were found either parallel to the circular muscle or forming concentric rings around the glands.

In the circular muscle layer the reticulum was either reduced or gone entirely and was substituted with collagen fibers up to 2 microns wide. Atrophy of the muscle cells was revealed by Masson preparations proportional to the degree of replacement of the reticulum with collagen. The process was so advanced in some cases that it was difficult to differentiate the circular smooth muscle from the endometrium. The longitudinal smooth muscle along with its reticulum was usually in a better state of preservation although there was some thickening of reticular fibrils and the collagenous trabeculae were usually thickened and numerous.

**Other investigations** Similar findings on the rat was published by Burack, et al. (1941) and Schaub (1964-1965). The latter in-
vestigator found that in the aging uterus of the rat the collagen content increased and its concentration became three fold. The amount of muscle tissue in the human uterus also decreased with advancing age (Sauramo, 1954).

In normal human uteri 3 age-related changes were observed (Woessner, 1963). The wet weight collagen and elastin contents increased to a maximum by age 30, remaining constant for the next 20 years and then declined to levels of about one-half the maximum values in the period 50 to 65 years. He also found that although uterine collagen was completely digested by bacterial collagenase at all ages the rate of digestion was much slower on the older collagen.

**Vascular changes**

Investigations on the porcine endometrium by Bal (1968) revealed that in 8 year old specimens excessive elastic tissue rendered it difficult to delineate the intima from the media. Bal indicated that the larger arteries of the mesometrium had more prominently thickened intima than was found in younger animals. As to the increase in elastic tissue, Moschow and Olds (1966) reported that part of the increase in elastosis was due to increasing numbers of pregnancies in older bovine. Speert (1949) observed that the characteristic coiled vessels of the human endometrium atrophied promptly after the menopause, a phenomenon probably related to the diminution in circulating estrogen. According to Maher (1959) the entire circumference of the arteries was affected uniformly and smooth muscle of the media was atrophic. This was differentiated from post-partum changes as the latter muscles were not atrophic, the lumen was often eccentric and excess elastic tissue was removed 25 to 30 days
postpartum (Maher, 1959). Some of the larger vessels of the vascular layer and even the main arteries supplying the old uterus underwent at least to some degree the "tanning" process described by LaBella and Lindsey (1963) for aging of aortic elastin. In this process a fluorescent substance accumulated in aortic elastin with age.

In the senile human uteri Speert (1949) and Noer (1961) reported that thin-walled veins filled with blood coursed through extremely superficial layers of the endometrium, often separated from the uterine cavity by only one or 2 layers of cells. He suggested that possibility of hemorrhage if the veins were in the area of a ruptured cyst.

Cervix

Although it was realized that the cervix, as Leeson and Leeson (1966) phrased it, was the lowest segment of the uterus, it was felt there were enough differences in morphology to warrant a separate section on this structure. The cervix was a sphincter muscle between the uterus and vagina. Nalbandov (1958) observed that it varied anatomically in different mammals. Nalbandov stated that in most mammals the lumen of the cervix was interrupted by transverse interlocking ridges or annular rings which were developed to different degrees of prominence. The mucosa was thrown into high folds according to Trautman and Fiebiger (1957) who indicated that the epithelium contained mucinuous cells. The muscularis of the cervix was rich in dense fibrous tissue, smooth muscle cells, collagen and elastic tissue (Nalbandov, 1958).

Embryology and development

The excavation of the Mullerian cords in the cranio-caudal direction to form a true tubule was discussed in the sections covering embryology of
the oviducts and uterus. This proceeded throughout the length of the cord including its fused caudal end (Allen, 1958). Allen indicated that early in the development, the lumina of the fused caudal ends were separated by a septum which broke down about the eighth week in the human and formed the uterovaginal cavity. In the 17 mm human embryo Davies and Kusama (1962) found that the single canal of the fused Mullerian ducts was lined by stratified columnar epithelium with nuclei oriented at right angles to the basement membrane. Some years ago, Fleming (1926-1927) reported that in the 15 cm human embryo the future cervix had a thicker wall than the uterus and was lined by a single layer of deep staining epithelial cells with large nuclei. No muscle fibers were present in the cervical wall. About this time (4 months development) in the human according to Arey, (1965) the fornices of the cervix appeared. Davies and Kusama found that in the 22 week old specimen the stratified squamous epithelium of the vagina extended deeply into the endocervical canal and ceased abruptly about its midpoint. They found that the smooth muscle was well differentiated in the body of the uterus but was absent in the wall of the cervix which contained a vascular, highly cellular mesoderm. At 30 weeks in the human fetus Davies and Kusama (1962) found that the junction between the stratified squamous epithelium of the vagina and the tall columnar epithelium of the cervix was lateral to the external os. They observed that in most areas the epithelium was pseudo-stratified, heavily mucified and rested on a basement membrane. At term the cervix was hypertrophied, took up two thirds of the total length of the uterus and was lined with stratified or pseudostratified columnar epithelium with irregularly mucified superficial layers (Davies and Kusama).
These investigators also indicated that at term the cervical struma was highly vascular and densely infiltrated with lymphocytic leucocytes. The glandular epithelium varied from mucified simple columnar to stratified. In a 22 month old child Davies and Kusama (1962) found that the cervical epithelium appeared quiescent, was lined with low columnar, non-mucified columnar epithelium and lacked a deep layer of clear cells that was conspicuous at term. In the calf the columnar epithelial lining cells were shorter than they were in the mature animal (Delonoschkin, 1946).

**Physiology**

According to Nalbandov (1958) the main function of the cervix was to protect the uterine lumen from microscopic and macroscopic intruders. He indicated that it was closed at all times except during parturition. Soranus, (Temkin, 1956) recorded in the second century, that the cervix opened at certain times as in 1) the orgasm of coitus to receive semen, 2) during menstruation that the blood may escape, 3) in pregnancy according to the growth of the embryo, and 4) in labor to the greatest extent until it would admit the full sized hand. The mucosa of the cervix did not take part in cyclic changes according to Bloom and Fawcett (1962) although an increase in cervical mucous was described by Papanicolaou (1954). Copenhaver and Johnson (1958) indicated that during pregnancy the cervical glands become more extensive and secreted copious amounts of mucus that hardened to form a cervical plug (Nalbandov, 1958). Just before parturition this plug liquified, probably under hormonal influence and the cervix relaxed. At parturition Guyton (1961) indicated that stretch or irritation of the cervix was very important in eliciting uterine contractions so that birth could be accomplished.
Histology

The mucosa of the canine cervix according to Harrop (1960) was thrown into high primary folds that bore secondary folds. Tubular glands were contained in the lamina propria which was composed of collagen and elastic fibers. Out from this was the inner circular and outer longitudinal muscle layers (Harrop). Structurally the mucosa of the bovine cervix was thrown into many folds that had a central core of cell-poor connective tissue and were lined with a single layer of mucoid epithelium that formed simple sacculated and/or branched tubular glands (Roark and Herman, 1950). It was found that not all investigators agreed with the statement of Bloom and Fawcett (1962) that the cervical mucosa did not take part in cyclic changes. Cole (1930) and Casida and McKenzie (1932) described cyclic changes in the cervix of the cow and the ewe respectively. In 1951, Herrick described cytological changes in the bovine cervix during the estrous cycle. Any cyclic changes that were found in the literature were described along with the histological description of the cervix.

Epithelium

The lining of the canine cervix contained tall mucus-secreting cells (Harrop, 1960). In the human the endocervical epithelium was composed of 3 kinds of cells according to Laguens, et al. (1967), mucous columnar, ciliated and reserve cells. In the more caudal portions of the cervix the epithelium was more stratified. Krantz and Phillips (1962) in describing the endocervical canal observed that this canal was lined with tall columnar cells, some of which were ciliated. Novak (1940) called them "picket cells" because of their uniform, single row
arrangement resembled a picket fence. Their nuclei were round or oval, basally located and the cytoplasm showed irregular vacuolization. The epithelium of the portio vaginalis was stratified squamous, contained varying amounts of glycogen and had 5 zones, 1) the basal cell layer composed of a single row of cuboidal-like cells with large dark staining nuclei and a high nuclear-cytoplasmic ratio, 2) the parabasal layer which contained a varying number of layers of polyhedral cells with large dark nuclei and cytoplasmic borders along which intercellular bridges were seen, 3) the intermediate cell layer which showed some flattening of the cells which had glycogen rich, frequently vacuolated, clear cytoplasm and light vesicular nuclei, 4) intraepithelial layer which was not well defined in the cornified type, but was distinct in the keratinized type with its closely packed, dark-staining cells with keratohyaline granules and 5) the stratum corneum, or functionalis which was composed of several layers of flat elongated cells with small pyknotic nuclei. According to Krantz and Phillips (1962) the thickness of this zone depended upon the phase of the cycle. They indicate that the line of junction between the two epithelial types varied from 1 to 10 mm, in width and call it the squamocolumnar transitional zone.

_Proestral_ In the bovine Cole (1930) reported that the cervical epithelium was very deep during proestrus, was composed of 1 to 5 layers, with 1 layer in the body of the cervical glands and 2 or 3 layers over most of the area. It increased slightly in depth according to Roark and Hanman (1950) and the cells changed from cuboidal to columnar. Cole observed that the superficial layer consisted of large, wide goblet cells between which were the nuclei of other goblet cells or interpolated cells
that were compressed and only occasionally extended to the surface. Mucicarmine staining showed that the cells started secreting mucus at this time (Roark and Herman, 1950).

Estral The tall mucus secreting cells of the canine cervical epithelium became active at estrous (Harrington, 1960). Cole (1930) found that the epithelium of the bovine cervix near the external annular fold was composed of a single layer of cells during estrous. The cells were described as tall columnar, with basally crowded nuclei that were arranged with their long axis perpendicular to the basement membrane (Roark and Herman). They were 15 to 24 microns in height, with 10 to 16 microns staining intensely with mucicarmine. In man O'Brien, et al. (1964) stated that the cervix had a filtering effect so that only healthy active sperm ascended and that the inflammatory effects of cervicitis appeared to regress toward ovulation so that sperm migration was not hindered.

Postestral Inasmuch as information regarding cyclic changes was not found for the canine other than the fact that the canal was open at this time (Andersen and Wooten, 1959), postestrum rather than metestrum was used to illustrate this phase for the bovine. At one day postestrum, Cole (1930) found a reduction in epithelial height with the cell nuclei oval and basal instead of elongated as during estrous, and the cell width at the base and apex was more uniform than during estrous. Roark and Herman (1950) found at 2 days postestrus in the bovine a superficial epithelium varying from tall columnar with crowded, basal and elongate nuclei in some areas to cuboidal, with oval, less basally located nuclei. They agreed with Cole that about 10 days postestrus, the epithelial cells were greatly reduced (12 to 20 microns in height) and
indicated that they stained only lightly at the periphery with mucicarmine, while in the deeper crypts, some of the cells were still columnar and stained intensely with mucicarmine. Cole (1930) found that the periphery of the superficial cells stained intensely with hematoxylin and that the free surface of the cells were more irregular than at estrous and gave a ragged appearance. In the ovine, sections taken from the midregion of the cervix showed some evidence of secretion 8 days postestrous and intense secretion 10 and 13 days postestrous, with very little evidence of secretion at other times, (Casida and McKenzie, 1932).

According to Reid (1966) the human cervix was partially everted during pregnancy and during the first pregnancy the most prominent portions of the everted columnar epithelium underwent squamous transformation immediately after contact with vaginal secretions. He indicated that in subsequent pregnancies a lesser degree of eversion occurred and that the everted mucosa was covered with mature squamous epithelium. Once instigated the columnar-squamous change was autochthonous and stable, with no to and fro movement evident (Reid, 1966).

During pregnancy, in the bovine, the cervical epithelium before the eighty-fifth day was similar to that observed 10 days postestrum, varied from cuboidal to columnar and was deeply stained with hematoxylin (Cole, 1930). From the eighty-fifth day to the end of pregnancy the superficial cells were taller and more active, although not secreting at a constant rate. Just prior to parturition in the sow according to Palmer (1965) the epithelial cells were tall columnar, measured 25 to 30 microns in height, contained large vesicular basal nuclei and aldehyde-fuchsin-positive distal cytoplasm in the non-ciliated cells. These findings
indicated the presence of mucoprotein-like material. Palmer (1965) found that one to 3 days after farrowing the epithelium resembled that of the prepartum structure. After the third day a gradual decrease in cell height was observed so that by 21 days the epithelium had decreased to 15 microns and was low columnar to pseudostratified in appearance. According to Palmer many vacuolated cells with pyknotic nuclei were found near the basement membrane and neutrophils and eosinophils which were numerous up to 14 days postpartum had decreased by 21 days. From 21 days postpartum until 21 to 28 days postweaning the cervical epithelium remained essentially unchanged and then the epithelium increased in height to a histological appearance nearly identical with that observed 1 and 3 days after farrowing.

Krantz and Phillips (1962) felt that changes in the human cervical epithelium appeared to be a matter of increased incidence rather than specific gestational changes and that any changes described could have been observed in the non-pregnant state.

Membrana propria

Dougherty (1961) and Warren, et al. (1966) indicated that a basement membrane was not demonstrated with consistency beneath the cervical epithelium in the human. Of six human specimens studied by Lamb, et al. (1960) one had no P.A.S. positive membrane, 3 showed a discontinuous membrane of variable thickness and 2 showed a continuous membrane. Song (1963) reported that the basement membrane of the human cervix first became demonstrable in the embryo at about the twenty-fifth day of gestation, and was a thin permeable structure composed of collagen
fibrils through which stromal and epithelial cells migrated. The P.A.S.
positive basement membrane observed under light microscopy was 0.4 to 0.5
microns thick, was composed of connective tissue especially reticular
fibers and failed to show up after incubation with collagenase. (Younes,
et al. 1965).

Lamina propria

According to Trautmann and Fiebiger (1957) only in carnivores did the
cervical mucosa contain glands to the level of the external os. Harrop
(1950) called them tubular glands. Roark and Herman (1950) indicated
that the plica of the bovine cervix formed simple sacculated and/or
branched tubular glands. During proestrous and estrous they indicated
in agreement with Cole (1930) that the stroma was loose, cell-poor and
slightly edematous. At one day postestrous Cole found that the stroma
became more dense and the blood vessels less prominent or less congested
than they were during estrous (Roark and Herman). The former investigator
found that 8 to 11 days postestrous the stroma became more dense and
showed no edema. He indicated that this appearance was also observed for
the first 85 days of gestation at which time the lumens of the glands
appeared filled with mucus for the first time. This mucus continued to
accumulate until the gland lumens become spherical or oval instead of
tubular and branched until on day 240 of pregnancy the branches were re-
presented by folds protruding into lumen of the gland (Cole, 1930). On
day 254 the glands contained no mucus and began to recede to this ori-
ginal form according to Cole.

In the human, Fluhmann (1957, 1958) indicated that there were no
compound tubular racemose glands in the cervix but that the extensive
folding of the mucosa gave a glandular appearance and the epithelium of
the cervical glands or clefts was identical with that of the surface.
Trautmann and Flebiger (1957) gave a similar description to the mucosal
foldings for domestic animals. Krantz and Phillips (1962) felt that in
the human cervix, no consistent cyclic changes were observed in the lamina
propria during pregnancy. Other investigators, Buckingham, et al. (1962)
found that in the non-pregnant human cervix the collagen appeared as dense,
tightly woven bundles of interlacing fibers, and that reticulin fibers
appeared as short segments irregularly dispersed or clumped in haphazard
relation to collagen bundles. In the cervix of pregnancy, the reticulin
fibers were more robust and frequently tended to parallel collagen fibers.
Buckingham, et al., explained that by this characteristic difference in
reticulin fibers it was possible to distinguish the pregnant from the non-
pregnant cervix. They reported that in late pregnancy the collagen appear-
ance was similar to that of the non-pregnant state except for a loosening
and appearance of clear spaces between the bundles that becomes more pro-
nounced after delivery. The explanation given was that the loosening was
due to dissociation of the fibers into their fibrillar components and that
the fibers ran in all directions. Reticulin fibers after delivery were
scattered and thin.

Lymphocytes were sometimes found in the cervical stroma of the bovine
(Cole, 1930). Demetrakopoulos and Green (1958) indicated that well circum-
scribed, round or oval follicles with primitive reticular and reticulo-
endothelial cells were found directly beneath the cervical epithelium or
deeper in the stroma of the human cervix. They concluded that lymph fol-
licles were not congenital, but appeared after birth, were usually associ-
ated with and probably resulted from chronic inflammation. Argyrophil cells were occasionally found in the cervix, but not in other areas of the female genital tract (Fox, et al. 1964).

Muscularis

Trautmann and Fiebiger (1957) stated that in the bitch where the vaginal portion of the cervix was fused to the dorsal wall, the inner longitudinal and circular layers of the vaginal muscle formed a loop around the external uterine orifice. The cervical muscularis in domestic animals according to Trautman and Fiebiger was rich in elastic fibers. In the cow and mare the circular muscle formed the body of the portio vaginalis. The prominent middle muscle layer of the uterus was practically absent in the cervix of the cow, sheep, pig, horse and human (Tourris, et al., 1963).

Tourris, et al. (1964) indicated that a lamellar muscular structure, separated by abundant "cotton-like" connective tissue stemmed from the external muscle layer of the uterus and vagina passed in an oblique direction from the external to the internal layer. They observed that the internal muscle layer of the cervixes of these species and man was poorly developed. Both Lierse (1960) and Krantz and Phillips (1962) described this inner layer as a longitudinal one. In the human the cervix was composed of predominantly fibrous connective tissue with some smooth muscle and some elastic connective tissue (Danforth, 1947 and Danforth and Chapman, 1950). Korte (1965) described 2 layers of muscle, a wide inner layer with an interwoven basket pattern diagonally directed and a narrow outer longitudinal layer. According to Krantz and Phillips (1962) the transition of the myometrium to the fibrous tissue of the cervix varied from abrupt to gradual, over a space of 5 to 10 mm. They indicated that at the internal
os there was a condensation of spiral smooth muscle (50 to 60 percent muscle) as a sphincter, while the middle portion of the cervix contained the terminal fibers of the central longitudinal uterine muscle and in the distal portion the portio vaginalis was devoid of muscle. The muscular lamellae were considered by Tourris, et al. (1963, 1964) to have played an active role in dilation of the cervix.

Vascularization and innervation

Blood vessels Both the endocervix and exocervix had a rich capillary vasculature according to Fanger and Barker (1963) who indicated that occasionally there was an extension of capillary loops into the subepithelial papillae. D'Ablaing and Mihato (1965) described the cervical vasculature in the human as a single to complex arcade with loops of variable tortuosity and complexity. Both sets of investigators indicated that there was no significant difference in the capillary pattern with respect to age, race, marital status or number of pregnancies. In both the aorta and peripheral vasculature of the pregnant animal, Danforth and Buckingham (1964) found decreased collagen, fragments, reticulum, alterations in elastic fibers and decreased acid mucopolysaccharide and concentration.

Innervation Krantz and Phillips (1962) indicated that the nerves supplying the cervix entered the uterus with the blood vessels and that the nerve fibers ran parallel to muscle bundles and frequently branched to form a syncytium before termination on the sarcoplasm as small free nerve endings. In the cervical area, they found occasional free nerve endings between the muscle fibers and a plexus supplying the blood vessels of the cervix beneath the epithelium. The endocervix and isthmis portion of the non-pregnant uterus according to Krantz and Phillips both contained the
highest number of nerves and blood vessels found in any part of the uterus.

Age changes

Epithelium

Although no mention was made of age changes in the epithelium of the cervix Wolfe, et al. (1942) reported that the stratified epithelium in the vertical portion of the rat uterus resembled that of the vagina, and that in the cervical canals there was a progressive decrease in thickness from caudal to cranial. They found cyclic changes occurred similar to those of the vagina. In the human Sauramo (1952) found that the epithelium underwent atrophy with increasing age. Retention cysts not only increased in number with age but grew deeper into the tissue and were larger in older specimens.

Lamina propria

Burack, et al. (1941) found that the connective tissue of the immature rat cervix was usually a closely interwoven collagenous meshwork, in which a number of fibroblasts containing large vesicular nuclei were embedded and indicated that an increase in connective tissue density was observed with advancing age. In the uterine portion of the cervix Wolfe, et al. (1942) reported that these changes were identical to those of the uterus in which the collagenous zone of the older specimens nearly replaced the reticular tissue. The increase in amount and density of the collagenous tissue was accelerated to the greatest extent in non-breeding rats to the extent that cervices of one year old virgins were larger than those of breeders of the same age which caused the outer lips of the cervical canals to project into the vagina (Burack, et al., 1941).
Muscularis

The inner longitudinal smooth muscle of the rat cervix according to Wolfe, et al. (1942) which appeared at 30 days of age was relatively thin in all age groups, in comparison with the circular muscle. In young rats the smooth muscle of the cervical lips was penetrated by narrow collagen fibers and as the animals aged there was an increase in the dilution of muscle by collagen fibers, which was always most advanced in the cervical labia. These investigators found that in markedly hypertrophied cervices of older rats the smooth muscles of this region were broken up into small aggregates of as few as 2 or 3 muscle cells, by collagen fibers up to 5 microns thick and that in part the reticulum about the muscle cells was apparently transformed into collagen to bring this about. In the very old rats there was often a complete disappearance of reticulum with atrophy of smooth muscle cells according to Wolfe, et al. (1942) who were of the opinion that a persistence of reticulum was necessary for the survival of muscle fibers.

Vasculature

With regard to small muscular arteries in general, and including a variety of species Detweiler and Luginbühl (1967) indicated that smooth muscle cells often formed the chief component of intimal thickenings along with varying amounts of elastic fibers, reticulin and sparse collagen fibers. They further stated that in some cases only a portion of the intima was thickened, whereas in others it was uniformly thickened all the way around. Often the distinction between the intima and media was lost though, due to disintegration of the internal elastic membrane. In other cases in canine coronary arteries the internal elastic membrane was frag-
mented and often was not demonstrable with elastic stains (Luginbühl, et al. (1965). They indicated that there was no significant increase of elastic fibers in these vessels.

Sauramo (1952) indicated that in the human arteriosclerosis in the cervical arteries increased with age. Subjects aged 61 and 73 years showed as well an increase in the numbers of capillaries in the cervical stroma which Sauramo felt was a possible cause of bleeding.

Vagina

The canine vagina was defined by Christensen (1964) as a musculo-membraneous highly dilatable canal that extended from the uterus to the vulva. Nalbandov (1958) indicated that the vestibule (normally considered as part of the vulva) was the caudal part of the vagina. According to Trautmann and Fiebiger (1957) the mucosa-submucosa, muscularis and serosa (cranially) or adventia (caudally) were the component parts of the mammalian vagina. In the human Arey (1968) described transverse folds in the mucosa.

Embryology and development

The most caudal portion of the fused Mullerian ducts represented the primitive vagina, the epithelium of which began as a solid column (Arey, 1965). Arey stated that the muscular wall of the vagina (as well as the rest of the tract) was forshadowed at 3 months by mesenchyme condensing about the primitive vaginal epithelial column which excavated at about 5 months gestation in the human and developed a stratified epithelial lining. At about mid-term in the ovine according to Bulmar (1964) the columnar Mullerian epithelium at the caudal end of the upper vaginal segment became converted into a stratified polygonal form similar to the epithelium of the
urogenital sinus. He concluded that the epithelia of Mullerian origin exhibited either a stratified squamous or a columnar form depending on their site in the genital tract and influences acting upon them. Wells (1959–1960) observed, however, that eventually all of the vaginal epithelium disappeared and was replaced by new epithelium which migrated cranially from the cloacal region. At birth in the mouse Forsberg (1965) found that the Mullerian vaginal epithelium was pseudostratified columnar and was differentiated from the vaginal epithelium derived from the urogenital sinus. Forsberg observed that 3 days after birth the pseudostratified columnar epithelium of the Mullerian vagina divided into superficial and basal zones resulting ultimately in the disappearance of the border between the urogenital sinus and the Mullerian vagina. Thus, in the mouse Forsberg found that the epithelium had dual origin, the posterior portion from the epithelium of the urogenital sinus and the anterior part from the Mullerian epithelium.

**Physiology**

The vagina, the female organ of copulation, served as a receptacle for spermatozoa and was the passageway for the conceptus (Velardo, 1958). Trautmann and Fiebiger (1957) indicated that the nonglandular mucosa of the vagina was composed primarily of longitudinal folds, some circular folds and bore a stratified epithelium whose appearance was altered to varying degrees in the different species by the estrous cycle. The vaginal changes reflected the physiological events of the estrous cycle that occurred in the ovary (Nalbandov, 1958).

According to Trautman and Fiebiger the epithelium was thickened during proestrous and estrous in all species. The bleeding during proestrous
in the canine was thought to originate from the uterus by diapedesis and subepithelial hemorrhage (Venkke, 1967). Donovan (1968) found however that after performing hysterectomies and taking care to leave one of the ovaries intact the animals still cycled and the bleeding was still present during proestrus. His conclusion was that with the uterus and cervix removed it must have originated from the vagina.

In most species the surface layers were stratified squamous and they were also cornified during estrous in carnivores due to the action of estrogen (Trautmann and Fiebiger, 1957). After the rapid proliferation of proestrus in the bitch according to Eckstein and Zuckerman (1956) the epithelium was transformed into a high stratified squamous lining. By the time the bitch first accepted the male the vagina was lined with 12 to 20 layers of low stratified squamous cells. According to Witiak (1967) only cornified cells and erythrocytes were found on a vaginal smear at estrous. Eckstein and Zuckerman found that desquamation of the superficial layers also took place throughout proestrus and estrous eventually resulting in a marked thinning of the mucosa.

Twenty-four hours after the last acceptance of the male, leucocytes rapidly infiltrated the vaginal epithelium and migrated into the lumen of the vagina according to Eckstein and Zuckerman (1956) and Witiak (1967). Trautmann and Fiebiger (1957) stated that the leucocytes appeared during heat, and agreed with Eckstein and Zuckerman that they were present throughout metestrus or pregnancy.

Eight to 10 days after estrous Eckstein and Zuckerman found that the canine vaginal epithelium changed from squamous to columnar. They stated that the columnar epithelium was typical of metestrus and Nalbandov (1958)
indicated that low cuboidal epithelium was typical of anestrous.

Vaginal smear techniques and their interpretation were described thoroughly for the dog by Schutte (1967) and for the human by Papanicolaou (1954) so they were not reviewed here. It was of interest to note, however, that in the human newborn neither leucocytes nor flora were present (Wied and Keebler, 1967).

**Histology**

The morphological picture of cyclic epithelial changes as discussed above were reviewed as well as the histological structure of the membrana propria, lamina propria, muscularis, adventitia and serosa, vascularization and innervation.

**Epithelium**

With the exception of the cow, Trautmann and Fiebiger (1957) indicated that the vaginal epithelium was composed of stratified squamous epithelium. Mulligan (1942) observed that in the immature bitch the vagina was lined by two layered epithelium, the outer layer had the appearance of slight pseudostratification due to the indistinct borders of, 1) cells with pale round nuclei in their mid-portions and abundant acidophilic cytoplasm and 2) the basal layer of cuboidal cells which had scanty cytoplasm and dense oval nuclei oriented perpendicularly to the basement membrane. Vaginal epithelium in the human was thicker than that of the cervix with more numerous and taller papillae penetrating the associated connective tissues (Velardo, 1958). There were three main layers in the human vagina according to Krantz (1959), 1) a basal layer, 2) an intermediate layer as described above for the immature dog, plus 3) a superficial layer of cornified cells with pyknotic nuclei, Velardo observed that the superficial
cells were flat and contained granules but did not become truly cornified in the human female as they did in lower mammals. Papanicolaou (1954) described 5 zones of cells for the human vagina, which briefly were: 1) a deep basal zone of cuboidal cells, usually found only in the ectocervix; 2) a parabasal zone composed of several layers of polyhedral cells with large nuclei and intercellular bridges; 3) an intermediate zone consisting of several layers of moderately flattened cells, also connected by intercellular bridges and sometimes referred to as navicular cells; 4) a zone corresponding to the stratum granulosum of the epidermis and the cells of which contained distinct keratohyalin granules and 5) the outer zone which consisted of several layers of flattened cells which appeared elongated in cross-section and contain small pyknotic nuclei.

**Proestral** Rapid proliferation of the epithelium in the bitch according to Eckstein and Zuckerman (1956) transformed it into a high stratified squamous lining with a well marked basal layer, a parabasal layer and a superficial cornified layer. Papilla from the basal layer extended down into the lamina propria (Andersen and Wooten, 1959). Mulligan (1942) explained that during proestrous in the canine the vaginal epithelium reached its greatest development. He observed that the basal cells 1) increased in size and number, 2) that their nuclei became pale and rounded, 3) that their cytoplasm became more abundant and 4) that mitotic figures were readily observed. According to Laguchev (1965), this change in mitotic activity was statistically significant and was the first rise of the normal estrous cycle. Mulligan stated that intercellular edema was found in the next 3 to 4 layers of cells and included cells with a stubby spindle shape and nuclei oriented parallel to the
surface. The outer stratum 8 to 10 layers in thickness showed flattened cells many of which were being sloughed from the surface. These, with many erythrocytes were seen on vaginal smears.

Cole (1930) found that in the bovine there was a variation in the epithelium of proestrous in different parts of the vagina. During proestrous in the bovine Roark and Herman (1950) found that the epithelium consisted of small, compact deeply staining cells and that small cysts or epithelial follicles, often filled with lymphocytes were seen. They stated that inasmuch as the surface cells were polyhedral in character the epithelium was stratified but not squamous.

Estral In estrous, the stratified squamous epithelium of the canine vagina sloughed away (Mulligan, 1942). This sloughing was described by Eckstein and Zuckerman (1956) resulted in a thinning of the canine epithelium as estrous progressed. Very little exfoliation of cells was observed in the sow during estrous according to McKenzie (1926) who found that from early to late estrous the vaginal epithelium increased from 10 to 15 cells in thickness to 14 to 26. In addition to the epithelial proliferation, Done and Heard (1968) found crypt formation at this phase.

Roark and Herman (1950) also found an increase in epithelial height of the bovine vaginal epithelium during estrous of up to 54 microns in height as compared with 46 microns during proestrous. Ovine epithelial cyclic changes were said to be obscured by variation in thickness of the lining epithelium in different regions of the same animal and so were not worked out (Casida and McKenzie, 1932).

Metestrual By the first day of this period in the canine, the epithelium was only 3 to 6 layers thick and in the first 10 days, (begin-
ning 24 hours after the last acceptance of the male according to Eckstein and Zuckerman, 1956) it was infiltrated with leucocytes, chiefly neutrophiles (Mulligan, 1942). This infiltration subsided by 20 days mestrous and Mulligan found that by this time the epithelium showed only a basal cuboidal layer and an outer columnar layer.

In the bovine Cole (1930) found that the vaginal epithelium thickened so that by 8 days after estrous it was 140 microns thick. The resulting increase in depth was brought about by hypertrophy of the cells. He indicated that true cornification of the cells occurred at 9 days postestrous. Roark and Herman (1950) agreed with the observation regarding the increase in epithelial height but stated that the cornification was not observed. These authors then described cyclic changes in the bovine vagina 2 to 3 cm. caudal to the cervix, and indicated that the epithelium reached its peak in this region 2 days after estrous. In the sow McKenzie (1926) found that the vaginal epithelium decreased from the 14 to 26 cells found at estrous to 14 to 15 cells observed in mestrous.

Anestral Mulligan (1942) stated that the canine vaginal epithelium of anestrous was the same as that of the immature bitch. Eckstein and Zuckerman (1956) indicated that it consisted of 2 or 3 layers of low cuboidal or columnar cells.

Gestational At this time the cells of the outer columnar layer contained mucin (Mulligan, 1942). This was especially marked in late pregnancy in the canine. In the bovine Cole (1930) indicated that epithelium 2 to 4 cm. cranial to the external urethral orifice was low and flattened, although it sometimes assumed a low columnar form, and usually contained lymphocytes. One to 2 cm. caudal to the cervix Cole found that
the epithelium was often reduced to a single layer of deeply staining columnar cells that contained some mucus. In the sow it became 2 or 3 layers of regularly arranged cells, cuboidal at first, but later flattened with densely stained nuclei.

**Postparturient and lactational** In the bitch after whelping the mucin secretion disappeared and the epithelium returned to the state found at late metestrous or anestrous (Mulligan, 1942). A similar change was described by Roark and Herman (1950) for the bovine.

One day after farrowing, the vaginal epithelium of the sow was composed of 5 to 8 layers of cells with large vesicular nuclei in the basal layers of cells and deeply staining nuclei in the superficial cells (Palmer, 1965). Some neutrophils invaded the epithelial layer at this time. Palmer observed sloughing of the epithelial cells 3 and 7 days after parturition and by 14 days after the epithelium was reduced to 2 or 3 layers of cells. A heavy infiltration of neutrophils were often observed in clumps in the lacunae of the epithelial layer. No marked changes took place up to 45 days after farrowing according to Palmer who stated that the epithelium then increased to 6 or 8 cells in depth and remained in that state until 3 and 4 days after weaning. At this time it increased to 70 to 80 microns in height or 12 to 15 cells in depth. Neutrophils were still observed in the epithelium at this stage. Palmer (1965) found that the cytoplasm of the superficial cell layers had less affinity for stain than the basal cells and that they had pyknotic nuclei.

During the latter half of lactation in the mouse Greenwald (1958) found that the upper 2 or 3 layers of the vaginal epithelium became mucified. This continued through lactation and up until estrous.
Membrana propria

Mulligan (1942) mentioned that there was a basement membrane under­lying the vaginal epithelium of the bitch, but did not give any description. It was described as being most distinct in the sow a few days before es­trous by McKenzie (1926) who indicated that it became invisible in places after estrous and remained in this interrupted condition throughout the metestrous period.

In the human Novak (1940) stated that the membrana propria exhibited an irregular wavy outline. Pundel, et al. (1967) described a basement membrane in the human and indicated that it was composed of scleroproteins of the collagen type.

Lamina propria

In the canine the lamina propria was papillated according to Harrop (1960), consisted of a cell rich superficial region and a loose connective tissue deep region. The nonglandular vaginal mucosa of animals (Trautmann and Fiebiger, 1957) indicated exhibited primarily longitudinal and some circular folds which bore the stratified epithelium described earlier. They stated that the lamina propria formed little or no papillary body and that its connective tissue was rich in cells near the epithelium and contained lymph nodules. In the bovine the folds of the mucosa were very deep, especially near the cervix, were longitudinally disposed caudal to the urethral opening and contained diagonal or cross folds between the urethral opening and the cervix (Cole, 1930).

Ricci, et al. (1949) stated that in the human vagina, the lamina pro­pria contained fibrous tissue and was devoid of muscle cells. Fine and coarse elastic fibers surrounded the vessels. According to Velardo (1958)
it was formed by a network of areolar tissue and was penetrated at its outermost surface by a group of thin vascular channels which gave it the appearance of erectile tissue. He explained that this richly vascular layer rested upon the muscular wall and was often referred to as the submucosa, perhaps similar to the loose submucosa described by Krantz (1959) for the human vagina and Trautmann and Fiebiger (1957) for the vagina of domestic animals. Velardo (1958) indicated that numerous elastic fibers were found directly beneath the epithelium but were rare in other parts of the vaginal wall and stated that lymphoid tissue was often observed in the vaginal mucosa. According to Krantz the lamina propria of the vagina was thicker than that found in other organs and contained a thick network of collagen fibers with an interlacing network of elastic fibers. Ricci, et al. (1949) stated that there were no erectile tissue elements, no lymphatic nodules and no glands in the vaginal wall.

**Cyclic changes** During proestrus in the bovine Cole (1930) the stroma was loose, cell-poor and slightly edematous, and the small blood vessels of the superficial stroma were congested. Cole stated that there appeared to be more arterioles and capillaries than occurred during midcycle or pregnancy. Roark and Herman (1950) found that lymphocytes formed a rarely continuous layer subjacent to the basement membrane. These investigators agreed that the histological picture described above continued through estrous in the bovine.

At one day postestrum in the bovine Cole found the stroma to be more dense and fewer blood vessels were observed. While Roark and Herman agreed with the increase in stromal density, they stated that at 2 days postestrous the smaller blood vessels of the superficial stroma were more
congested than during estrous, and then on the next page stated that congestion was reduced during postestrous. At 8 to 11 days postestrum in the bovine (Cole, 1930) the stroma was more dense, edema was absent, the blood vessels were small and few in number. Cole indicated that lymphocytes and leucocytes were few in number at this time. Edema of the lamina propria was also least in the ovine about day 11 to 14 according to Casida and McKenzie (1932).

Especially in the postparturient canine cervix, lymph nodules were a common finding (Harrop, 1960). Following parturition in the bovine Roark and Herman (1950) found that the stroma was dense and fibrous and that stromal vascularization was slightly less than it was during the inter-estral period.

Muscularis

In the canine the tunica muscularis was composed of a very thin inner layer of longitudinal muscle, a thick circular layer and a thin outer longitudinal layer according to Christensen (1964) who stated that the inner longitudinal and the circular layers encircled the external cervical orifice while the outer longitudinal layer blended with the muscularis of the corpus uteri.

Ricci, et al. (1949) stated that there was no definite layer of muscle in the human vagina but that a few circular, oblique and longitudinal fibers were observed. According to Velardo (1958) and Krantz (1959) the muscularis of the human vagina was composed of an inner circular and an outer longitudinal layer and Krantz cautioned against confusing the striated bulbocavernosus and ischiocavernosus muscles with the inner circular muscle layer of the vagina.
Serosa and adventitia

The serosa covered only the cranial portion of the vagina and had its own smooth muscle which was continuous with that of the broad ligaments (Trautmann and Fiebiger, 1957). Velardo (1958) stated that the outer fibrous part of the vagina which was presumably the adventitia was made up of highly compacted areolar tissue that was well supplied with elastic fibers. The elastic tissue element which Ricci (1949) found was the most important component of the vaginal wall, followed the blood vessels as they approach the cervicovaginal area. As the vessels descended into the vagina elastic fibers diffused and intermingled with the fibrous and muscular elements.

Vascularization and innervation

Blood vessels Velardo (1958) found that the fibrous coat of the vagina contained a plexus of blood vessels and lymphatics. He stated that several branches from this plexus reached the muscular coat and the mucosa where they formed more well defined plexuses.

Innervation Peculiar sensory localities 3 to 5 mm. in diameter were plainly evident in the canine vaginal canal at estrous (Evans, 1928). Evans stated that they consist chiefly of cells whose long axis was perpendicular rather than parallel to the basement membrane. The mucosa according to Evans was entered by small cables of nerve fibers which subdivided and spun elaborate fibrillar plexuses about each epithelial element. Krantz (1959) occasionally found Vater-Pacinian-type corpuscles in the adventitia surrounding the vagina but more within the organ itself. An extensive nerve network, including spinal and sympathetic fibers, among which were many small ganglia were prominent in the
fibrous coat of the vagina (Velardo, 1958). Ganglia were described by Krantz (1959) along the lateral walls of the vagina adjacent to the blood vessels. Krantz explained that the wavy pattern of the nerves running along and in the vagina was thought to act as a protective device to the nerves during the distention of the vagina at parturition.

Age changes

Leathem (1958) indicated that in the postmenopausal human, the vagina became a short narrow organ. He found that the vaginal tissue also exhibited a loss of elasticity.

Epithelium

In the porcine Dal (1968) observed that cystic epithelial invaginations were a regular feature of the vagina of 6 year old sows. According to Leathem, the vaginal epithelium became modified in old age and Kretzschmar and Stoddard (1964) explained that there was a decrease in cornified cells resulting from lack of estrogenic stimulation. The latter authors stated that the inner walls of the vagina developed a dry glistening surface, and were easily lacerated and irritated.

At the first stage of menopause in the human Giacomini and Lucchini (1965) found that there was atrophy of the vaginal epithelium, then at 60 to 74 years of age a new epithelial proliferation occurred. Later on at about 70 years of age he found atrophy of the mucosa again. Merker (1965) found that multivesicular inclusion bodies occurred sporadically in the vaginal epithelium of old or pregnant rats.

Lamina propria

Leathem (1958) and Kretzschmar and Stoddard (1964) agreed that there was a loss of mucosal rugae with age in the human.
Wolfe, et al. (1942) found that reticular tissue which was so prominent in the lamina propria of the rat uterus was absent from the vagina. They indicated that in most areas collagen fibers fused together to form short papillae which were in direct contact with the basal cells of the vaginal epithelium. These papillae took on an argyrophyllic stain and due to their position, Wolfe, et al. thought that they possibly functioned as a counterpart to the basement membrane. Age changes according to these investigators consisted of an increase in thickness and density of the collagenous fibers of the mucosa associated with a decrease in cellularity which was also found in the aging hamster (Rolle and Charipper, 1949).

Wolfe, et al. found that in the mature rats the majority of the fibers were arranged in a circular or radial direction while in the older rats they were longitudinal or more obliquely oriented. The papillae became shorter and although in most of the old rats they retained their capacity for silver impregnation, in some they became collagenous.

Burack, et al. (1941) reported that in the rat differences in number and nature of fibroblasts in general characterized the young and old vagina. In the young, the cells were abundant and large with typical vesicular nuclei while in the old they were relatively few in proportion to the amount of collagen and appear predominantly flattened and shrunken.

Muscularis

Burack, et al. (1941) reported that in the rat differences in number and nature characterized the young and old vagina. In the young the cells were abundant and large with typical vesicular nuclei while in the old they were relatively few in proportion to the amount of collagenous tissue and appeared predominantly flattened and shrunken. In older rats Wolfe, et al,
(1942) found that there was a marked tendency for dense collagen fibers to penetrate the longitudinal smooth muscle and divide it into widely isolated units. They indicated that in some instances this resulted in a pronounced thickening of the vaginal wall.

**Vascularization**

Bal (1968) found that in the porcine, the vaginal arteries were not affected as severely as those of corresponding age groups in the cervix, uterus or oviduct. Intimal thickening was found in a few arterioles of the muscularis in the older specimens studied.

A specific change although not stated to be age related was the transformation of collagen fibers into elastin as described by Balo (1965). He indicated that both collagen and elastic fibers contained 2 mucopolysaccharide components one of which was a sheath mucoid and that on the molecular level the collagen and elastic fibers were analogous.
MATERIALS AND METHODS

All of the genital tissues used for this study were collected along with other tissues and organs as a part of an overall departmental gerontology project. This project since its inception over 11 years ago has been continually expanded to cover more and more facets of the aging phenomenon.

Dr. Getty has headed this project in the Department of Anatomy at Iowa State University over the years. The importance and necessity of an understanding of normal tissue changes, and the lack of such knowledge has been emphasized (Getty, 1966). Support was obtained for gerontological studies by grants from the National Institutes of Health and the Gaines Division of General Foods Corporation. Both canine and porcine tissues have been studied in the overall project. Only the canine, however, was used in this study. These animals were obtained from 2 sources, 1) from the purebred Beagle colony which has been maintained at the Department of Veterinary Anatomy since 1957 and 2) from Gaines Research Kennels at Saint Anne, Illinois. Tissues were systematically collected from these animals so that presently many tissues have been studied and many are available for study, covering a wide age range. With the completion in the future of all areas for which tissues are available it is then the goal to compare the age changes of the tissues and organs within each of the 2 species concerned and also to compare the age changes between the 2 species.

Historical Sketch of Animals

Tissues from 115 females of the canine species from birth (12 hours of age) to 19 years of age were used in this investigation. The environmental conditions under which the dogs had been raised was known.
these, 73 were raised on the purebred Beagle colony of the Department of Veterinary Anatomy, Iowa State University. Others including 10 Beagles and dogs from 14 other breeds were from the Gaines Research Kennels, Saint Anne, Illinois. Data on age, identification, breed, diet, body weight and the month tissues were taken was prepared in tubular form (Table 2). Abbreviations used in this table were prepared on Table 1.

The diet of the Anatomy Department dogs consisted of dry commercial dog food. Even prior to weaning the pups were started on this food and were allowed free access to it throughout their lives. Some of the dogs from the Gaines Kennels had been on the regular dry Gaines Dog Food, whereas others had been on various experimental diets. Records were maintained of all diets fed.

The general health of the colony was maintained by daily cleaning of the quarters with the use of hot water under pressure, by worming after fecal examinations showed the need, by routine innoculations for canine distemper and infectious canine hepatitis and by isolation from other animals.

Total body weight was recorded before tissues were collected. In most instances the dogs were killed by electrocution, using 2 spring electrodes and 110 volt current. However, when tissues were to be collected for electron microscope studies intravenous sodium pentabarbital was used. In either case the animals were then exsanguinated prior to tissue collection for lightmicroscopic studies.

1 Supplied by Gaines Dog Food Division, General Foods Company, Kankakee, Illinois.
Collection and Preparation of Specimens

The tissues were usually all collected within less than 30 minutes after the death of the animal. They were then fixed in buffered neutral formalin which was prepared as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde, 40 percent</td>
<td>100 ml.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>900 ml.</td>
</tr>
<tr>
<td>Sodium Phosphate, dibasic</td>
<td>7 gm.</td>
</tr>
<tr>
<td>Sodium Phosphate monobasic</td>
<td>4 gm.</td>
</tr>
</tbody>
</table>

The tissues collected from the female genital system included the ovaries, oviducts, uterus, cervix and vagina. Generally each ovary was carefully removed from the bursa, weighed and then put in formalin. In 3 cases the ovary, bursa and oviduct were removed and fixed intact for orientation. Usually only one oviduct was taken. A section from the middle one third of the right uterine horn was taken from each tract. The cervix was then removed as well as the cranial portion of the vagina. In some cases the other portions of the tract, other than those mentioned above were also fixed to aid in a more complete histological picture. On 24 of the animals multiple blocks of tissue, 2 or 3 sections from the oviduct and 3 sections respectively from the uterus, cervix and vagina were collected for this purpose.

Tissue processing

After fixation for a minimum of 48 hours the blocks were trimmed and then washed in tap water, dehydrated and cleared and infiltrated as indicated in this schedule:

<table>
<thead>
<tr>
<th>Component</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, 80 percent</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ethanol, 95 percent</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ethanol, 95 percent</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ethanol, absolute</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ethanol, absolute</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ethanol, absolute-xylene(equal parts)</td>
<td>2 hours</td>
</tr>
</tbody>
</table>
Then the tissues were embedded in routine manner and sectioned on a rotary microtome. Most of the sections were cut at 5 microns but on some of the older blocks it was necessary to section at 7 microns.

Staining

For the histomorphological observations 4 stains were used; 1) hematoxylin and eosin, 2) Verhoeff's elastic stain and Van Gieson counterstain, 3) silver impregnation for reticulin and 4) periodic acid Schiff (PAS).

Harris hematoxylin and eosin were used for general overview purposes and to compare with observations made by other investigators. In some cases measurements were made from these sections. Preparation of stains and staining were as follows:

Solutions used

Harris hematoxylin

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Alcohol, 95%</td>
<td>50.0 ml.</td>
</tr>
<tr>
<td>Aluminum Potassium sulfate</td>
<td>100.0 g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0 ml.</td>
</tr>
<tr>
<td>Mercuric oxide</td>
<td>2.5 g.</td>
</tr>
</tbody>
</table>

Dissolve the hematoxylin in the alcohol, the aluminum potassium sulfate in the water by heating. Mix the 2 solutions, bring mixture to a boil; remove from heat, add mercuric oxide. Reheat until it becomes a

---

dark purple (1 min). Solution is ready to use when cool.

**Acid alcohol**

<table>
<thead>
<tr>
<th>70% ETOH</th>
<th>1000 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>conc. HCl</td>
<td>10 cc.</td>
</tr>
</tbody>
</table>

**Lithium carbonate**

1 g. in 100 ml. dist. water

**Eosin**

stock solution--1 g. in 100 ml 95% ETOH
Dilute 1:1 stock solution and 95% ETOH for use

**Procedure used**

1. Xylene
2. Xylene
3. Abs. ETOH
4. 95% ETOH
5. Tap water
6. Harris Hematoxylin
7. Tap water
8. Acid Alcohol
9. Tap water
10. Distilled water
11. Lithium carbonate (1%) or sat. sodium bicarbonate
12. Distilled water
13. 5% Eosin with acetic acid
14. 95% ETOH
15. 95% ETOH
16. Abs. ETOH
17. Abs. ETOH
18. 1:1 Abs. ETOH : xylene
19. Xylene
20. Xylene
21. Mount

Verhoeff's elastic stain with Van Gieson counter stain aided in the differentiation of the collagen, elastic tissue and smooth muscle. Changes in amounts of these tissues and alterations in the tissues themselves were of prime importance in the aging phenomenon. This stain was also used to determine the presence of amyloid. Preparation of stains and staining were done as follows in partial accord with the report of Lillie, et al. (1967).
Solutions used

Verhoeff's elastic stain

5% fresh, unripened alcoholic hematoxylin... 10 ml.
10% fresh, aqueous Ferric chloride... 4 ml.
Lugol's Iodine
2 gm iodine
4 gm Potassium iodine
100 ml distilled water

Van Gieson's stain

1% acid fuchsin... 10 ml.
saturated Picric acid... 90 ml.
distilled water... 100 ml.
Boil 3 min. to ripen

Procedure used

1. Xylene... 5 min
2. Xylene... 5 min
3. Abs. ETOH... 3 min
4. Abs. ETOH... 3 min
5. 95% ETOH... 3 min
6. 70% ETOH... 3 min
7. Tap water... 3 min
8. Verhoeff's elastic stain (until black)... 15 min
9. Tap water... rinse
10. 2% FeCl₂ (until only elastic fibers and nuclei are stained)... 12 sec
11. Distilled water... rinse
12. Van Gieson's stain... 30-60 sec.
13. Distilled water... rinse
14. 95% ETOH... 1 min
15. Abs. ETOH... 1 min
16. Abs. ETOH... 1 min
17. 1:1 Abs. ETOH : xylene... 30 sec
18. Xylene... 2 min
19. Xylene... 10 min
20. Mount

To aid in the study of basement membranes, secretions and their changes with growth and aging the PAS stain was used as follows:
Solutions used

Schiff's Reagent (McNamus)

- basic fuchsin: 1 gm.
- sodium bisulfite: 1 gm.
- boil distilled water: 200 ml.

Add basic fuchsin and stir
Cool to 50°C
Filter
Add 1N HCl: 20 ml per 1000 ml volume
Cool to 25°C
Add sodium bisulfite
Store in dark until colorless (straw-colored)

Sulfurous acid rinse

- 10% sodium metabisulfite: 6 ml.
- 1N HCl: 5 ml.
- distilled water: 100 ml.

Procedure used

1. Xylene: 5 min
2. Xylene: 5 min
3. Abs. Ethanol: 3 min
4. 95% Ethanol: 3 min
5. 70% Ethanol: 3 min
6. 50% Ethanol: 3 min
7. Tap water: 5 min
8. 0.5% periodic acid for: 5 min
9. Distilled water rinse
10. Schiff's reagent: 15 min
11. Sulfurous acid rinse: 2 min
12. Sulfurous acid rinse: 2 min
13. Sulfurous acid rinse: 2 min
14. Running tap water: 5-10 min
15. Harris hematoxylin: 15 sec
16. Running tap water until blue
17. 0.1% light green: 15 sec
18. Distilled water rinse rinse
19. 95% Ethanol: 1 min
20. 95% Ethanol: 1 min
21. Abs. Ethanol: 1 min
22. Abs. Ethanol: 1 min
23. 1:1 Abs Ethanol : xylene: 30 sec
24. Xylene: 2 min
25. Xylene: 10 min
26. Mount
The silver impregnations for reticulin as outlined by Pearse (1961) was used to compare the reticulin basement membrane with the PAS basement membrane. It also aided in determining changes with age in the reticulin component of the tissues studied.

Measurements and Statistical Analysis

Collection of data for analysis of changes with age involved (Table 2); 1) the weight of the dog prior to tissue collection, 2) weight of the fresh ovary as soon as it was removed from the animal, 3) measurements with an eyepiece micrometer of epithelium and the various components of the uterine wall. Body and ovarian weights were made on all of the dogs whereas the detailed uterine cross-sectional measurements were made only on 7/4 from 12 hours to 13.1 years of age.

The data were then analyzed with the linear and curvilinear regression method on the IBM 360/50 computer, Computer Center, Iowa State University, Ames, Iowa.

Other data such as determination of collagen infiltration of the tunica media of vessels were obtained simply by observation and although given in percentages were not measured with instruments. Percentages were used to aid the reader in easily interpreting the relative infiltration of the tunica media and thickening of the tunica intima.

Age Groups

In order to evaluate the growth and age changes it was necessary to categorize the specimens into groups. The breaks were made primarily where major changes had occurred in the tissues.
Group | Canine | Comparative for man (Getty, 1967)  
--- | --- | ---  
Prematuration | Birth (12 hrs.) to 1.7 mo. | Birth to 4 yrs.  
I | 2.5 mo. to 11 mo. | 2.5 yrs. to 11 yrs.  
II |  
Postmaturation | 1.0 yrs. to 4.1 yrs. | 12 yrs. to 32 yrs.  
III | 6 yrs. to 10.4 yrs. | 40 yrs. to 57 yrs.  
IV | 11 yrs. to 19 yrs. | 60 yrs. to 92 yrs.  
V |  

The first group included those in early puppyhood when maternal hormonal influences were still evident on the genital tract and the time of early growth and differentiation of tissues. During the span of time covered by the second group growth and development of the ovaries and tubular tract as influenced by intrinsic hormones were evidenced. In this group functional maturity of the genitalia was achieved. Dogs from the third group covered a span from early adulthood to middle age. This was the time of greatest reproductive efficiency. The fourth group spanned the time which compared with middle age in man and the last group included those of old age. The oldest specimens studied were from a 19 year old, from which only ovaries and vagina were available, and the next oldest was a 13.1 year old from which all parts were studied.

Seventeen of the bitches from the Beagle colony had whelped at least one litter. Table 5 gives the diet of the bitch, her age when tissues were collected, the number of litters whelped, total number of pups, whelping span and the date that the tissues were taken.

The stage of estrous in the specimens was determined through histological appearance of the ovaries and various parts of the genital tracts. This was supplemented in some cases by known histories.

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OBSERVATIONS

Ovary

Statistical evaluation of weight changes with age

There was linear relationship (Graph 1) between the ovarian weights and age, and a curvilinear relationship between relative ovarian weights and age in the Prematuration Age Group (0.02 to 11.20 months). In the 12 hour old animal that weighed 0.4 kg, the combined weight of the ovaries was 0.15 gm. and the relative ovarian weight 0.38 gm. per kg. Mature weights were reached by 11.2 months. Maximum body weight, ovarian and relative ovarian weights were 11.1 kg, 2.22 gm and 0.49 gm per kg, respectively. In this group the ovarian weights did not increase as rapidly as did the body weight; although both increased as the animal matured. The relative ovarian weight showed a decline to 6 months of age and then increased up through the growth period.

In the Postmaturation Age Group (1 to 13.1 years) there was a linear relationship with a significant regression coefficient between ovarian weight, body weight and age (Graph 2). The increase was parallel, compared to the more rapid increase in body weight observed in the Prematuration, going from 4.6 kg, to 88.2 kg, body weight and 0.56 gm, to 9.42 gm, ovarian weight. No significant regression was found for the relative ovarian weights of the Postmaturation Age Group.

Germinal epithelium

It was found by using Mallory's triple connective tissue stain or Verhoeff's elastic tissue stain counterstained with Van Gieson's stain that the germinal epithelial cells were easily differentiated from the underlying tunica albuginea. In the age groups studied, the germinal epithelial
cells varied in cytoplasm content. Their nuclei varied in size, shape and
stainability. The nucleolus and chromatin granules were often not ob-
served, and even when present varied in their prominence.

Chronological appearance

Birth to 2 months of age In the ovary from the 12 hour old
specimen, the germinal epithelium was composed of flattened cuboidal cells
(Figure 2). By 2 days of age (Figure 5) the cells averaged 7 microns in
length by 3 microns in height in the hilus area, to typical cuboidal cells
5.5 by 3.5 microns high covering the remainder of the ovary. The nuclei
of these cells were large and comprised about 90 percent of the total cell.

At one week of age (Figure 6), the germinal epithelium varied from one to
2 layers in depth. Relatively little change was observed in the 2 week
old specimens. In some areas the epithelium was flattened but in general
it was cuboidal. By one month, however, the margin of the germinal epi-
thelium was slightly wrinkled whereas it had hitherto been smooth. It
was double layered in some areas although uniformly cuboidal. Also at this
age the nuclei appeared as smaller, more discrete structures, while in the
5 week old specimen they were again large and darkly stained.

Two and one half months of age to 11 months of age In the
2.5 month old specimen (Figure 8), the germinal epithelium was still low
cuboidal and varied from one to 3 cells in depth. By this time large
cells or clusters of cells with clear cytoplasm were observed in or im-
mEDIATELY deep to the epithelium. They were especially numerous in the
depressions and in areas where the epithelium was cuboidal, but absent
where it was flattened. Up to this time the nuclei had been round and
essentially centrally located, or oval and horizontally located. In the
2.75 month old specimen (Figure 10), low columnar cells were observed to make up portions of the germinal epithelium. They contained vertically oriented, centrally located, elongate nuclei. These cells measured from 4 to 8 microns in height while in other places the low cuboidal epithelial cells were approximately 7 microns long by 3 microns in height. Of the 3 month old specimens studied, one had a tall columnar germinal epithelium while in the other it was cuboidal. By 5 months (Figure 12), the epithelium was mostly cuboidal and in many areas 2 cells in depth. At 6 months the germinal epithelium was flattened, low cuboidal, but not squamous. Secretion-like material was observed at the apical end of many of the epithelial cells. Indentations in the surface of the ovary had become more and more prominent by 7 months. It was found that a double layered germinal epithelium was often found in the indentation areas. Some areas contained cuboidal cells, but most of them were columnar. The secretory-like material was a prominent feature of the epithelial cells of this age group. The germinal epithelium from 8 and 9.25 month old specimens (in early proestrus) varied from low cuboidal with pyknotic nuclei and scanty cytoplasm to double layered cuboidal or columnar in type. In these germinal epithelium cells the apical cytoplasm was uniformly darker staining than the basal. The epithelia of 10 month old specimens in metestrous were composed of low cuboidal to flattened cells with dark, round to oval nuclei.

Of the 11 month old animals, 3 were in late anestrous, one was in metestrous and one was pregnant. The germinal epithelium of those in anestrous was predominantly columnar and had nuclei that varied in stainability. In some areas 2 or more layers of cells were observed. In and
beneath the germinal epithelium of these were many large bi- and multi-
nuclear cells with clear cytoplasm (Figure 22). Columnar cells with
light staining nuclei and dark apical cytoplasm were found in the germinal
epithelium from the animal in metestrous.

One year of age to 19 years of age During this span, the
only observable changes in the appearance of the germinal epithelium cells,
were cyclic changes.

In proestrous the germinal epithelium was light staining, columnar and
contained many clear cells in or beneath the epithelium.

Those in the metestrous phase of the estrous cycle varied from one to
12 years of age. In 70 percent of the metestral ovaries the germinal
epithelial cells had moderately dark staining apical cytoplasm, light
staining basal cytoplasm, and dark staining nuclei (Figure 22). Most of
these cells were cuboidal in shape. The other 30 percent had light stain-
ing germinal epithelial cells, many of which were columnar.

Of those in various stages of anestrous, covering a range of 1 to 19
years, two thirds of the ovaries showed predominantly dark staining epi-
thelial cells that had extremely dark staining nuclei (Figure 36). Many
pyknotic nuclei were seen among these. The remainder of the ovaries had
light staining germinal epithelia.

Even though the changes with age of the germinal epithelium were
minimal, there was a gradual changes in the contour of the ovary. At one
year of age there were indentations (Figure 23). By 6 years of age the
ovaries were definitely lobed and depressions became more prominent
(Figures 27, 28). Most of the cellular activity (Figure 32) was in de-
pressions formed by the lebation; although occasional mitotic cells were
seen in the outer contours. At 9 years of age the lobes were even more noticeable (Figure 39), and the germinal epithelium itself had wrinkled and gave the appearance of an increase in cells to cover the same surface area. In general the nuclei of the cells on the elevated areas were apical and those in the depressions were basal. These wrinkles covered the ovary except for the areas stretched out by regressing corpora lutea. A typical simple low cuboidal germinal epithelium covered the latter.

**Tunica albuginea**

As in the case of studying the germinal epithelium the connective tissue stains were most important for this phase. In contrast to the cyclic dynamics of the germinal epithelium the changes in the tunica albuginea were more subtle, gradual, and more definitely related to changes with age.

**Chronological appearance**

**Birth to 2 months of age** The tunica albuginea in the ovaries from a 12 hour old and a 2 day old specimen consisted of a layer of fibroblasts that varied from one to 6 cells or 9 to 24 microns in depth (Figures 2, 5). The elongate nuclei of these cells were oriented parallel to the outer surface of the ovary except at the intervals where septate fingers were directed toward the medulla (Figure 1). These fingers of collagen fibers varied in density and formed a criss-cross pattern. Later in life they appeared to become incorporated with the tunica albuginea.

At a week of age this structure varied in depth from a few collagen fibers to about 2 cells or 15 microns, but was generally more dense than in the younger specimen. By 2 weeks of age the tunica albuginea varied from 8 to 21 microns in depth and the fibroblast nuclei were not as prom-
inent as in younger specimens. The whole ovarian cortex was divided into islands of cells by the branching criss-crossing bands of collagen fibers which extended from the tunica albuginea to the medulla (Figures 1, 3). The broad triangular bases of these bands were incorporated into the tunica albuginea thereby adding to its lack of uniformity in thickness. An increase of density of these collagen structures was observed in the 5 week old specimen in which the tunica albuginea averaged 22 to 24 microns in depth.

Two and one half months of age to 11 months of age Although an overall increase in density was observed in the outer connective tissue structure of 2.5 and 2.75 month old specimens, the most prominent feature was that the tunica albuginea was over twice as thick at the poles of the ovary (Figure 9), than on the other borders. In the 2.75 month old specimen it was 175 microns in depth on one end and 45 to 60 microns deep on the other borders. The tunica albuginea was disrupted by the invagination of the germinal epithelium that was beginning to take place at this age (Figure 8). A few small morula-like groups of epithelial cells that had become completely isolated from the germinal epithelium were observed within the tunica albuginea (Figures 10, 22). It appeared that these epithelial nodules migrated into the cortical areas in the septa that penetrated the cortex, because in these areas the nodules were often found down in the cortex. In areas where all of the fibers of the tunica albuginea were parallel to the surface, the epithelial nodules appeared to be trapped in the tunica albuginea near the surface of the ovary.

The tunica albuginea from a 5.5 month old specimen was a dense, nearly
unbroken structure, 8 to 30 microns in thickness along one border and
about 38 microns in thickness along the other. A prominent cap of colla­
gen fibers at one pole was also observed on this specimen. It was found
that in areas where the most active invagination was taking place, the
fibers of the tunica albuginea were perpendicular to the surface. Whether
the invagination of the epithelium caused this reorientation or whether it
was the result of the perpendicularly oriented fibers was not known. Also
beneath these areas of invagination there appeared to be a dissolution of
the collagen fibers and the appearance of small vacuoles.

The 6 and 7 month age groups showed a definite trend for the criss­
crossing septa from the outer cortex to become an inseparable part of the
tunica albuginea (Figure 8). Germinal epithelial invagination activity
determined the amount of discontinuity observed in the tunica albuginea.

Minor variation of thickness and varied density of this structure
were observed in the ovaries from the 8 to 11 month old dogs. Some of the
older group showed a lack of parallel orientation of fibers whereas others
showed areas in which there were definite layers of fibers forming the
tunica albuginea (Figures 18, 22).

One year to 19 years of age

There were 13 dogs in the one
to 1.4 year old age group. The tunica albuginea of the ovaries from
these specimens was generally increased in density of structure and
a fusion of the outer parallel and inner criss-cross septal layers was
complete. Thickness of this total structure ranged 12 to 234 microns
averaging 60 to 100 microns in depth. Little change was observed up to
4 years of age (Figure 25). In specimens of this age there were areas of
the ovarian surface covered with a pseudostratified appearing germinal
epithelium. Under these areas but few epithelial nodules or epithelial cords were observed in and beneath the tunica albuginea. A low power field revealed dense swirls of tissue extending from the cortex into the fibers of the tunica albuginea or perhaps the fibers from the tunica albuginea were infiltrating the stroma. Under oil immersion it was observed that these swirls were masses of large elongate nuclei probably remnants of degenerating follicles, or stroma cells. These follicular remnants or stromal cells were found in increasing numbers among the collagen fibers of the tunica albuginea of the 6 to 10 year old specimens (Figures 28, 30, 34, 45). From 10 years old on, the ovaries contained increasingly large areas where it was difficult to demarcate the tunica albuginea from the cortex (Figures 58, 61).

In one ovary from a 10.2 year old dog, cysts as wide in diameter as the thickness of the tunica albuginea were found (Figure 51). These were also found in an 11 year old specimen. They were composed of a thin collagenous wall lined by a single layer of flattened cells of which only the long, flattened nuclei were observed. Cells that made up some of the epithelial nodules were undergoing degeneration and were in a vacuolated state. It appeared that these may have been stages in the formation of the cysts.

Between 11 and 12 years of age there were increasingly large areas of the tunica albuginea that contained no epithelial nodules. The tunica albuginea consisted of a dense belt of collagen fibers. Invagination of the tunica albuginea had become confined to the crests and folds of the ovarian surface. Portions of the tunica albuginea of one of the ovaries from a 12 year old bitch contained a solid mass of epithelial nodules and
cords that appeared to be hyperlastic (Figure 58). Due to the density of degenerated follicle cells as well as the epithelial cells it was difficult to delineate the tunica albuginea.

The tunica albuginea of the specimens which were between 12 and 19 years of age were all dense, with much intermingling of cortical cells. There was a decrease of epithelial nodules in the tunica albuginea of these older ovaries (Figures 60, 61).

**Cortical stroma**

It was the original intent to include in this phase of the investigation subepithelial structures which had originated by invagination from the germinal epithelium and interstitial cells. After reviewing the literature again and studying the slides again it was felt that these structures were more closely related to follicular development and so they were discussed in that phase and only the connective tissues were discussed here.

**Chronological appearance**

**Birth to 2 months of age** In the ovary from a 2 day old Beagle the cortical stroma consisted of embryonic fibroblasts and interlacing strands of collagen fibers which separated the primordial follicles into islands and cords of tissue (Figures 3, 5). The cortical stroma appeared to be an extension of the tunica albuginea and the medullary connective tissue each of which sent septae into the cortex from opposite directions (Figure 1). The nuclei of the fibroblasts were numerous, large, usually oval structures, moderately light staining and contained well dispersed granules of chromatin.

At this early age (12 hours to one week) the cortex made up approxi-
mately 15 percent of the thickness of the ovary. In the 2 to 5 week age groups it was not possible to determine the cortico-medullary boundary. There was a gradual increase in the staining intensity of the collagen fibers as well as an increase in amount of cortical stroma. The fibroblast nuclei stained more intensely and became more elongate in the older specimens. At 5 weeks of age some fuchsin staining cells with dark staining nuclei and cytoplasm were observed among the collagen fibers. These stromal cells were definitely unlike the cells of the developing primordial follicles and often appeared in small groups.

Two and one-half months of age to 11 months of age

By 2.5 months of age the ovary had increased considerably in overall size and the cortex was composed predominantly of collagenous tissue. The fibroblasts were elongate and mature in appearance. The cells described above as first appearing in the 5 week old specimen were now easily observed and appeared much like the primordial follicle cells but were darker staining (Figure 9). Invagination of the germinal epithelium and resulting increase of epithelial cords caused an apparent decrease in the overall connective tissue of the cortex. The septae extending into the cortex from the tunica albuginea were infiltrated with epithelial cords and cells. Cortical width of the 3 month old ovary made up 30 percent of the depth of the ovary. There was an overall increase in ovarian size by this time with an increase of more mature collagenous connective tissue which although oriented in bands parallel to the ovarian surface also had many that were cross-hatching from band to band. This criss-crossing effect was not as prominent in the 5.5 month old specimen, although the parenchymal structures of the cortex were isolated into large islands of tissue which were further subdivided by narrow
strands of collagen fibers. Embryonic fibroblast nuclei were still a prominent feature of the actively changing cortical region, whereas in the area of the tunica albuginea only a few fibroblast nuclei were seen and they were of the long mature type.

In the 6 and 7 month old ovaries the cortex made up 25 to 60 percent of the depth of the ovary. The cortical stroma, by virtue of the follicular growth was forced to reorient around the follicles and the bands previously so prominent were no longer seen. It was noted that the degree of development observed in ovaries from this age period was highly variable. Those that showed but little follicular development had much connective tissue and conversely those that showed all stages of follicles had more compressed and condensed collagen fibers comprising the stroma. Some of these ovaries had prominent chains of invaginated epithelial cells that extended into the cortex.

In specimens from the 6 to 9 month age groups, it was increasingly easy to delineate the cortico-medullary junction. At this junction the connective tissue fibers ran perpendicular to each other (Figure 16). With the appearance and the growth of follicles this line became increasingly prominent in most but not all ovaries. The follicular growth of ovaries of the 8 and 9 month age period caused an overall increase in the weight of the ovary and a resulting increase in the thickness of the ovary.

But little change was observed in the 10 and 11 month old specimens except a general increase in density of the septa entering the cortex from the medulla and the tunica albuginea. In the functional cortex slender collagen fibers that infiltrated the cortical substance were a prominent feature (Figures 20, 21).
In relating the amount of cortical stroma to the estral cycle it was found that the ovaries from dogs that were in anestrous at the time tissues were collected contained the greatest amount of connective tissue. They even contained prominent criss-crossing bands as were described for some of the 2 to 3 month old specimens. The converse was true for the ovaries from dogs that were in proestrous and estrous.

One to 19 years of age

Due to the variation of ovarian cortical width observed with normal cyclic changes, measurements were meaningful only from anestrous dogs. In the ovaries of these dogs from the one to 2 year age group, the cortex made up from 20 to 30 percent of the depth of the ovary. This percentage was much higher in ovaries that contained many large follicles and corpora lutea.

From one year on, there appeared in the stroma of most ovaries an increasing number of cells and clusters of cells associated with remnants of follicles (Figures 25, 26). These cells, some of which were of epithelial origin, some from degenerating theca and others from atretic follicles and from old corpora lutea, therefore diluted the connective tissues of the cortical stroma (Figures 28, 30, 34, 35, 36, 37, 38). The latter cells gradually increased in amount so that in ovaries from animals over 6 years of age they were a very prominent feature. Under high magnification collagen fibers were always observed to interweave through them (Figure 36). In general all of the cortical-stromal tissues underwent a gradual increase in density (Figures 29, 31).

With the change in the overall shape of the ovary from a bean shaped structure at birth to a semi-lobulated structure at 8 years of age (Figures 1, 36), it became increasingly difficult to evaluate changes in the cortical
thickness and in the stroma itself. From 8 years on the number of dense bands of connective tissue and finer infiltrating strands varied with the pattern of the parenchymal cell masses. In the ovaries where the various stage of follicles were distinct structures, connective tissue surrounded each. In an increasing number of ovaries beyond 8 years of age the cortex was predominantly composed of hyperplastic cells of one or more cell types. These cells were often arranged in whorls (Figures 40, 45, 50, 51). Some were infiltrated to a great extent with collagen fibers while in others it was very minimal. Overall there was an increase in amount and density of cortical connective tissue up to 19 years of age. In general the cortex became narrower with age (Figure 61), and the type of parenchymal cells making it up changed as well.

Follicles and related structures

In this phase of the investigation attention was given to the study and description of: invaginating, structures from the germinal epithelium, ovi- gerous cords, oogonia, primordial follicles, primary follicles, growing follicles, mature Graafian follicles, various stages of atretic follicles and all stages of corpora lutea from developing to corpora albicans. The cellular structures resulting from invagination of the germinal epithelium, balls of cells, long epithelial cords and the chains of oogonia which were observed to extend into the cortex of the ovary were described separately due to difference in morphological appearance. It was realized that the latter may have arisen from the germinal epithelium but no evidence was found in this study to support or disprove that assumption. Oogonia were of various sizes (Figures 4, 5), but all were larger than the surrounding cortical stromal cells, had clear cytoplasm and large round to oval pale
staining nuclei, often with no evidence of chromatin.

The next structure that developed after the oogonia was the primordial follicle, which was simply a primary oocyte, similar in appearance to the oogonia, perhaps a little larger and surrounded by one layer of flattened cells (Figure 7). Next was the primary follicle in which the layer of enclosing cells had become a layer of plump cuboidal cells (Figure 7). Growth and development from this stage included the growing follicle which had over one layer of surrounding granulosa cells (Figures 11, 12), a theca interna and an antrum (Figures 12, 13, 14). With maturation the Graafian follicle developed a complexly wrinkled granulosa layer and 2 thecal layers (Figure 19).

Because atresia was observed in all stages of growing follicles, descriptions of various types were given (Figures 17, 20, 21). Finally the corpora lutea were described as they appeared in the ovaries of different age groups and different stages of the estrous cycle (Figures 20, 21, 24).

Detailed descriptions of all of these different structures were not made in this investigation. Descriptions were found in textbooks and in the literature. They were described sufficiently well in this study for proper identification and for purposes of designating the changes that took place with age.

**Chronological appearance**

**Birth to 2 months of age**  In the 2 day old specimen the ovarian cortex, a moderately broad rim of tissue that separated the germinal epithelium and tunica albuginea from the deeper medulla was composed of many germ cell nests or clusters. Each of these nests or clusters were in turn made up of a few too many oogonia. These oogonia generally had large
round to oval pale staining nuclei, often with no evidence of chromatin, but with abundant, pale staining, lacy, cytoplasm. Various stages of mitosis were clearly visible. These oogonia varied in size. The larger ones were more pale staining that those of lesser size.

In germ cell nests throughout the cortex, some of the larger cells had been surrounded by an indistinct layer of flattened cells and thus had become primordial follicles. Often in the same nest 2 or 3 oogonia were observed with shrunken pyknotic nuclei and no cytoplasm, indicating cell death even at this early phase in the life of the ovary (Figure 5).

By one week of age many of the germ cell nests had been replaced by ovigerous cords (Pflüger tubes). These cords or tubes extended from the tunica albuginea through the cortex and varying distances into the medulla. They were composed of chains of oogonia outlined by a few collagen fibers. The remaining cell nests were also surrounded by interlacing strands of collagen. Much mitotic activity was observed in the oogonia.

Changes observed in the 2 week old ovaries, were the disappearance of many of the ovigerous cords and the retention of germ cell nests around the periphery of the ovary. Most of the nuclei of the oogonia now contained numerous small clumps of chromatin dispersed through them. This was especially noticeable in the larger nuclei.

By 4 weeks of age, only a relatively few oogonia were observed compared to the 2 week ovary. Clusters of primordial follicles, however, were now much in evidence. The oocytes varied from 12 to 15 microns in diameter and contained nuclei measuring 5.5 to 6.5 microns. Most of the primordial follicles were about 13 microns in diameter and their nuclei were about 10 microns in diameter.
An overall increase in weight of the ovary (Table 3) with a simultaneous increase in size was observed in the 4 week age group. Of the follicular elements, about 70 percent were oogonia and about 30 percent were primordial follicles. The nuclei of the latter structures contained one to 2 prominent nucleoli, and numerous small granules dispersed in the vesicular karyoplasm. The cytoplasm was easily visible and had a fine netlike appearance. Also seen in the 4 week old specimens were primary follicles, the first seen in the ovaries up to this age. The primary oocyte was 6.6 microns in diameter, its nuclei 4 microns and the surrounding cuboidal cells 2.5 microns deep with nuclei measuring 1.2 by 3 microns.

Two and one half months of age to 11 months of age In the 2.5 to 3 month series, the cortex was mostly connective tissue stroma. A few cell nests were observed as well as a few primordial follicles and primary follicles. In the 2.5 month old ovary the primary oocytes were 7.5 microns with 2.5 micron nuclei and the surrounding granulosa cells were 1.4 microns in diameter with one micron nuclei. The first follicle with more than one layer of granulosa cells was found in a 2.75 month old ovary. It contained a primary oocyte that was 21 microns in diameter and was surrounded by 2 to 3 layers of granulosa cells. Other primary multilaminar follicles of similar size were observed in the 3 month series, but were located in the medulla.

In addition to the follicles, the 2.75 month old specimen contained some short ovigerous cords and germ cell nests in the outer cortex. Also observed in the 2.5 to 3 month series were structures that appeared to result from invagination of the germinal epithelium, subsurface epi-
thelial structures, (SES, O'Shea, 1966). They were found in the shape of balls and cords (Figure 10). These structures were observed in varying numbers throughout the cortex and in some instances in the medulla. The cords had no specific orientation and were often 2 to 3 cells in width. The SES were darker staining than the ovigerous cords.

In the 5.5 month old ovary, the largest ovary studied thus far, germ cell nests and SES were observed in the inner cortex and medulla. Some of the SES contained prominent lumen. In the outer cortex, just beneath the tunica albuginea clusters or groups containing many primordial follicles were seen. Immediately deep to these were primary and growing follicles. Seen for the first time up to this age group was a follicle which contained an antrum (Figure 13). The antrum was oval shaped and was 300 to 420 microns in diameter. Granulosa cell depth varied from 5 to 7 nuclei, or 50 microns, while the theca interna and theca externa were each about 65 microns in thickness. The granulosa gave the appearance of pseudostratified columnar epithelium. In the smaller growing follicles this impression was very evident.

In the numerous growing follicles of the 5.5 month old ovaries a definite membrane just over 2 microns in thickness, the zona pellucida, appeared around the primary oocyte (Figure 12). Immediately surrounding the zona pellucida was a layer of granulosa cells, 2 to 3 cells in depth. These cells had a limited amount of cytoplasm. Surrounding the basement membrane of follicles over about 125 microns in diameter (including granulosa cells in the measurement), the theca interna was observed. At first only one or two layers of fusiform cells were seen and later more layers of the typical thecal cells were added.
In the 6 month age group numerous bi- and tri-ovular follicles were seen in various stages of growth and atresia (Figure 15). One of the larger atretic follicles (250 microns in diameter) had a prominent hyaline membrane which replaced the inner part of the theca interna (Figure 17). These membranes often contained granulosa cells, appeared in various shapes, and comprised the remains of atretic follicles (Figure 21). Other follicular structures, SES were as described for the 5.5 month old ovary.

The ovaries from 6 month old Dachshunds were not as advanced in development as those from the Beagles of the same age. (All of the ovaries described up to this point were from Beagles). The ovaries from one of the Dachshunds had many oogonia and primordial follicles and very few primary follicles. They had many subepithelial cords traversing the cortex and part of the medulla. There was no evidence of atresia in these ovaries. The ovaries from another 6 month old Dachshund were more advanced and contained many growing and atretic follicles. One of the growing follicles contained 5 oocytes. In the cortical stroma of the ovary, mild interna thecosis was observed (Figure 16). From this age on, ovaries also contained structures referred to in this study as granulosa cell islands (GCI) (Figure 21). These consisted of various sized structures comprised of granulosa cells which had pinched off and become isolated from a large follicle undergoing atresia. The nuclei were smaller than in normal granulosa cells. GCI were observed in varying sizes and numbers in the remainder of the age groups studied.

There were no additional age or growth changes per se observed in the 7 and 8 month age groups beyond those described thus far. A study of the slides from this group, of 3 Beagles and 4 Springer Spaniels revealed a wide
variation in stage of maturity with regard to follicular development. No breed distinction was observed.

In one ovary from a 9 month old specimen that had numerous primordial follicles, a few primary follicles and only 2 to 3 larger growing follicles there was evidence of stromal thecosis in the cortical stroma (Figure 18). These cells differed from those of the 6.5 month specimens which showed interna thecosis, because in the 9 month old specimen there was no association between the theca interna of the growing follicles and the thecal type cells found throughout the cortical stroma.

The first corpora lutea in this study were observed in the ovaries from a 10 month old Beagle. Three of these large functioning structures comprised most of the ovary so that there was but little other cortical tissue evident. An ovary from another 10 month old Beagle contained a large follicle that was apparently near maturity or had just ruptured because it contained highly developed folds and convolutions of the granulosa and theca interna (Figure 19). The innermost layers of the theca interna were highly vascular. Granulosa cell islands were prominent cortical features of these 10 and 11 month old ovaries (Figures 21, 24). As explained earlier the nuclei of the cells in these islands of tissue were in general pyknotic, others simply smaller than the normal granulosa cells, were oval and contained evenly dispersed chromatin granules. The cytoplasm appeared as a reticular network. Large vacuole-like spaces appeared centrally in the GCI, similar to those described earlier in the granulosa cells between the oocyte and basement membrane of the granulosa layer. The cellular and nuclear membranes were very dense in these GCI (Figure 24). Some structures, identical in appearance to the GCI, were
found in the theca externa of large Graafian follicles. They were thought to be of similar origin but in an earlier stage of regression.

All of the 11 month old specimens showed varying numbers and kinds of follicular development and atresia. Atretic follicles were divided into 4 general categories as follows:

1. those with some form of hyaline membrane in the theca, that contained granulosa cells which were being phagocytized and were also often vacuolar (Figure 17).

2. as #1 but without the hyaline membrane (Figure 20, right).

3. a gradation between #1 and #2 with a partial hyaline membrane (Figure 20, left).

4. with hypertrophied granulosa cells similar in appearance to luteinized cells of a corpus luteum (Figure 34).

One year of age to 1.1 years of age During this age span all sizes and stages of function were seen in the ovaries depending on the time of year that the tissues were taken and the stage of the estrous cycle that the animal was in. There were no age changes observed in the follicles or corpora lutea from ovaries of this age group. A gradual overall increase in interna thecosis and combined interna and stromal thecosis, however, was observed in all but 3 of the 22 sets of ovaries from one year to 3 years of age. One ovary showed swirls of stromal cell hyperplasia (Figure 18). Numerous bi- and tri-ovular follicles were observed in many of the ovaries.

Follicular cysts were seen in the ovaries of a 3.3 year old Beagle, the youngest animal in this study in which such structures were found. Three of these cysts in one ovary measured 250 by 420 microns, 170 by 670
microns and 420 by 500 microns in cross-sectional diameter. The cysts were lined generally with a layer of granulosa-like cells which varied from one to 3 cells in depth. The granulosa cells were flattened and contained flattened but otherwise normal appearing to pyknotic nuclei. Using Verhoeff's and Van Giesson's stain the content of the cysts was a netlike greyish mass. One of these cysts was encircled with a narrow hyaline membrane, and the other two by cortical stroma. Both of these ovaries contained all types of growing and atretic follicles, old regressing, and early regressing corpora lutea and had combined interna and stromal thecosis (Figures 25, 26).

Six years of age to 10.4 years of age Larger cysts were observed in the ovaries from two 6.1 year old Dalmations. One cyst was sufficiently large to fill the scanning field. All of the ovaries from this group had combined interna and stromal thecosis which showed increased density and amount of age (Figures 28, 30, 54, 36, 37, 58). The "combined" classification was given in some instances as (M29, African Basenji, 9 years of age) even though there were no atretic follicles with abundant interna thecosis, but because the hyperplastic thecal cells were of 2 definite types, one type typical of theca interna cells; the other of stromal cells. In one ovary from a 10.3 year old Labrador Retriever only stromal thecosis was observed, while in the other dense combined interna and stromal thecosis was found.

Granulosa cell tumors were observed in the ovaries from the 9 year old African Basenji. Some were located within the outer cortex and one was a peduncular structure attached to the cortex by a broad neck of collagenous connective tissue. Cellular arrangement was in the form of whorls and the
cells each contained an elongate intensely stained dark nuclei with a central round nucleolus and fine granules of evenly dispersed chromatin. The cytoplasm was netlike in appearance and was moderately dark staining (Figures 43, 44).

Other cellular structures found in the ovaries were SES, found in abundance in ovaries from 5 dogs 9 to 9.7 years of age. In 2 of these sets of ovaries the SES had prominent lumens. Granulosa cell islands observed in the ovaries from most of the animals over 6 years of age (Figure 33) but were especially prominent in 3 dogs 9 and 10 years of age respectively (Figures 47, 48, 49). Up through this age period all types of follicles were observed in most of the ovaries studied.

**Over 11 years of age.** In this age group there were a number of changes seen that either were not found in younger ovaries or were not as prominent in younger ovaries. The dogs from 11 to 19 years of age represented 8 different breeds. From this group, all ovaries showed varying degrees of combined interna and stromal thecosis. In 12 of these it was very dense. In some cases it was whorled in appearance, while in others it was moderate in amount (Figures 40, 49, 50, 51). Although the thecosis of these ovaries was in general more dense than found in ovaries under 11 years of age the increase in density from 11 to 19 years was not progressive. For example in the ovaries from the 19 year old animal there were only small areas of combined interna and stromal thecosis.

Ovaries from 3 dogs 11.2, 12 and 13.1 years of age respectively (the first 2 were Fox Terriers and the last one a Golden Retriever) had SES tumors. In the 11.2 year old animal they were small and in one case combined with a thecal cell body which was part of a fluid filled cyst. Two
of these SES tumors contained much hyaline tissue (Figures 53, 56). The
small SES tumors found in the ovaries of the second Fox Terrier showed both
hyperplasia and hyalinization of the subepithelial cells and were usually
but not always enclosed by a few collagen fibers. They often contained
and/or partially surrounded by varying amounts of hyaline tissue. There
appeared to be no uniformity as to location of these structures, because
they were observed in all parts of the cortex. The ovaries from the Golden
Retriever showed some large definite SES tumors, typical of those described
above but much larger. In other parts of the cortex the SES had lost
their acinar character and just appeared as sheets of cells intermingled
with fine collagen fibers. A few GCI were scattered among the above
structures.

The next most common feature of the older ovaries was the appearance
of cysts, which were found in 7 dogs varying in age from 11 to 19 years and
representing 8 different breeds. Most of these cysts were either follicular
cysts or germinal inclusion cysts. Some cysts were found in the 9
and 10 year old specimens of the younger age group as well as in this
group (Figures 46, 51, 58, 61).

Hyalinization of tissue was a common finding in most older ovaries,
as well as some younger ones including animals from Groups IV and V. It
often replaced the inner portions of the theca interna of atretic follicles.
As the dogs aged the amount of hyaline tissue was found in increasing
amounts. In 4 of these dogs (10 to 12 years of age) it was especially
prominent as part of the theca interna. One of these a 9 year old African
Basenji also had large amounts of hyaline and amyloid located in the
cortical stroma (Figures 41, 42). Ovaries from 2 other dogs 11 and
12 years of age (both Fox Terriers) also had much hyaline tissue throughout the cortex (Figures 55, 56, 59).

Primordial, primary and growing follicles, various stages of atretic follicles and of corpora lutea were found in most of these ovaries. There was a general decrease in the numbers of growing follicles seen in the older ovaries, and not quite so prominent a decrease in the atretic follicles and corpora lutea. The decrease in the normal follicular and luteal structures was especially noticed in the ovaries from dogs over 11 years of age. Often only one or 2 growing follicles were found in a section (Figure 50). In one 12.4 year old Beagle there was a lack of normal cortical tissue except for a few small GCI in dense collagenous connective tissue and no evidence of follicles of any type. Thus, there was a gradual replacement of normal growing follicles with connective tissue and with the various cells differentiating from the cortical stroma. Cysts and hyalinization of collagen also replaced normal growing follicles.

**Medulla**

Since the medulla was essentially a supportive structure, its size and contents depended upon the more dynamic changeability and needs of the functional cortical elements. Its contents were in addition to the ever present vessels, portions of the paroophoron tubules, rete ovarii, migrated epithelial cord elements, follicular elements and often regressing or regressed corpora lutea, smooth muscle and nerve elements. Although some arteries and veins were always present, their numbers, size and tortuosity varied with cyclic changes as well as with age. In most cases at least a small portion of rete ovarii was found imbedded in the depths of the medulla usually between some large muscular arteries. The other structures
were or were not found depending upon the stage of the estrous cycle and upon how the ovary was sectioned.

**Chronological appearance**

**Birth to 2 months of age** In the 12 hour and 2 day old ovary the medulla was large. It was composed mainly of a collagenous connective tissue network which contained some strands of oogonia, **rete ovarii** and **blood vessels** (Figures 63, 64, 65). The tubular structures in cross section were 30 to 40 microns in diameter and lined with large cuboidal to wedge-shaped cells of sufficient size to nearly fill the lumen. Some longitudinal sections were observed with a branching arrangement. These cells were dark staining and contained large oval nuclei in which clumps of chromatin were observed. The bulk of the medulla was composed of strands and islands of oogonia; large cells with netlike cytoplasm and light staining nuclei which contained chromatin clumps. Many of these oogonia were in a degenerating condition, contained vacuolated cytoplasm and pyknotic nuclei. In the hilus area proper only a few small veins and muscular arteries were observed embedded in collagen fibers. The 2 week old ovary was much larger but the medulla was very similar in basic appearance to that described above. Most of the oogonia were gone, however, and only large theca-like cells were observed scattered among the collagen fibers and fibroblasts. Some areas of the medulla of this age were less dense than others and gave the appearance of having been slightly edematous. By 4 weeks of age the medullary connective tissue was more dense and contained numerous nests of oogonia in various stages of migration through it. The only change by 5 weeks of age was a decrease in cellularity and an increase in collagen.
Two and one half months of age to 11 months of age

A further increase of connective tissue was observed in the 2.5 month old ovaries. By this time a migration of epithelial structures was seen. These cells had granular cytoplasm, dark staining oval to round nuclei and were found in rows one to 2 cells wide or in clumps. A structure thought to be rete ovarii consisted of acinar like bodies, lined by light staining columnar cells with dark staining elongate basal nuclei, was also found. A number of these acini were found in close relationship with each other. Some single scattered ones were found in the area with dense collagen fibers enclosing and infiltrating between them. The epithelial cords mentioned earlier persisted in the 2.75 month ovary but by 3.0 months, collagen fibers had separated them into smaller rows or clumps and often into individual cells. Additional cords and clumps of epithelial origin structures, as well as a few primary follicles, were found embedded in the semi-dense medullary tissue. The rete ovarii by now was infiltrated by strands of collagen and in the centers of some were peculiar hyaline-like structures. By 6 months of age many of the epithelial cord cells contained pyknotic nuclei. The connective tissue of the medulla was in general more dense than was observed in younger age groups, while the rete ovarii remained about the same (Figure 66).

With the increase in overall size of the ovary from 6 months to a year there was a resulting increase in size of the medulla and so also of the connective tissues which comprised it. The density of the medulla varied from slide to slide from very dense to semi-dense with areas that appeared to have been edematous. There were often a large number of pyknotic nuclei found among the collagen fibers. From 7 months on there were often signs of
various sizes in the medulla. They were usually elongate and composed of columnar type cells with dark basal nuclei. In ovaries that contained large corpora lutea the medulla was compressed into a relatively small dense collagenous structure. It expanded again as the corpora lutea regressed. The regressed corpora lutea were often found in the medulla, and as they regressed they were replaced by collagen which itself regressed quite rapidly in the younger ovary (Figure 20).

Up to about 11 months of age the rete ovarii was a healthy cellular structure. After that it often had low columnar instead of tall columnar cells and the cells were vacuolated and contained pyknotic nuclei. There was an increase in the connective tissue that infiltrated this structure.

Over one year of age observed in some of the ovaries 1.7 years of age were hyaline-like extracellular structures which when stained with Verhoeff's and Van Gieson's stain light brown. These were thought to be of an amyloid nature and were seen in older specimens (Figures 90, 91, 92, 93, 94, 95). The age changes observed in the ovarian medulla from ovaries that were over 2 years of age were difficult to discern. There appeared to be an overall increase in collagen resulting in a more dense structure in the older ovary. Found in varying but generally increasing amounts with age were short elastic fibers which were seen between the collagenous connective tissues of the medulla. They were usually found in focal masses and first observed in a 6.1 year old Dalmation. They were prominent in an 8 year old Beagle (Figure 89). In the medulla of most ovaries over 10 years of age there were prominent areas of these elastoid remnants. Much of the increase in collagen of the older ovaries was due to the thick bands that formed around the
muscular arteries. There were less dense areas in some Figures 52, 53, 54).

The hyaline-like brown or pink staining structures described previously in a 1.7 year old ovary were found in increasing numbers in older ovaries. They were found in only one ovary up to 1.7 years out of 45 sets of ovaries studied from dogs under that age. From these ovaries remnants of the epoophoron or rete ovarii were observed in 15. They were probably present in all but due to their size and the fact that the ovaries were not sectioned serially they were observed in only 15 out of 45. Between 1.7 and 6 years, 4 embryonic remnants were found out of 8 sets of ovaries studied and in only 1 of these were the hyaline structures observed. Thirty-eight sets of ovaries from dogs over 6 years of age were studied. Portions of embryonic tubules were observed in 24 of these and hyaline structures in 22 out of the 24. Whether or not there was a quantitative increase in hyaline in the embryonic structures was not determined by this study; only that it was found more frequently in the ovaries from older dogs (Figures 55, 56, 57, 84, 90, 91, 92, 98).

In one set of ovaries from a 10 year old Irish Setter numerous GCI were found in the medulla most of which were embedded in hyalinized tissue (Figures 47, 48). A leiomyoma was found in one ovary from a 9 year old specimen (Figures 95, 96).

Vasculature

Since the spiral muscular arteries of the hilus region were the most prominent vessels observed in the ovary and were present in nearly all of the sections studied, they were described first. The next most outstanding arteries were those related to corpora lutea, especially regressing corpora
lutca. Smaller arteries and capillary networks were observed around the larger follicles but few changes were observed in these vessels. As to veins, those of the hilus were the most prominent and morphological changes with growth and age were observed in them. In most cases large venous and lymphatic sinuses were observed in the ovarian hilus, although changes with age were not prominent in these structures.

**Chronological appearance**

*Birth to 2 months of age* Only a few small arteries were observed in the hilus of the 12 hour and 2 day old ovary. Large endothelial nuclei, due to the collapsed condition of the artery appeared to be **jammed together with some standing on end.** Surrounding them was a fine internal elastic membrane (IEM). Around this was a layer of smooth muscle 2 to 5 cells in thickness (depending upon the size of the artery) then a few sparse elastic fibers and **finally a layer of collagen fibers in which** fibroblast nuclei were seen (Figure 69). These arteries were found throughout the medulla. Smaller arteries were observed near the cortico-medullary junction and numerous capillaries were found in the cortex around the nests of oogonia. A few large veins, consisting of an endothelium enclosed by pale staining fibroblasts were seen. By one week of age an increase in size was noted in the arteries. Fine elastic fibers were observed, which formed incomplete rings in the tunica media, close to the IEM. In some of these arteries no IEM was found. No other changes were observed in the 2 and 1/4 week old specimens. In the ovaries from the 5 week old dogs prominent elastic fibers were found between the smooth muscle cells of the tunica media. The endothelial cells still contained large plump oval nuclei. Most of the arteries of this specimen had a lumen size of about
70 microns, and a tunica media 7 to 9 microns or 3 to 5 cell layers thick. No changes were observed in the veins of this specimen.

Two and one half months of age to 11 months of age  By 2.5 months of age the arteries had a lumen size up to 100 microns but with no increase in thickness of the tunica media (Figure 66). Large venous and lymphatic sinuses were observed in this specimen. The 2.75 month old ovary was more vascular than any observed up to this time including various sized arteries, veins, venous and lymphatic sinuses. The arteries 40 to 60 microns in diameter had thicker walls than had been observed in younger specimens. In the smaller arterioles with only one layer of smooth muscle around them the IEM appeared as a series of dots. Many engorged arteries were observed in this specimen next to some that were completely empty (Figure 67).

By 3 months of age an incomplete external elastic membrane was observed in the larger arterioles as well as the complete IEM. Strands of elastic fibers from this extended out into the collagen fibers of the adventitia. A mild infiltration of collagen fibers was found in the muscle layers all the way to the IEM. The arterioles of the 5.5 month old specimen were similar but had thicker tunica media, up to 12 cells (13 to 27 microns) thick and contained elastic fibers throughout. An increase of venous sinuses near the cortico-medullary junction was observed. Elastic fibers were found infiltrating the collagen venous walls and surrounding tissues.

The vessels of the 6 month old age group were similar in general morphology to the 5.5 month old ovary except that splitting of the IEM was observed in the older specimens (Figure 69). Smooth muscle cells from the
tunica media were found in the area of splitting of the IEM. Elastic fibers found in the walls of the veins were much more prominent than the 5.5 month old ovary. In the 6.5 month old specimen splitting of the IEM was again observed. The smooth muscle cells in the split area were oriented in a longitudinal direction (Figure 70). About half of the arteries of this specimen showed splitting of the IEM resulting in nearly one third of the thickness of the arterial wall being in the divided membrane. In these arteries the membrane was split uniformly all around the artery. Similar splitting of the IEM was found in the 7 month old specimens as well as some evidence of intimal thickening. Out of the 6 sets of ovaries in the 7 month age groups, 4 showed various stages of intimal splitting or thickening, in at least some of the arteries. One ovary of another pair showed localized intimal thickening while the vessels in only one pair were free of any intimal changes. In the 8 month old specimen the intimal changes were slight and in the 9 month old set they were very prominent, including up to one third of the thickness of the vessel wall. The arteries from one ovary of a 9 month old dog showed only a split IEM, while in the other ovary disruption of the IEM was more pronounced and intimal thickening was observed in most of the larger arteries. The arteries of the 9.25 month old specimen showed splitting of the IEM and slight collagen infiltration of the tunica media from the tunica adventitia. Many elastic fibers were observed in the collagenous tunica media of the larger veins (Figure 71). A plaque-like structure was observed in an artery (Figure 72).

The presence of large corpora lutea observed for the first time in the 10 month old ovary compressed the medullary tissue of the ovary. These corpora lutea were very vascular as well as was the cortical and medullary
stroma which surrounded them. Even large venous sinuses were observed next to the corpora lutea. The large medullary arteries from 10 month old specimens with large corpora lutea showed very little evidence of intimal damage. Of the ovaries from 11 month old dogs only one showed evidence of intimal splitting of the larger medullary arteries. Three of the 5 showed prominent intimal changes in the arteries of the inner cortex and 2 in arteries that supplied a regressing corpora lutea. The arteries that supplied the most regressed corpora lutea all showed some degree of intimal thickening, splitting or collagen infiltration, cyclic sclerosis (Figures 73, 74).

One year of age to 4.1 years of age Ovaries were taken from 10 Beagles between one and 1.4 years of age. Intimal thickening and splitting was observed in one of the ovaries from the 1.4 year old specimen. Definite intimal thickening was found in one ovary of a one year old and one from a 1.3 year old (Figures 75, 76). The vessels of the other ovaries of these animals had intact IEM. Collagen infiltration was prominent in the arteries of the older ovary. Both ovaries from 4 of these animals had some degree of splitting of the IEM. One artery from another pair of ovaries showed slight splitting of this membrane, while the vessels of the other ovary showed no intimal change. No change was observed in the medullary vessels of either ovary in only 4 out of the 10 pairs studied in this age group. Two of the ovaries had old regressing corpora lutea which were supplied by arteries with prominent intimal changes (Figure 79).

In most of the arteries observed up to this time in which changes were observed, the intima was uniformly affected around the inner wall of the vessel. Such was the case in most of the vessels of the ovaries from
a 1.7 year old Beagle, including prominent collagen infiltration of the tunica media as well. In one of the larger arteries, a large intimal plaque nearly filled the lumen. It was composed of smooth muscle fibers but contained much collagen and elastoid.

Ovaries from 13 Beagles were used to cover the age span from 2 to 4.1 years of age. Eleven had been on control diets, one on medium protein and one on high protein. All except one of the ovaries from 3 dogs showed the large muscular arteries of the medulla with some degree of intimal thickening. In 4 of these, intimal plaque-like structures were observed. The thickening was localized in another and generalized in the remaining ovaries of this group. The severity varied from just a few of the arteries being involved with early intimal thickening, to about half of the vessels being prominently thickened, some with elastoid. Collagen infiltration of the tunica media was observed in 4 of these (Figures 77, 78). While splitting of the IEM was observed in only 2 (Figure 80). In one ovary from one and both ovaries from another animal, there was no evidence of intimal change in the hilar arteries. Ten of the 13 pairs of ovaries of this age span contained old corpora lutea in varying stages of regression. The degree of intimal thickening, collagen infiltration and elastoid formation of the corpora luteal arteries was dependent upon the regression stage of the corpora lutea. When the corpora lutea were in advanced regression the intimal changes, cyclic sclerosis was severe. Near occlusion of lumens was observed in some cases.

Other changes noted in the 2 to 4 year age span were pronounced thickening of the collagenous tunica adventitia of the arteries. It was often thicker than the tunica media and interna combined, and blended with the
medullary stroma. The tunica media of the larger veins was also observed to be thicker and more intensely infiltrated with elastic elements. In those arteries undergoing early intimal thickening, a reorientation of the smooth muscle layers nearest the intima, from circular to longitudinal, was observed. This change appeared to precede the actual breakdown of the intima and subsequent migration of the cells to the subendothelial space (Figure 80). In most of the ovaries of this series even the smaller hilar arteries and cortical arteries showed some degree of intimal change. Many of the small arterioles had thickened tunica media with collagen infiltration of the tunica media and no thickening of the intima.

Six years of age to 10.4 years of age This age span contained 24 dogs. Only 2 of the Beagles had been on special protein diets, one on high and the other on low protein. Vascular lesions from the ovaries of these dogs were not significantly different from those of the previous group. The most prominent feature of the ovarian vessels from these dogs was the intimal thickening found in 23 out of the 24 sets of ovaries. In 8 of these it severely affected the majority of the arteries of the hilus as well as the smaller vessels. In only 2 was the thickening of mild nature. Varying degrees of collagen infiltration was observed in 12 of the 24 sets of ovaries (Figures 83, 85). It was more prominent than in previous age groups studied. In the ovaries from the African Basenji, a 9 year old animal, a thick collagen ring was observed just outside of the internal elastic membrane. So, from the lumen the arterial wall was composed first of a thickened intima, IEM, a wide ring of collagen fibers, a band of circular smooth muscle fibers and finally a heavy collagenous tunica adventitia which blended into the medullary stroma. This trend for
localization of collagen near the IEM was observed in many of the arteries of this age span but was most prominent in the ovaries from this particular dog (Figure 91). Collagen infiltration of the thickened intima was also found in some of these (Figures 86, 97). Intimal plaque-like structures were observed in the ovarian arteries of only 3 dogs, from a 7.8 year old Beagle, a 9 year old Golden Retriever and a 9.7 year old Dachshund. As described previously, the arteries supplying regressing corpora lutea appeared to undergo age changes on their own plane. In the corpora lutea that were in advanced stages of regression the arteries had very small, or in some cases no lumen left, due to the severe intimal thickening, collagen infiltration and elastoid content. In only one animal from this age span were the ovarian arteries free from intimal changes. They were from a 6.1 year old Dalmation. The veins contained much elastoid (Figures 81, 82).

The veins were more prominent structures in this age span than in the younger age groups. Intimal plaque-like structures were observed in the ovarian veins of an 8.6 year old Beagle (Figures 97, 98). The walls of the veins were thicker than observed previously and composed of more dense collagen fibers, often near hyaline in appearance. Elastic fibers and elastoid was also a prominent component of the wall (Figure 88).

Over 11 years of age Fifteen dogs from 8 different breeds made up this age span. Breed difference did not appear to be a factor in the vascular changes found in the ovaries from these animals. Severe intimal thickening with varying degrees of collagen infiltration were found in some of the arteries from each specimen of this age group. Intimal plaque-like structures were seen in 7 out of the 15 sets of ovaries studied. The presence of elastoid and the breaking down of the IEM were prominent.
features of the arteries of 3 sets of ovaries, although early stages of this breakdown were observed in most of the other ovaries (Figures 99, 100, 101, 102, 103, 104). Two ovaries with regressing corpora lutea were found in this age span, from a Labrador Retriever and from a German Shorthair Pointer. The vessels associated with these structures were severely affected.

Prominent arterial changes of this age span were the increase in elastoid in the thickened tunica intima and the increase of collagen fiber infiltration into the thickened intima (Figures 102, 103, 104). The smaller arterioles in all ages studied underwent thickening of the tunica media rather than changes in the tunica intima. Most of these smaller arterioles were cortical vessels.

Oviduct

Epithelium

The number and appearance of epithelial cells that lined the canine oviduct was dependent on the developmental state of the underlying lamina propria (La Pr) and its rugae. The rugae were fully described in the La Pr section. It was observed that the longitudinal folds were well developed complex structures. They often contained secondary and tertiary folds in the anterior portions of the oviduct, while they were decreased in complexity toward the uterus. The epithelial cells covering these folds also varied in their appearance depending on the area of the oviduct from which the section was taken, so as these observations were discussed, the specific areas infundibulum, ampulla, or isthmus were designated.

Chronological appearance

Birth to 2 months of age The sections from the 2 day old
specimen were taken from the isthmus. Epithelial cell height was rather uniform, 17 to 22 microns, and the cells varied in shape as they were wedged together (Figure 10). Most of the nuclei were vesicular, contained chromatin strands and a large vacuole-like structure near the center of the nucleus. Numerous cells with densely stained nuclei, some with mitotic figures, were observed perpendicular to the epithelium in the basement membrane region. It was felt that these were epithelial precursor cells. Plasma cells were also found in the basement membrane area. No cilia were observed on the free border of the epithelial cells although tissue debris or extruded cytoplasm was seen. By one week of age the epithelial cells were shorter and varied from 4 to 13 microns in the isthmus, 4 to 6 microns in the ampulla and 9 to 13 microns in height on the region of the fimbria. In the isthmus 2 types of cells were found, the cuboidal cells with dark staining nuclei on the tips of the rugae and the lighter staining longer, larger cells along the sides and bottom of the crypts between the folds. Invagination of the epithelium in the latter areas was observed. These types of cells were also observed in the ampulla and fimbria, as were numerous precursor cells in the basement membrane region. Mitotic figures were more prominent in the epithelial cells of the fimbria than in the other regions of the oviduct. The fimbria of the 2 week old specimen was similar to that in the one week old oviduct. In the oviducts from the 4 and 5 week old dogs the fimbrial epithelium showed numerous areas of apparent invaginations into the La Pr. The cells were from 9 to 18 microns in height and varied from simple columnar to pseudostratified in appearance. Some of the precursor cells appeared to be in stages of migration from the basement membrane up into parallel relationship with the epi-
Epithelial cell nuclei were central to basilar in location. The cytoplasm was light staining and appeared to be extruding from the cells in some instances. Cilia were not observed on any of the epithelial cells up through 4 weeks of age but were observed on some of the non-secreting fimbrial cells in a 5 week old specimen. Some of the interciliary cells showed large droplets of secreted material on their free border (Figure 108).

**Two and one half months of age to 11 months of age**
The epithelial cells lining the fimbria of a 2.5 month old oviduct were similar to, but showed greater activity than those of the 5 week old specimen (Figure 110). They were 5 to 13 microns in height and were divided into four categories: (1) those that were large and round with light staining cytoplasm and round basilar nuclei which contained finely granular karyoplasm, (2) those as number one but with cilia, (3) those darker staining than number one, in various stages of secretion and (4) tall columnar peg cells with long dark staining nuclei. Ampullar and isthmal cells from this specimen varied from 5 microns on the top and sides of the rugae to about 13 microns in the crypts. Except for the fact that no cilia were observed on it, the epithelium of the ampulla was very similar to that of the fimbria. In the latter 2 areas clear cells were observed near the basement membrane. Some portions of both the ampulla and isthmus of the 3 month old oviduct contained pseudostratified columnar epithelial cells while the remainder of the cells were simple columnar to cuboidal. Cell height varied from 5 to 17 microns in the ampulla to 5 to 9 microns in the isthmus and no ciliated cells were found. The only additional finding in the 5.5 month old specimen was increased height of the epithelium which was typical of estrous
(Figures 111, 112). Of the 3 specimens studied in the 6 month age group, the isthmus epithelial cells were tall columnar and up to 19 microns in height. In one of these specimens much cellular activity was observed in the basement membrane area including mitosis, cell destruction, phagocytosis by neutrophils and some neutrophils in stages of migration through the epithelium.

Oviducts from 7 month old Beagles were similar in appearance to those found in the 6 month old specimens. Others from Springer Spaniels were lined with shorter epithelial cells, 5 to 9 microns in height, with scanty secretion in evidence and very little cellular activity in the basement membrane area (Figure 113).

An increase in size and activity was observed in the 8 specimens studied in the 9 to 11 month age group. In the mid-ampulla region of the 9 month old specimen much invagination and complex infolding of the 10 to 16 micron high columnar epithelium was observed. Cilia were observed on some cells and secretory material was observed on others. Rings of epithelial cells resembling glands were observed in the La Pr. In one of the 10 month old specimens the ampullar epithelium varied from 15 to 24 microns in height and contained all types of epithelial cells, those migrating from the basement membrane, clear cells, peg cells, ciliated cells and secreting cells. All of the 10 month old oviducts had much pseudostratification of the epithelium (Figure 116). In one it was up to 39 microns in height, light staining and appeared hypertrophied to the extent that it nearly occluded the oviducal lumen (Figure 115). That of another was less than 20 microns in height and was mostly low columnar and ciliated. In all of the 10 month old specimens the complex infolding was observed.
The epithelium of the 11 month old specimens was 6 to 15, 8 to 15, and 12 to 20 microns in height respectively in the region of the ampulla. Especially on the tips and sides of the rugae, the epithelial cells were dark staining, had decreased cytoplasm, pyknotic nuclei and presented a palisade appearance. The crypts and invaginated rings of epithelial cells were lighter staining and showed some evidence of secretion. A few were ciliated.

One year of age to 4.1 years of age

The oviducal epithelium from 9 dogs that were one to 1.2 years of age when tissues were taken, was 5 to 20 microns in height, contained no cilia and had only minimal secretion (Figure 118). Epithelial rings of the La Pr and crypt epithelial cells were lighter staining than the epithelium on the tips of the rugae.

Secretory activity was observed in a section taken through the isthmus of a 1.3 year old specimen in which the epithelium was 8 to 13 microns in height, cuboidal to low columnar, with basal nuclei and which contained granular strands in the cytoplasm. The cytoplasm of some cells was in stages of extrusion from the apical end of the cell. In the 1.4 year old specimen the epithelium from both ends of the ampulla was in an inactive state as described above for the one to 1.2 year old oviducts. A section from the cranial ampulla of the 1.7 year old specimen contained tall pseudostratified ciliated columnar epithelium which was 60 to 80 microns in height. The nuclei were generally in 2 rows with darker-staining ones midway in the cell, and the lighter-staining granular nuclei in the apical one third. No secretory activity was evident in this specimen.

Secretory activity was evident in one ampulla of the 9 specimens from the 2.7 to 4.1 year age range. Pseudostratified, ciliated columnar cells were observed in 4 of these. The remainder were in an inactive
state. The epithelium from the latter specimens was 4 to 15 microns in height while in the active ones it was 8 to 28 microns in height.

**Six years of age to 10.4 years of age** Tissues from 11 dogs were studied in this age group. Cilia were present in varying degrees in all except one of the 10 year old specimens. The epithelium varied from pseudo-stratified ciliated columnar to low ciliated cuboidal to a shrunken palisade epithelium with pyknotic nuclei. Epithelial cell height from 6.4 to 10.4 years of age was generally 5 to 19 microns in height with most of the cells around 10 to 14 microns high, except for one 9 year old specimen. In this oviduct they were uniformly 40 microns in height and of all types previously described even some that were in a secretory stage. The epithelial cells from the 4 animals that were from 9.1 to 10 years of age when tissues were taken were 4 to 11 microns in height. Through this whole age span, 6 to 10 years of age, there appeared to be a general decrease in epithelial cell height. There also was a decrease in the number of epithelial rings observed in the La Pr from specimens over 9 years of age. This was very noticeable as these structures were numerous in younger specimens (Figure 124).

**Over 11 years of age** The epithelium of the 8 oviducts studied in this age group did not show the smooth trends described in the above series. One of the 11.2 year old specimens had a 10 to 14 micron high epithelium with ciliated cells, secretory stage cells, all of which had basal nuclei. Many rings of epithelial cells were also observed. In other specimens 11.2 to 12.8 years of age the cells were 5 to 15 microns in height and usually formed a cuboidal epithelium. Cilia were found in only one of the 5 specimens of that group. In that one, the cilia were found only in the
rings of the epithelial cells. Rings of epithelial cells were few in number in 6 of the 8 oviducts. In the 12.4 year old specimen many of the rings were cystic with a 4 micron high epithelium (Figures 134, 135, 136), while those which were not cystic had a 10 micron high epithelium. A thick layer of epithelial rings was observed in the oviduct from the 12.8 year old specimen in which many mitotic figures were found. Mitotic figures were also observed in the oldest specimen studied, 13.1 years of age. It contained an epithelium which was 20 to 27 microns in height, tall pseudo-stratified, ciliated, columnar in appearance and had many cells in a secretory phase. Thus, it appeared that a general trend of decreased height of epithelial cells and decrease in rings of epithelial cells continued up to 11.5 years of age, after which each had to be described on its own merits.

Lamina propria

Between the epithelium and muscularis of the oviduct, forming some simple primary folds near the uterine end and very complex primary, secondary and often tertiary folds near the ovarian end, was the La Pr. It varied from a densely cellular connective tissue structure to a loose structure, depending on the area of the oviduct from which the section was taken and upon the stage of cycle. Small vessels, usually of capillary size were a constant feature of the La Pr.

Chronological appearance

Birth to 2 months of age In the isthmus of the 2 day old specimen (Figure 105), the La Pr consisted of disoriented masses of embryonal type cells. These cells had round to oval nuclei that contained strands of chromatin or chromatin granules. The cytoplasm was light staining and
fluffy. It was often difficult to discern the cell membrane. Loose strands of collagenous tissue was interwoven through the La Pr. Capillaries lined with large endothelial cells were especially prominent beneath the epithelium. Only a few thick primary folds were evident in this section. In the week old specimen the isthmus had 5 blunt primary rugae, while the ampulla had 8 slender, primary folds with beginning secondary folds. The fimbria contained numerous fingerlike primary folds and developing secondary folds. Although the collagen fibers were still loose, they were more dense than observed in the younger specimen and evidence of mitosis in the La Pr was abundant. Plasma cells, lymphocytes and large mast cells were observed. By 2 weeks of age 2 definite types of connective tissue cells were prominent, (1) the plump embryonal type and (2) the typical fibroblast cell with a dark nucleus. Moderately dense collagen fibers with some elastic fibers were seen for the first time in the fimbria of a 4 week old specimen. Complex primary and secondary rugae gave an edematous appearance in the fimbria portion of an oviduct from a 5 week old dog (Figure 108). There was still no directional orientation of the fibers.

Two and one half months of age to 11 months of age An increase in density was found in the isthmus fibers, in contrast to the loose collagenous and elastic fibers observed in the ampulla and fimbria portions of a 2.5 month old oviduct (Figures 109, 110). This was the earliest age at which increased density was observed. There appeared to be an increase in mesenchymal type tissue toward the fimbria in contrast to the more mature fibroblasts found in the isthmus. Definite evidence of circularly arranged fibers was observed in the isthmus of this specimen and evidence of early circular orientation in the ampulla.
The only changes observed up to 9 months of age were a gradual increase in density of the connective tissue elements, an increase in complexity of the rugae and an increase in mature fibroblasts (Figure 111, 113). In 2 of the 10 month old specimens there was a decrease in fold complexity with a resulting condensation of the connective tissue elements as the La Pr was displaced by enlarged epithelial cells or by edema (Figure 114). The 11 month old specimens had prominent densely cellular La Pr similar to the 9 month group.

One year of age to 4.1 years of age: The La Pr of the majority of the oviducts in this age range were composed of varying amounts of consistently dense collagen fibers, some elastic fibers, various cellular elements and capillaries. Determining the amount of connective tissue or depth of the La Pr and thickness of the rugae was the size of the epithelial cells. If they were large and hypertrophied then the La Pr was sparse.

Six years of age to 10.4 years of age: A general increase in density and fibrosity of the La Pr was observed in this age span. There was even some evidence of cellular degeneration, such as pyknotic nuclei and vacuolization of cytoplasm in some of the other specimens.

Over 11 years of age: Characterizing this age group was a dense but generally narrow La Pr (Figures 131, 132). The folds of the ampulla were often less complex than in younger age groups and the epithelial rings of the crypt areas were often surrounded by dense rings of collagen fibers. The oldest oviduct studied was 15.1 years of age and its La Pr was sparse and loose in contrast to the dense La Pr of the others.

Muscularis: Inasmuch as the canine oviduct was imbedded in the ovarian bursa it
was often difficult to determine which tissue belonged to the oviduct proper and which belonged to the bursa. It was felt that some was common to both. Typically the muscularis and serosa consisted of four layers: (1) a prominent inner circular muscle (ICM) surrounding the La Pr (2) out from this, composed of varying sized rings of collagenous tissue, and smooth muscle fibers was the vascular layer (VL), (3) then some scattered bundles of longitudinal or oblique smooth muscle (ELM), intermingled with collagen fibers, elastic fibers vessels and adipose tissue and finally (4) the serosal lining of mesothelium. At least a few elastic fibers were seen in all 3 muscle layers. The serosa was often missing due to the manner in which the oviduct was collected or processed. Thus, even though the sections were taken from varying locations along the oviduct growth and age change trends were established.

Chronological appearance

Birth to 2 months of age  Even in the 2 day old specimen there was a definite boundary between the La Pr and future muscularis (Figure 105). Large venous sinuses of the vascular layer separated what was to become the ICM and ELM. The nuclei of the future muscularis were more elongate than those found in the La Pr, were circularly oriented in the inner layer and contained chromatin in granules or strands. Many fine collagen and a few elastic fibers were interwoven among them. Surrounding all of this was the bursal serosa. The oval nuclei of the bursal cells were spaced one nuclei length apart as they covered the outer portion of the bursa. The only change observed in the muscularis of the week old oviduct was an increase in density of undifferentiated smooth muscle nuclei and an increase in prominence of the collagen and elastic fibers.
Two and one half months of age to 11 months of age

In the 2.5 month old oviduct (Figure 109), a wide ring of predominantly collagenous connective tissue surrounded the La Pr. The VL was also mainly collagen fibers as well as the tissue beneath the serosa. The only mature smooth muscle seen was a few well defined bundles beneath the serosa, which formed part of the bursae. What appeared to be collagen with some elastic fibers in the muscle areas of the oviduct increased in density in specimens up to 5.5 months of age but contained chiefly large embryonal type fibroblast nuclei (Figure 111). In the latter oviduct an occasional smooth muscle cell was found in the circular layer but none were differentiated in the future longitudinal muscle area. By 6 months of age a narrow band of circular muscle fibers was observed around the La Pr. They were still well infiltrated with collagen fibers all of which blended into a wide band of dense connective tissue that surrounded the circular muscle ring. Out from this was the VL, a second broad band of dense connective tissue that contained a few longitudinal muscle cells. Covering this was a cuboidal serosa. The appearance of the developing smooth muscle was similar in all sections of the oviduct, but it increased in width toward the isthmus. Thus, there was less collagenous tissue in sections from the isthmus than from the more cranial portions. The ELM in the isthmus of the 5 to 6 month age groups consisted of a band one to 2 cell layers wide beneath the serosa. In the 9 to 11 month interval the smooth muscle cells continued to mature and increase in number (Figures 113, 117). By 11 months of age a prominent wide band of circular muscle was found even in the cranial ampulla. Many muscle bundles were found in the vascular layer and outer areas near the serosa. Although a decrease of collagen was observed during this growth period in
the ICM area, collagen was still a prominent part of the other areas. For the first time the intrinsic musculature of the oviduct appeared similar in development to the smooth muscle bundles of the ovarian bursa, except for more collagen fibers in the former (Figure 117). It had lost its embryological appearance.

**One year of age to 4.1 years of age** Nine of the 12 specimens studied for the one to 1.7 year age group showed a trend of increased density of the muscularis, resulting mainly from collagen infiltration but also in part by smooth muscle growth and development. The other 5 oviducts had varying amounts of vacuolization, pyknotic nuclei and in general, degeneration of the musculature. From 2.7 to 4.1 years, oviducts from 10 dogs were studied, and in all cases the muscularis was composed of healthy appearing cells (Figure 120). In the isthmus, the ICM was composed of about 90 percent muscle cells and 10 percent connective tissue, while in the ampulla the proportions were 50:50. This fact was observed in younger specimens but was definitely more prominent in these specimens.

**Six years of age to 10.4 years of age** The 6 year old oviduct caudal fimbrial region, contained no ICM. Only connective tissue was observed in its place. Embedded in the outer portions of circular connective tissue fibers, near the VA were a few longitudinal muscle bundles that comprised the ELI of this portion of the oviduct. The bursal musculature in this area was very abundant and well developed. In the specimens from this age span the ICM of the ampulla region was increased in density. There appeared to be a decrease in cytoplasm with a resulting increase in numbers of nuclei observed in the microscopic field (Figure 129). Often the older specimens had some smooth muscle fibers extending into the rugae of the
ampulla. In all of these older oviducts, the serosa was missing. Only a few sparse longitudinal muscle bundles were found out from the VL and surrounding this, only connective tissue, adipose tissue and vessels. From the musculature alone, however, it was difficult to differentiate oviducts between one and 10 years of age due to the subtleness of the changes.

Over 11 years of age In one of the 11.2 year old oviducts the ICM was narrow, infiltrated with collagen and degenerating (Figure 130). The other intrinsic muscles were nearly non-existent while the bursal muscles and connective tissues were prominent. The second 11.2 year old specimen and a 11.5 year old specimen had dense, wide, fibrous circular muscles, in which the layers adjacent to the La Pr were infiltrated by collagen fibers from the La Pr. In the older of these 2 there appeared to be a loss of muscle cytoplasm as the large dark staining nuclei were very prominent. The ICM was composed of dense but light staining vacuolated wide cells in which the nuclei were sparse as compared to the preceding specimen. The next 3 were all dense, dark staining structures, with prominent nuclei (Figure 135), while the last one from a 13.1 year old dog had only a few strands of smooth muscle embedded in collagen.

Vasculature

The vasculature of the canine oviduct consisted of: (1) capillaries located beneath the epithelial basement membrane and a few capillaries or arterioles interspersed throughout other areas of the La Pr, (2) capillaries and arterioles in the ICM especially near the La Pr, (3) arterioles, venules and venous sinuses in the VL and (4) larger arteries and veins in the bursal tissues adjacent to the oviduct (VA). The latter arteries probably supplied both the bursa and the oviduct. Much variation was observed in both size
and numbers of vessels located in the different areas. Often the vessels from the VL and/or the VA were not included on the slide as they had been lost during tissue collection.

**Chronological appearance**

**Birth to 2 months of age** In the 2 day old specimen a few capillaries were observed in the inner La Pr area beneath the epithelial basement membrane. Arterioles with one to 2 layers of muscle cells were prominent in the ICM near the La Pr muscularis junction, as well as being interspersed throughout the musculature. The VL contained capillaries, arterioles, venules and larger venous sinuses (Figure 105). By one week of age the essential differences were (1) in the fimbria, an increase in capillaries throughout the La Pr, (2) in the ampulla, a vascular increase in all the layers of the ampulla and (3) in the isthmus, an increase of capillaries, especially beneath the basement membrane. Some of the arterioles of the isthmus ICM had an incomplete internal elastic membrane (IEM) and up to 3 muscle layers. Large veins with a few smooth muscle cells in their collagenous connective tissue walls were observed in the outer adjacent tissues. The only change noted in the 4 week old specimen was the appearance of small arterioles and venules in the central portion of the primary rugae. These were especially prominent in fimbria of the 5 week old oviduct.

**Two and one half months of age to 11 months of age** An increase in number of vessels was noted in the 2.5 month old specimen, including the arterioles and venules in the middle of the primary rugae, as well as in the secondary rugae in the ampulla. There were few vessels in the dense connective tissue of the La Pr. The isthmus of this oviduct was much less
vascular than the ampulla and the vessels that were present were smaller. Many arterioles and venules were observed in the dense tissue of the La Pr in the 3 month old specimen, in contrast to the 2.5 month old specimens. The arterioles of the VL of the ampulla, now had a complete IEM, up to 4 muscle layers and a few elastic fibers blending into the adventitia. Large venules with collagen and elastic fiber was also found. As in the younger specimens the vasculature of the isthmus was not as prominent as in the more cranial portions of the oviduct. An increase in size and number of vessels of the VL was observed in oviducts from 5.5 and 6 month old dogs. In a 6.5 month old specimen a large muscular artery adjacent to the oviduct had a split IEM. An artery of similar appearance but with the addition of a plaque-like formation was found near the oviduct from a 10 month old dog. An increase of elastic tissue was observed in the larger veins of the older specimens of this age group. In a 10 month old specimen there was an overall increase in vasculature, throughout the La Pr of the ampulla, and a decrease in the isthmus. Of the 5 specimens in the 11 month age group, 2 showed decreased vascularization and the other one increased. In some of the adjacent larger arteries of all 3, changes were observed, 2 with thickening of the intima and the other with early splitting of the IEM. Some of the larger veins had walls composed of collagen and elastic connective tissues and some smooth muscle fibers.

One year of age to 4.1 years of age Of the 9 specimens studied in the one to 1.2 year age group, collagen infiltration of the muscular wall was observed in 2. No intimal changes were seen in this group. In the 1.3 year old oviduct, the adventitia of the larger arteries found in the vascular layer and near the oviduct was much thickened by dense rings of collagen.
An increase in vasculature, with most of the vessels engorged was a feature of all layers of the oviduct from a 1.4 year old dog which was in proestrous when tissues were taken. One of the nearby veins of the latter oviduct had a high percentage of elastic tissue in its walls (Figure 119).

Little change was noted up to 3 years of age except intimal thickening that affected 10 to 30 percent of the intima in some of the neighboring arteries. In one such adjacent oviducal artery from a 3 year old dog in metestrous, splitting of the ECM and collagen and elastic fiber infiltration of the muscle wall was found. Intrinsic oviducal vessels of this dog were engorged. Elastoid was observed in a large artery from a 5.3 year old specimen, in which up to 30 percent of the intima was involved in change (Figures 121, 122). A plaque-like structure was observed in a large vein near the oviduct from a 3.6 year old dog. Staining variability was seen in an artery from a 2.7 year old animal (Figure 123). In the outer VA of a 4.1 year old specimen many elastic fibers were found.

Six years of age to 10.4 years of age Although some fluctuation of vascularization was observed, depending upon cyclic changes, there were no changes in the walls of the arteries of 7 of the 11 oviducts of this age span. In the other 4 there were varying degrees of collagen infiltration, elastoid formation, intimal splitting, intimal thickening and plaque-like formation (Figure 128).

Over 11 years of age Of the 7 specimens studied in this age span, the intrinsic musculature of only one was involved with degenerative changes. It was a 11.9 year old Beagle (Figure 133), in metestrous when tissues were collected. All of the vessels were engorged as well as having 50 to 90 percent intimal thickening, elastoid, and collagen infiltration.
Some of the collagen infiltrated bands were nearly hyaline in appearance.

Ten to 30 percent intimal thickening and some collagen infiltration and elastoid formation was observed in some vessels of each specimen in this age group while many of the vessels were free from change. Thus it was difficult to discern any real age change trend in these older specimens.

Uterus

**Statistical evaluation of dimensional changes with age**

**Epithelium of uterine lumen**

The mean heights of the uterine epithelial cells (Table 4) decreased shortly after birth, then increased up to middle age and finally decreased again. A curvilinear decrease and subsequent increase was observed up to 11.2 months of age (Graph 3), with a significant regression coefficient. The decrease in the middle and old age groups, however, was not statistically significant.

There was no significant increase or decrease in the epithelial height of the uterine glands from birth to 13.1 years. The mean height was 14 microns with a range of 8 to 35 microns in various specimens.

**Lamina propria**

A significant curvilinear regression showed an increase in La Pr thickness with age (Graph 3). The mean thickness of the La Pr was 257 microns for Age Group I and 534 microns for Age Group II. This bore out the growth increase for this time period. In Age Group III after growth and during the productive years the mean La Pr thickness was 694 microns. A subsequent decrease was observed in the middle and old age groups (IV and V respectively) although the regression coefficient was not significant (Table 4).
Muscularis

During the prematuration period there was a curvilinear relationship between the VL and age (Graph 2). The muscularis had developed from a 35 micron structure in the younger specimens up to 300 to 500 microns in the older animals from the Prematuration Age Group. Upon breaking this into 2 subgroups the total muscle thickness increased from 171 microns in Age Group I to 534 microns for Age Group II (Table 4). The regression coefficient for VL increase was significant whereas it was not significant for the other two muscle layers. A linear relationship did, however, exist between the total muscle thickness and age as well as between the total uterine wall thickness and age (Graph 4). The latter had the greatest increase. In both cases the regression coefficient was significant.

In the Postmaturation Age Group the regression coefficient was not significant for the increase observed in wall thickness with age. There appeared to be a decreased thickness in the wall thickness of Age Group V, but there were not sufficient animals in the group to (Tables 3, 4) render a valid conclusion.

Statistical evaluation of dimensional changes other than with age

Body weight

In the Prematuration Age Group there was a curvilinear increase in both La Pr thickness and total uterine wall thickness with respect to body weight up to 5 kg, and then a slight decrease up to 11.1 kg. body weight. The regression coefficient was significant for this age group (Graph 5), whereas it was not for the Postmaturation Age Group.

However, when both Prematuration and Postmaturation age groups were considered together, with respect to body weight there was a significant
regression coefficient in which the increase was linear for ovarian weight, VL thickness, ELM thickness and muscle total thickness. A nearly parallel increase was noted for the latter 3, and a more rapid increase for ovarian weight (Graph 6). In contrast to statistical predictions these weights and measurements were in some cases greater on dogs of a lesser body weight than with those of a greater body weight. Thus, cyclic variation as well as individual variation were considered pertinent.

**Ovarian weight**

When uterine gland epithelial height, La Pr, ICM, VL, ELM, MT, and WT thicknesses of Prematuration Age Group dogs were analysed with respect to ovarian weight, there was a linear increase as ovarian weight increased. The regression coefficient were significant. It was noted that the increase of all measurements was nearly parallel except for the height of the glands found in the uterine La Pr. Although the height of the glands did increase, it was not as rapid as were the other increases (Graphs 7, 8, 10). When similar analysis were run on data from both Prematuration and Postmatureation Age Groups the results were similar (Graph 9, 11).

**Uterine epithelial height**

In the Prematuration Age Group there was no significant regression between the various uterine wall data and the epithelium that lined the uterine lumen. It was noted, however that in the Postmatureation Age Group there was a significant linear increase of ICM, VL and ELM with respect to the measured increase in epithelial cell height (Graph 12). The most rapid, yet nearly parallel increase was in the muscle layers per se (ICM, ELM) and the slower increase was in the VL thickness. The regression coefficient was significant.
Vascular layer thickness

It was found that in both the Prematuration Age Group, Postmaturation Age Group and in both combined there was a linear increase in ELM, MT and WT thickness with an increase in VL thickness (Graphs 13, 14, 15). The validity of the ELM was considered most significant as in the other 2 measurements the thickness of the VL was included. In each case the regression coefficient was significant.

Epithelium

The epithelium which lined the canine uterus varied from cuboidal to tall columnar (9 to 60 microns) and was in some cases ciliated. All variations of stainability were observed. Usually the epithelium was simple in morphology, but during early metestrous and pp it underwent extremely complex proliferation. This resulted in a fluffy popcorn appearance microscopically, as the cells were very light staining. For most of the cycle the epithelium varied from columnar to cuboidal, generally 15 to 20 microns in height, except during metestrous and pp when it became pseudostratified, and very complex. The most obvious changes were those that related to the stage of the estrous cycle that the dog was in when tissues were taken.

In none of the specimens studied was there any sign of blood escape into the uterus during proestrous although congestion of vessels was seen.

Chronological appearance

Birth to 2 months of age A tall (23 to 28 microns) light staining pseudostratified columnar epithelium with numerous areas of invagination lined the uterus of the 12 hour old and the 3 day old dog (Figures 138, 140). Secreted material was observed on the epithelium. The epithelium that lined the crypt areas was taller than elsewhere in the...
uterus. Beneath it there was a definite basement membrane (Figure 140). The epithelium was still the same height in the week old specimen but the nuclei were more basal and infolded areas more numerous. By 2 weeks of age the epithelium had decreased to 10 to 14 microns in height with no changes apparent (Figure 141).

Two and one half months of age to 11 months of age In the 2.5 months old uterus a low columnar to cuboidal (10 to 14 microns) epithelium composed of cells with large central nuclei and light staining cytoplasm formed the lining (Figure 143). Some large mononuclear cells were found in the lumen of the 2.5 month old uterus and many migrating leucocyte-like cells were in and beneath the epithelium. Varying numbers of these structures were observed in all age groups studied. An increase in infolding of the epithelium was the only change noted in the 3 month old specimens. Increased complexity of this was observed in the uteri from the 5.5 and 6 month old dogs (Figure 144). A slight secretion was first observed on the epithelial cells in this age group (Figure 147), as well as numerous leucocyte-like cells as described above. No other changes were observed until 10 months of age. In these uteri, the epithelium had reached a height of 19 to 23 microns, the cells were ciliated, contained basal nuclei and moderately dark stained apical cytoplasm. Two of these were observed to be in estrous at the time tissues were taken (Figures 151, 152). In two of the 11 month old specimens studied the epithelium was 9 to 12 microns in height and appeared like that of the 7 to 9 month old series. The other specimen, from which tissues were taken 14 days pp contained a tall (12 to 16 microns) highly proliferated, complexly folded columnar epithelium (Figures 153, 155). Neutrophils and mononuclear cells filled the
epithelium. This uterus was from a dog that had severe mastitis, pyometra, and a heavy ascarid infection at the time of death. She had died 14 days after a C-section had been performed.

One year of age to 4.1 years of age  The lumen of a 3 weeks pp specimen contained collagenous masses, leucocytes, cell debris and epithelial cells that had sloughed off the 23 to 32 micron high epithelium (Figures 156, 157, 159). It was similar to the last one described for the 11 month age group except that it lacked the complex infolding. The nuclei of these cells were light staining and basally located. In another one year old specimen with tall columnar epithelium the nuclei were stained darkly and were uniformly apical in location. Of the 13 dogs studied in the one to 1.3 year age group, 9 had tall columnar epithelium but lacked the proliferation of the specimens described above. The others were lined with cuboidal cells 7 to 9 microns in height, and had shrunken, infolded areas. One of these (Figure 161) was taken one month pp.

This trend was continued in the 13 uteri studied for the 1.7 to 4.1 year span. Seven were in various stages of proliferative complexity (Figure 168) while the remaining 6 were in regressing phases which had no relationship to increasing age.

Six years of age to 10.4 years of age  The 6.4 year old, 4 month pp uterus contained a 12 to 14 micron high, light staining epithelium in early stages of proliferation. Numerous leucocytes were observed in it. According to records, the uterus from the 6.6 year old dog was taken one week pp. Literally filling the uterine lumen were: conglomerates of leucocytes and cell debris, collagen fiber masses and larger, fairly intact but detached masses of the complexly folded epithelium in various
stages of degeneration.

Uteri from 18 dogs were studied for this 6 to 10 year age period. Three were in early proliferative stages, 4 in more advanced proliferative stages and 22 in various stages of regression. The variations appeared to be due to the phase of the estrous cycle that the dogs were in when the tissues were collected. Some showed degrees of endometrial hyperplasia (Figures 177, 179, 183). Even in this state a bm was visible (Figure 179).

Over 11 years of age The three general categories as described above, early proliferative, proliferative and regression contained 4, 2 and 4 specimens respectively. Ten uteri were studied for this age span which covered from 11 to 13.1 years of age. The oldest specimen from which a highly active proliferative epithelium was taken was a 12.1 year old Fox Terrier. The epithelium were low columnar to cuboidal in the regression state (Figure 190) even though the underlying glands had tall columnar lining cells.

Lamina propria and glands

As was found with regard to the uterine epithelium, the La Pr and glands of the uterus varied with the stage of the estrous cycle. Mononuclear or lymphoid-like cells were observed in all cyclic phases. Four layers of the endometrium, as described by Bloom (1954) were evident in most of the sections studied: (1) the outer or crypt layer, (2) the stromal layer of connective tissue, (3) the tubulo layer and (4) the innermost or basal gland layer (Figure 168). Thus the development or regression of these various components determined the thickness of the La Pr. Calculations were made to determine what proportion the La Pr plus epithelium was with respect to the total uterine wall. The epithelium was included
in these measurements for comparisons with the study done by Hadek and Getty (1959) on the sow uterus.

**Chronological appearance**

**Birth to 2 months of age** The total endometrium, epithelium and La Pr of the 2 day old canine uterus comprised 51 percent of the uterine wall of 240 microns total thickness (Figure 138). No glands were found in the La Pr of this specimen, although there were indentations in the epithelium. Just beneath the basement membrane (Figure 140), of the uterine epithelium were a few generally circularly disposed loosely arranged collagen fibers. The free ends of these splayed out into the La Pr. The La Pr consisted of stromal cells that contained large, moderately dark staining, round to oval nuclei that had nets and clumps of chromatin. Outer portions of the La Pr contained an aggregation of loose collagen fibers that blended into the muscularis. By one week of age the endometrium comprised 52 percent of a 322 micron uterine wall. A few shallow epithelial invaginations were still present, but no glands. Subepithelial collagen fibers were more prominent than in the younger specimen and had a definite perpendicular orientation with the outer uterine wall. These fibers although sparse were found throughout the La Pr.

In the 2 week old specimens, the endometrium made up 62 percent of a 467 or 244 micron uterine wall (Table 3). Infolding of the epithelium to form gland crypts was observed for the first time (Figure 141). A few rings of epithelial cells 14 to 19 microns high were found in the subepithelial La Pr, and in some cases the developing glands extended half way through the La Pr. An increase in collagen, elastic fibers and stromal cells resulted in a more dense La Pr. The connective tissue fibers had a
gentle swirl-like orientation perpendicular to the muscularis. Large mono­
nuclear cells with granular nuclei were observed in the La Pr.

Two and one half months of age to 11 months of age By 2.5
months of age the endometrium made up 57 percent of the uterine wall which
was now 630 microns in thickness (Figure 143). Gland components, crypts,
rings and tubes, were more common than previously observed and the epithelium
of these structures varied from 10 to 12 microns in height. They were
light staining. Some of the glands extended nearly to the muscularis.
Stromal nuclei of the La Pr were more numerous than in younger specimens
and the La Pr appeared in general more dense. Plasma cells, neutrophils
and mononuclear cells were observed.

In one of the 3 month old specimens the major change observed in the
La Pr was a 3-fold increase in gland development over that observed in the
2.5 month old uterus. The other was as the 2.5 month old uterus. The
gland cells were taller (10 to 12 microns) than the epithelial cells (7 to
10 microns), and lighter staining. By 5.5 to 6.5 months of age the endo­
meterium of the 4 specimens studied still comprised about 60 percent of the
uterine wall which had now increased and had a mean total thickness of 980
microns (Figure 144). The glands were more numerous and in some cases the
tubular portions extended nearly to the muscularis. The connective tissue
elements including reticulin (Figure 145), although still oriented per­
pendicularly to the muscularis were of greater density than in the younger
specimens.

Uteri from 6 dogs were examined in the 7 month age group. The uterine
endometrium from 2 Beagles made up 61 percent of the mean total wall thick­
ness (970 microns) compared with 55 percent of mean thickness (670 microns)
for 4 Springer Spaniels. Even the glands in the Beagle uteri were longer (17 microns compared with 11 microns) and appeared to have been more active than those of the uteri from the Springer Spaniels. No changes were observed in the connective tissues of the La Pr.

Nine specimens were studied that were between 8 and 11 months of age. Cyclic changes had begun to effect the size of the La Pr, (Figures 150, 151, 153). It had a range of 500 to 1600 microns or a mean of 760 microns and made up 24 to 67 percent of the total uterine wall thickness. The 2 specimens that were known to be in estrous at the time tissues were collected had the thicker La Pr, which in both cases comprised 50 percent of the uterine wall (Figure 151). The La Pr from a 14 day pp uterus, in an interzonal area made up only 24 percent of the thickness (Figures 153, 154). Thus it was observed that growth changes had ceased to be a major factor of the size and relative width of the La Pr by this age, as cyclic changes had become more prominent. In conjunction with these changes the gland cell nuclei varied in stainability and in location of nuclei which varied from basal to apical or lacked a pattern completely.

One year of age to 4.1 years of age From one to 1.7 years of age, 13 uteri were examined. The proportion of La Pr varied from 30 to 60 percent of the uterine wall with a range of 1200 to 2300 microns in thickness (Table 3). The glands varied greatly in number and size. Their epithelial height ranged from 8 to 20 microns. When the glands were numerous the orientation of collagen fibers changed from perpendicular to parallel, to the muscularis or were without definite orientation. An increase of collagen was seen surrounding the arterioles of the La Pr. Increased reticulin (Figure 158) was observed in a 3 week pp specimen
as well as many degenerating cells and leucocytes. Cysts large enough to fill a low power field were observed near the muscularis in a 1.1 year old specimen in metestrous (Figure 160). They were lined with a layer of flattened epithelial cells and were filled with debris. Ciliated cells were found in the glands of a 1.2 year old specimen that was from a dog in late anestrus when tissues were collected.

The La Pr from the 12 specimens between 2.7 and 4.1 years of age comprised 25 to 50 percent of the uterine wall. The range of 1100 to 3900 microns total wall thickness (Table 3) was slightly greater than observed in the younger specimens. The overall mean of uterine wall thickness for the one to 4.1 year age group was 2000 microns, and the La Pr 700 microns (Table 4). Cyclic changes were still the predominant changes observed. Not only were the glands varied in number and appearance but also the connective tissue stroma varied in density and content. Although dense in most cases, it was loose and appeared edematous in some, especially in the La Pr from a dog that was in late anestrus. Hemosiderin was observed in the La Pr of pp specimens (Figures 163, 164). Large blood filled sinuses were found in the cellular, pigment containing La Pr from a 3 year old uterus. Collagenous structures, replacing sizable portions of the endometrium were found in a 4.1 year old Beagle. The remainder of the La Pr was composed of scattered collagen and elastic fibers as well as masses of pyknotic nuclei.

Six years of age to 10.4 years of age  The La Pr of the 16 specimens in this age group comprised from 25 to 60 percent of the uterine wall that had a range of 200 to 1500 microns in thickness (Table 5) and a mean thickness of 550 microns. The total thickness of the uterine wall was
from 1000 to 3000 microns, with a mean of 1800 microns (Table 4). Thus, it was found that the mean La Pr thickness was between that of the 8 month to 11 month age group and the one year to 4.1 year age group. The total uterine wall was thickest in the specimens that were 6 to 10 years of age. In the 6.6 year old specimen from which measurements were not included in the statistical analysis, tissues were taken one week pp. The La Pr and total wall thickness were 1200 to 3650 microns respectively, which was much greater than in any of the other observed. The 6.4 year old specimen was 3.5 months pp when tissues were collected and the measurements were 500 to 1700 microns respectively (Figures 169, 171). Except for excess development resulting from pregnancy, the glandular, stromal changes were typical of those seen with normal cycling. When only a few glands were present, as in anestrous or early proestrous, the collagen fibers had a definite perpendicular orientation to the muscularis. As glandular proliferation took place during later proestrous, estrous, metestrous or pregnancy the fibers were either disoriented completely by virtue of displacement, or oriented in a circular manner, parallel to the muscularis. In early metestrous, a typical 3 layered La Pr was observed. It had a superficial crypt and gland layer, a middle one third of aglandular edematous connective tissue and a deep comparatively thin-walled gland portion.

The uterine La Pr from a 9 year old African Basenji was nearly filled with various sized cystic glands and was diagnosed as cystic endometrial hyperplasia (Figures 177, 178, 179). Glands large enough to fill a low power field and lined with simple columnar to tall, ciliated pseudo-stratified, columnar epithelium nearly filled the lumen of the uterus.
Some of the glands contained a PAS positive secretion. A dense collagenous stroma filled in the spaces between the cysts. The outer half of the La Pr next to the muscularis was filled with smaller structures, nearly typical of the uterine glands of pregnancy, but larger. Superficial to the latter thin walled glands were those with smaller lumens. These were lined with tall, ciliated pseudostratified columnar epithelium.

**Over 11 years of age** The La Pr of this age group had a mean thickness of 500 microns and a uterine wall thickness of 1600 microns (Table 4). Glands found in these La Pr showed the different functional phases previously described from low columnar epithelial cells that had pyknotic nuclei to tall ciliated columnar cells with light staining characteristics. Stromal features were as described earlier also. Cysts were found in the La Pr of 6 of the 10 specimens studied in this age group (Figure 191). There were usually at least 2 in a section and they varied from 30 to 200 microns in diameter. Some were lined with flattened to cuboidal epithelium, some with ciliated pseudostratified epithelium and others with a combination of both. Secreted substance was usually found in the lumen. The cysts were observed in uteri that were in various cyclic states. In some of them only few glands were present and most of the La Pr was composed of connective tissue stroma, while others had many glands and had been in a secretory state.

**Myometrium**

The myometrium of the canine uterus was generally composed of 5 distinct layers of tissue (Figure 143). Innermost was a layer of circular smooth muscle, the ICM. Although in some cases bundles of oblique fibers were observed out from the ICM, the next layer was the VL. It consisted
of circularly oriented collagen fibers, some muscle bundles, arteries, veins and lymphatic vessels of various sizes and nerve bundles. Numbers of muscle bundles, longitudinally or obliquely oriented were highly variable from specimen to specimen. The outermost layer of the muscularis consisted in most cases of a narrow layer of ELM bundles, which was interspersed with bundles of collagen fibers. The whole organ was surrounded by a collagenous band of fibers covered by the serosa.

All three of these layers varied in thickness and density in different specimens, depending upon growth, development, cyclic state and age. Increases in collagen content, elastic or elastoid products were observed with age, although cyclic variation of these elements were in evidence as well.

In addition to the above structures, the myometrium showed some change in fiber direction especially involving the 2 outer layers of the mesometrial attachment and the aggregation of tissue directly opposite the mesenteric attachment (Figure 150). This disorientation of fibers was not an important part of the investigation, but it presented some difficulty in taking uniform measurements of the muscularis components.

**Chronological appearance**

**Birth to 2 months of age** In the 2 day old specimen there was no distinct line of demarcation between the La Pr and muscularis but rather a blending of tissues (Figures 137, 138). The embryonic smooth muscle cells were circularly oriented, contained large, light staining, oval nuclei and were embedded in circularly directed, fine collagen and reticular fibers (Figure 139). Out from the ICM was a narrow band of collagen fibers and stromal cells with a few blood vessels and nerves covered with
a serosa. No ELM was discernable. By one week of age the ICM layer was more cellular and at 2 weeks was a condensed band of muscle fibers. A thick collagenous, reticular and elastic VL was observed in this specimen and a few bundles of longitudinal muscle cells were observed under the serosa (Figures 141, 142).

Two and one half months of age to 11 months of age In the 2.5 month old specimen the ICM was a well differentiated structure with finite boundaries. It was composed of densely packed nuclei, circularly oriented collagen fibers and a few perpendicularly arranged elastic fibers (Figure 145). This layer was 90 to 100 microns in thickness and comprised 17 to 33 percent of the muscularis. The VL, also 90 microns thick, was made up of collagen, reticular and elastic fibers and contained the usual structures. The outer layer, the ELM layer was not as uniform in width as the inner layers of the muscularis and contained more elastic tissue than was found in the ICM. In some of the 3 month old specimens studied, an increase of collagen fibers was observed in the ICM and the VL, while in others they were also increased in a prominent ELM. This latter muscle was but poorly developed in one of the 3 month old specimens.

In most of the 5.5 to 6.5 month old specimens the muscularis had doubled in size from the 3 month old series (Figure 144). The ICM of this group were generally dense and infiltrated with collagen and reticulin (Figures 145, 146). Obliquely oriented bundles of smooth muscle were observed for the first time in the VL among the increasing amount of elastic fibers (Figure 146). No significant changes were noted in the 7 month age group, but in the 8 month old specimen the obliquely oriented fibers of the VL formed a definite, complete, though narrow ring of muscle. The VL of
the 9.25 month old specimen had 2 such rings of smooth muscle in the VL, one on each size of the vessels.

By 10 months of age the muscularis had grown in thickness to near 1000 microns, from the 400 micron thickness of the younger age groups. Growth *per se* had apparently reached its peak by this time as wide fluctuations in the thickness of the muscularis were seen in older specimens. The amount of connective tissue elements infiltrating the muscularis was also variable. Tissues had been collected 2 weeks pp from one of the 11 month old bitches (Figure 153). As involution had not been completed, the uterine wall was still very thick, 2250 microns. The ICM, VL and ELM made up 53, 18 and 29 percent respectively of the uterine wall, so it was readily observed that the ICM had increased the greatest amount during pregnancy.

One year of age to 4.1 years of age The most prominent change observed in the 14 specimens from one to 1.7 years of age, was a definite increase of collagen infiltration of the muscle tissues, especially the ICM and to a lesser degree in the ELM. Thickness of the muscularis varied from 400 to 1400 microns with the ICM forming 35 to 50 percent of this in most cases. A continued increase in collagen infiltration of the muscularis was observed in the 12 uteri of the 2 to 4.1 year age span, but was especially prominent in 2 of the 2.9 year old specimens, in which it comprised 80 to 90 percent of the muscle layers. In one of these the circular orientation of the ICM had been altered and had a criss-crossed effect. The VL was nearly non-existent in this specimen, while in some of the others with infiltration the VL's were very well developed.

Cyclic changes were observed, although they were not as prominent in
the muscularis as in the endometrium. In the 3-year-old Beagles in late
estrus or early metestrus, the muscle cells were hypertrophied and had
large nuclei. The muscle layers were thickened and the VL more fully de-
veloped than in other specimens of the same age that were in anestrous
(Figure: 167). The 4.1-year-old specimen was in anestrous and the muscle
cells of the ICM were in a disorganized state, shrunken and with vacuolated
cytoplasm. This phenomenon was also observed in the ELM but was not as
extensive.

Six years of age to 10.4 years of age  Tissues were taken
from a 6.4-year-old Beagle 3.5 months pp. Involution was complete and the
uterus was in an anestrous state (Figures 169, 170). The muscle layers
were dense, well defined but not concentrated. Very little collagen was
observed in the ICM. The muscles from a uterus that was in its first week
of involution, from a 6.6-year-old animal were still hypertrophied (Figure
174), but undergoing dissolution. They were vacuolated, in disarray and
heavily infiltrated with collagen. Other than collagen infiltration no
degenerative changes were observed in the VL or the ELM.

Varying degrees of collagen infiltration, especially of the ICM
(Figure 175) were observed in 9 of the 18 specimens studied in this age
group. Ten to 20 percent infiltration was observed in 3, 40 to 60 per-
cent infiltration in 5, and 95 percent infiltration of dense, coiled
collagen fibers in one. In one with 60 percent infiltration, from a 9
year-old African Basenji much disorientation of the ICM was seen and in a
10.3-year-old there was a disorientation of all the muscle layers (Figure
181). The endometrium from these uteri were cystic. The specimen with
the 95 percent infiltration of the ICM also had an estimated 80 percent
infiltration of the muscles in the VL and ELM and was from a 9 year old Golden Retriever.

Within the ICM of the uterus from a 10.2 year old Norwegian Elkhound were 3 glands. The 2 larger ones had 25 to 35 micron lumens and epithelial cells 60 microns in height with basal nuclei (Figure 182). They were similar in appearance to uterine glands of the La Pr and surrounded by stromal tissue also similar to that of the La Pr. They were cone-shaped structures 40 microns long and 20 microns at the base. Four other smaller focal areas were also seen.

It was observed that the VL comprised a mean of 38, 29 and 29 percent of the muscularis in the younger age groups (I, II, III) while it was up to 38 percent of the muscularis again in Group IV. This latter increase was due to increases in collagen tissue as well as in larger and more numerous vessels, especially thick walled arteries and veins (Figure 180). The walls of these veins were composed of a high percentage of elastic tissue.

Over 11 years of age The mean thickness of the muscularis for the 10 specimens in this age group (11 to 15.1 years of age) was 1060 microns as compared with a mean of 1200 microns for the 6 to 10 year age group (Table 4).

Collagen infiltration of the muscularis was observed in 9 of the 10 uteri (Figure 189). It was minor infiltration, about 10 percent in 5, 20 percent in 2, 30 percent in one and 85 percent in one. In all except 2 cases in which the uteri were in a metestrous state (as determined by ovarian appearance) the uteri were in anestrous. In these 2 cases the collagen infiltration was most prominent. One of these, an 11.2 year old
Fox Terrier, had a greatly disoriented ICM with bundles and masses of fibers directed in numerous oblique directions as well as circularly. In one portion of the ICM was a 150 micron wide rupture that lamina propria contents had filled. The other, an 11.9 year old Beagle that had whelped 5 litters of pups in the first 6 years of her life had not whelped any in the last 5 years, had about 30 percent collagen infiltration and pronounced disorientation of the ICM. In the uterus of a 11.5 year old Wire Haired Terrier a small 300 by 350 micron leiomyoma composed of hypertrophied muscle fibers was found embedded in the ELM in the ear of tissue opposite the mesometrial border (Figures 185, 186). It was encircled with a narrow broken band of collagen. The surrounding muscles of both ICM and ELM were atrophied and had pyknotic nuclei. A larger mass of swirled, and criss-crossing collagen and muscle fibers 2200 by 2400 microns in size was found embedded in one wall of the uterine muscularis of a 11.9 year old Beagle (Figures 187, 188). This leiomyoma displaced the entire thickness of the ICM at one point, bulged into the La Pr of the uterus on one side and into the VL and ELM on the other. It was enclosed by a band of collagen, 50 to 150 microns thick that varied from a dense band to a more loose structure that infiltrated the surrounding muscle cells. The stromal cells of this structure were large and light staining. Large mononuclear cells (2 to 3 per oil immersion field), the nuclei of which contained interspersed clumps of chromatin were observed throughout the mass.

The VL of these older specimens was not a uniform layer as it had been during the first few years of life. It was often very wide for half to three fourths of the way around the uterus and then nearly non-existent the rest of the way (Figure 191). An increase in both collagen, elastic
tissue and elastoid was observed in the VL of older specimens as well as an apparent concentration of the cells so that the muscle layers were masses of nuclei with very little cytoplasm (Figures 191, 192).

**Vasculature**

Histologically the vasculature of the canine uterus consisted of: (1) subepithelial capillaries, (2) capillaries, arterioles and venules of the endometrium, (3) arterioles and arteries of the ICM, (4) vessels of the VL. The ELM and serosa were supplied by the vessels from the VL and had no significant vascular system of their own. Arteries of the ICM appeared to be primarily concerned with getting through the ICM to the endometrium (Figure 148), and to be only secondarily concerned with vascularization of the ICM per se.

The state of vascularization of the canine uterus depended upon several factors and the interrelationship of these factors, namely: (1) growth changes, (2) cyclic changes, (3) pregnancy changes and (4) age changes. Although primary concern in this study was only for (1) and (4), the others were described as they were interrelated.

**Chronological appearance**

**Birth to 2 months of age** In the 12 hour and in the 2 day old specimens the vascular system consisted of numerous capillaries under the epithelium, few capillaries in the La Pr and ICM areas, a few capillaries, arterioles and numerous venous sinuses in the VL. The arterioles had only one layer of smooth muscle. By one week of age an increase in vessels was observed in all layers of the uterine wall. Considerable elastic tissue elements were observed in the adventitial area of one of the larger arteries found in the VL. This artery was located at the point of junction of
the uterus and mesometrium. No change was observed in the endometrial or ICM vessels of the 2 week old specimen, but capillaries and arterioles were more numerous in the VL. The arteries had up to 2 layers of muscle cells and definite IEM. Veins were observed, the walls of which were composed of smooth muscle cells, collagen and much elastic tissue.

**Two and one half months of age to 11 months of age**

Still no change was seen in the endometrial or ICM areas, of the 2.5 month old specimen. Up to 3 muscle layers were observed in the arterioles of the VL. The larger arterioles were generally grouped in the VL at opposite poles of the uterus as it appeared in cross section. One was in the mesometrial attachment area and the other opposite this in the embryological ridge that contained tissues of the VL and smooth muscle aggregations from the ELM. The IEM of these arterioles was complete. In the larger veins, 2 to 4 fairly constant rings plus additional incomplete rings of elastic fibers were observed.

Only capillaries were found in the endometrium and ICM up to 5.5 months of age, at which time small arterioles, with one muscle layer were observed in the ICM. Numerous arterioles were found in the VL by this age. The larger ones contained up to 4 smooth muscle layers and had prominent complete IEM.

In some of the 6 and 7 month old specimens studied, the vasculature was as described above, while in others a subepithelial plexus of capillaries had formed in the endometrium and the VL contained more and larger vessels. In one of these more vascular uteri, from a 6.5 month old specimen, arterioles extending from the ICM up through the endometrium to the subepithelial area were observed for the first time (Figure 148). Early
localized intimal change was observed in an arteriole found in the uterine VL from a 7.1 month old Springer Spaniel (Figure 149).

A gradual increase in numbers and size of the vessels was observed up to 10 months of age. Early intimal splitting was found in one 10 and one 11 month old specimen. In a uterus from one 10 month old dog many capillaries and arteries were observed in the La Pr, and numerous arterioles had penetrated the ICM from the VL. Arterioles with 2 to 3 muscle layers were found in 11 month old specimens. In this age group the VL was composed chiefly of small arteries with up to 3 or 9 muscle layers enclosed by a thick band of collagen. Early IEM splitting and up to 10 percent intimal thickening was observed, as well as collagen infiltration of the tunica media from the adventitia area. Changes in the arterial walls were observed in only one or 2 vessels per specimen. Large veins in which the smooth muscle cells and elastic tissue overshadowed the collagen content of the walls were observed.

One year of age to 4.1 years of age In 11 out of 14 specimens that were between one and 1.7 years of age when tissues were taken, some degree of early intimal thickening and/or collagen infiltration of the tunica media was observed. In most, these changes were mild, 10 to 15 percent involvement (Figure 162). In 2 it was more severe with 40 to 60 percent collagen infiltration of the thick walled arteries. Some were embedded in collagen (Figure 161). In others degeneration of the IEM was observed. Two of those with more severe changes had been in metestrous and proestrous when tissues were collected. The vascular system of the one in proestrous was well developed. An intimal plaque-like structure was observed in one artery from a one year old high protein Beagle and
from a 2.3 year old regular protein Beagle. Ten out of 12 of the 2.3 to 4.1 year old specimens had some degree of IEM hypertrophy or disappearance, intimal thickening or collagen infiltration of the tunica media and/or intima (Figures 165, 166). The collagen infiltration varied from 5 to 15 percent in most to 50 to 80 percent in 2. While intimal thickening was generally 10 to 20 percent, there was one case of 90 percent involvement. The more severely affected uteri were from 3 and 4.1 year old Beagles that were in metestrous at the time of tissue collection. Numerous thickened, collagen infiltrated arterioles were found in the La Pr and ICM of these, as well as many larger vessels in the VL. The other dogs from which tissues were taken for this age group were in anestrous. Thus, although cyclic changes affected the appearance of the vessels, changes were observed even in the vessels from anestrous dogs.

Six years of age to 10.4 years of age Eleven of the 16 specimens of this age group were in anestrous when tissues were collected and these generally had only minor vascularization of the endometrium but wide well developed VL. The arteries of the VL had 10 to 40 percent collagen infiltration often in conjunction with 10 to 90 percent intimal thickening and varying amounts of elastic tissue and elastoid (Figures 169, 170, 171, 172). Minor vascular changes were observed in two 10.3 year old specimens that were in late metestrous at the time of tissue collection. Four early metestrous specimens showed consistently well developed endometrial vasculature with 40 to 80 percent collagen infiltration and/or 40 to 90 percent intimal thickening (Figure 164). Less severe changes, no thickening of the intima, and only 10 to 20 percent collagen infiltration of the tunica media were observed in the arteries from a 6.6 year old Beagle that was
one week pp. Varying amounts of elastoid were found in the tissues around the vessels as well as varying degrees of IEM degeneration. Only a minimum of elastic tissue was found in the one week pp specimen. The veins with the thickest walls and the most elastoid were found in the uteri that also showed severe arterial change (Figure 173). Intimal plaque-like structures were found in only 2 specimens, 9 and 10 years of age respectively. Thus vascular changes that included varying degrees of intimal thickening, collagen infiltration or elastoid formation (Figure 176) were observed in all 18 specimens from this age group.

Over 11 years of age All 10 of the specimens of this age group showed vascular changes (Figures 189, 191, 192). Intimal thickening involved 10 to 20 percent of the arterial wall in the arteries of 5 specimens and 20 to 50 percent in the other 5. Collagen infiltration of the tunica media was lacking in only one specimen, was mild in 3 others, varied from 10 to 20 percent in 4 specimens and from 20 to 80 percent in the remaining 3. Elastoid content of the arterial wall, venous wall and surrounding tissues was a prominent feature of 6 out of the 10 specimens of this age group. Cyclic variations were not as prominent in this group, so the vascular changes that were observed were chiefly due to aging.

Cervix

Epithelium

The cervical epithelium of the canine varied from pseudostratified columnar or stratified cuboidal to stratified squamous, depending upon location in the cervix, stage of growth or stage of estrous cycle. The stratified squamous epithelium in the caudal portions of the cervix ranged
from 2 to about 12 cells in depth. The nomenclature of the different layers was varied as reviewed by Krantz and Phillips (1962) but that of Papanicolaou (1954) was used as follows for this study: (1) basal layer or zone (bz) a single row of cuboidal cells, (2) parabasal layer or zone (pbz) comprised of a varied number of layers, in which adjacent cells were joined by intercellular bridges, (3) intermediate cell layers or zone (imz) which usually consisted of a few layers of flattened cells, (4) intraepithelial cell layer or zone (ielz) the narrowest of the 5 zones, was a distinct independent zone of keratinized epithelium composed of closely packed dark-staining cells with keratohyaline granules, and (5) the outer layer or superficial zone (sz) which was composed of several layers of flat, elongated cells with small, pyknotic nuclei. Most of the sections for this study were from the cranial one third of the cervix.

In general the lumen of the cervix formed a half-moon shaped structure with the points of the moon projecting dorsad in normal position of the cervix. The epithelium that lined the greater curvature of the lumen was said to be related to the body of the cervix and those lining the lesser or inner curvature were said to be related to the suspended, ventrally directed, cervical pillar.

**Chronological appearance**

**Birth to 2 months of age** Cervical epithelium in the 12 hour, or 2 day old specimen (Figures 195, 196), consisted of 2 to 3 layers of moderately dark staining cells that lay on a PAS positive basement membrane. The bz was composed of a single layer of cuboidal cells with large nuclei and the pbz, made up of cuboidal to columnar cells, comprised the remainder
of the epithelium. At one week of age numerous shallow, but abrupt, invagina­
tions of the epithelium into the La Pr were observed (Figure 197). It was lighter staining and contained narrower, more columnar type cells that appeared much more crowded than in the previous specimen. The finding of mitotic activity in the bz increased the validity of this observation. Just beneath the basal layer was a narrow band of collagen that appeared to connect the epithelium to the La Pr. By 2 weeks of age there was a loss of cytoplasm and many pyknotic nuclei were observed. No evidence of mitosis was seen and the layer of collagen fibers was still present in the basement membrane area. There were no changes observed up through 2 months of age.

**Two and one half months of age to 11 months of age** The first changes observed in this series were in a 3 month old specimen in which the epithelium varied from 2 to 3 cells in height, was composed of moderately light staining cells and contained numerous migrating mononuclear cells (Figures 201, 202). The nuclei of these cells were large, usually oval and varied in appearance from dark staining uniform masses to very granular structures. Another 3 month old specimen was lined with a typical tall pseudostratified epithelium. Many shallow invaginated gland areas which in turn were lined by cuboidal to tall columnar cells were also observed. In the cervix from a 5.5 month old dog, the 6 to 8 cell deep epithelium consisted of light staining cells (Figures 203, 204). Columnar cells made up the bz, upon which were 2 to 3 layers of plump polygonal cells of the pbz covered with a layer of flattening cells which formed the imz. Numerous migrating leucocyte-like mononuclear cells were observed in the epithelium of nearly all of the sections studied. No condensed underlying layer of
collagen was found in this specimen. A PAS positive basement membrane although discontinuous was observed. Most of the 6 and 7 month old speci­mens were lined by epithelium similar to that just described, which varied only in number of layers and stainability (Figures 205, 206). An increased number of invaginated areas were observed in the latter specimens.

In the cervix from an 8 month old animal, many neutrophils as well as mononuclear cells were found in the 4 layered epithelium. The cells lining the cervical lumen contained basal nuclei (Figure 207), and dark staining apical cytoplasm that appeared in some cases to be extruded into the lumen of the cervix (Figure 208). The epithelium that lined the cervix of a 10 month old animal that was in estrous when tissues were taken was over 20 cells in depth and many epithelial papillae were observed (Figures 212, 213, 214). The crowded BZ cells contained large vesicular nuclei. Nuclei of the 4 to 6 layered PBZ were round, light staining with one prominent nucleolus and some lesser staining lacy chromatin clumps. The cytoplasm was also light staining. Another 4 to 6 cells in varied stages of flat­tening formed the IMZ, over which was the fourth or narrow IEZ and finally the sz composed of 10 to 12 layers of flattened, laminated-appearing cells with elongate pyknotic nuclei. No leucocytes were observed on this epi­thelium. The epithelium of other 10 month old specimens which were in early meteostrous (Figures 209, 210, 211, 215, 216), lacked the IMZ, IEZ and sz, but the deeper zones were similar to those described above ex­cept for the presence of cells with vacuolated cytoplasm, numerous neutrophils and mononuclear cells.

The cervix from one of the 11 month old specimens which was in mid­pregnancy when tissues were taken had a 2 to 4 cell deep, moderately dark
staining epithelium (Figures 217, 218, 219, 220). It was better developed on the cervical pillar and contained numerous invaginations. Only cells forming the bz and pbz were present, and the apical cytoplasm from some of the cells appeared to have been secreted into the cervical lumen. Other cervixes, from 11 month old anestrous specimens were lined by a ragged appearing atrophic epithelium generally 2 cells in depth. Large areas of autolysis were observed in the latter epithelium.

One year of age to 4.1 years of age The first specimen studied for this age group was from a one year old Beagle that had whelped 3 weeks before tissues were collected (Figures 223, 224). The epithelium had partly sloughed off. Some of the cells were of a tall columnar type with apical nuclei and light staining lacy cytoplasm (Figure 224). Because of their puffed out unique appearance, they were termed popcorn cells, and were similar to cells found in the pp uterus. The epithelium that replaced the sloughed areas was composed of intensely stained tall columnar cells with basal nuclei. It presented a palisade appearance. Stringy secreted material, which almost looked like cilia was observed on some of these cells from the 3 week pp specimen. At 4 weeks pp the epithelium contained no popcorn cells, but was a dark staining pseudostratified structure.

The cervical epithelium from 8 anestrous specimens in the one to 1.3 year age group were similar to those described for the 11 month anestrous group. Some variations in epithelial depth were seen in older groups but no significant differences were observed.

The 1.7 year old specimen, taken at late estrous or early metestrus was lined by a wide stratified squamous epithelium. One cell layer of
light staining healthy appearing columnar cells made up the bz. The pbz was a 2 to 4 cell layer of plump cells. The imz consisted of 4 to 5 layers of flattening cells, covered by a narrow almost imperceptible condensation zone. Adjacent to the uterine lumen was the 10 to 14 layers of flattened cells with dark elongate nuclei which made up the laminated appearing sz. This epithelium rested on a PAS positive basement membrane under which were numerous epithelial papillae that indented the La Pr.

Twelve specimens were studied in the 2.3 to 4.1 year age range. These showed all of the cyclic variations observed in the younger specimens but with no particular visible age changes.

Six years of age to 10.4 years of age The 6.6 year old specimen, taken one week pp, was lined by a 2 to 3 cell thick convoluted layer of tall, light staining, pseudostratified columnar epithelial cells with basal nuclei. In the cervical lumen were clumps of light staining, tall columnar popcorn cells with apical nuclei and numerous neutrophils. The bz in some areas had undergone degeneration to such an extent that large sections of epithelium appeared ready to be sloughed off. In a specimen collected 3.5 months pp, from a 6.4 year old animal there were a few, shallow, epithelial papillae. The smooth baseline supported a 2 cell layer of cuboidal cells with dark nuclei and scanty cytoplasm. Counting the one just described there were 11 out of the 17 specimens studied for this age group with nearly identical cervical epithelium. Variations in stainability made up the only visible difference in these anestrous cervixes.

Of the remaining 5 specimens, 2, an 8.3 year old Golden Retriever and an 8.6 year old Beagle, were lined by a proliferative appear-
ing epithelium that consisted of light-staining, plump basal and parabasal cells with some areas of the lumen cells flattened to form an imz. The cervical epithelium from a 9 year old African Basenji was darker staining, contained the 3 zones described above in a well differentiated state (Figure 233). In some areas the surface cells were flattened, while in others they were cuboidal, had basal nuclei and dark staining apical cytoplasm.

One of the two remaining specimens, a 9 year old Golden Retriever had an epithelial lining and glandular development, especially of the cervical pillar, typical of cystic endometrial hyperplasia (Figures 231, 232), as was found in the uterus from this animal. The glandular epithelial rings were lined with tall columnar secretory cells, while the cysts were lined with low cuboidal to flattened cells. Lining the junction between the cervical pillar and body and lining most of the body was a thick proliferative stratified squamous epithelium with numerous epithelial papillae. Its free border consisted of 2 layers of flattened cells that made up the imz. The cervical epithelium of the other 9 year old Golden Retriever contained all 5 layers in its wide stratified squamous epithelium. Numerous epithelial papillae formed the base (Figure 235). Beneath the epithelium were foci of mononuclear cells (Figure 237).

Over 11 years of age Of the 11 specimens in this age span, 11 to 16 years of age, only one, an 11.9 year old Beagle, had a wide healthy appearing proliferative epithelium with many epithelial papillae and light staining cells. Many neutrophils had infiltrated the epithelium. According to the ovary, this animal was in metestrous when tissues were taken. One other specimen from an 11.2 year old Beagle was in metestrous, but a much later stage and although the epithelium was light staining it had a
vacuolated ragged appearance. Another 11.2 year old Beagle was in late anestrous when tissues were taken and had an epithelium in early stages of proliferation. The cervical epithelium from the 13.1 (Figure 245) and 16 year old specimens (Figure 246) was proliferative and over 6 cell layers thick. Underlying the epithelium was a prominent PAS positive basement membrane (Figure 245). A quiescent, 2 to 3 cells deep epithelium lined the rest of the cervixes of this age group.

Lamina propria

Inasmuch as the La Pr of the canine cervix was essentially a connective tissue structure it was composed of reticulin, collagen and elastic fibers. It contained a vascular system and nerves, and served as a cushion between the epithelium and musculature. The dynamic variability in thickness, density and composition of the cervical La Pr depended upon the maturity of the animal, the area of the cervix from which the section was taken, stage of the estrous cycle, pregnancy, and age of the animal. Although this investigation was concerned primarily with growth and age changes, it was recognized early that the other changes had to be described and differentiated so that the primary concerns could be resolved.

Because the specific area from which the sections were taken was not predetermined and because this affected the width of the La Pr measurements of the latter were not attempted. Only general terms such as wide or narrow, with respect to the other cervical wall components were used to give indication of the thickness of the La Pr (Figures 203, 206).

Chronological appearance

Birth to 2 months of age  The La Pr of the 2 day old canine cervix was a uniformly wide structure that contained large, round to oval
stromal nuclei, capillaries, blood elements and a limited amount of collagen (Figures 193, 194, 195). Even at this early age the density varied, being prominently less dense and nearly devoid of collagen fibers near the epithelium but with a definite basement membrane (Figure 196). The increased density of the outer half of the La Pr was due to the collagen content. In the one week old specimen (Figure 197), a number of large mononuclear cells were observed in the La Pr. By 2 weeks of age (Figure 199), it was a less dense structure with regard to numbers of nuclei per unit area but had an increase of loose multidirectional collagen fibers. From 2 to 5 weeks of age a gradual increase in total size of the cervix, number of stromal cells and especially in density of the collagen fibers was observed.

Two and one half months of age to 11 months of age The less dense subepithelial and more dense outer or deeper La Pr were prominent features of the 2.75 month old specimens. Many mononuclear cells were found in this and the 3 month old specimens, especially in the subepithelial zone (Figure 201, 202). Although the cervices from the 5.5 and 6 month old specimens were more than 4 times larger than those of the 3 month old specimens, the La Pr was relatively narrower, and only moderately dense. Vacuolated cytoplasm was a common feature of the subepithelial stroma cells. This area appeared edematous in the 5.5 month old (Figure 203) and some of the 7 month old cervices while the deeper zone was more dense and composed of mature wavy or spiral collagen fibers, elastic fibers as well as stromal cells. Two of the 7.1 month old specimens were as those just described, while 2 others had a shrunken degenerated appearance with many pyknotic nuclei and migrating mononuclear cells (Figure 206). The
edematous subepithelial areas and narrow La Pr prominent in the 8 and 9 month old specimens (Figures 207, 208) were not observed in some of the 10 month series (Figure 209).

Two of the 10 month old cervices were from dogs that were in estrous when tissues were taken and were more than twice as large as any found in the younger groups. The La Pr was a wide structure and composed of uniformly dense, helical collagen fibers, with generally sparse stromal nuclei. A narrow subepithelial zone that contained an increased concentration of these stromal nuclei was the only variation found in these specimens.

In the cervix from another 10 month old dog, in early metestrous, the La Pr in the cervical body was very similar to that described above as found in the estral phase, while in the area of the cervical pillar, a narrow edematous appearing zone was observed. The deeper connective tissues had lost their uniformity and had become more concentrated into bands so that tissue spaces were observed between them. The concentration of stromal nuclei was greatly increased. In an estrous this degenerative, atrophic phenomenon continued as observed in the cervixes of 2 other 11 month old specimens. The La Pr were still wide structures. That contained dense stringy collagen fibers and a few shrunken stromal nuclei. A narrow edematous zone was found in the cervical body, while it was much wider in the cervical pillar. Stromal nuclei were so abundant as to appear dense in some areas (Figure 216). The cervical body of a specimen that was in midpregnancy when tissues were collected (Figures 217, 218, 219), contained moderately dense connective tissue and stroma, while the whole cervical pillar was of edematous appearance and the connective tissue elements formed only a sparse net throughout.
One year of age to 4.1 years of age  

The first one year old specimen studied was from a 3 week pp animal. The La Pr consisted of masses of pyknotic stromal nuclei decreased cytoplasm, degenerated, shrunk collagen fibers and in general a ragged appearance (Figures 223, 224). A 4 week pp cervix was even more severely degenerated than the 3 week one just described. Nine of the one year age group specimens were in anestrous and the La Pr of these was generally dense. A slight decreased density of the cervical pillar tissues was observed in these. Masses of pyknotic nuclei in the subepithelial areas were often, but not always observed in the anestrous specimens. A narrow subepithelial zone of edematous like tissue was observed especially in the cervical pillar, but also to lesser extent in the cervical body of those specimens that were in late anestrous.

In all 12 of the specimens studied from 2.3 to 4.1 years of age, cyclic changes were observed. Variations in the width of the edematous appearing subepithelial areas, in density of the collagenous tissues and in cellular density were found. The latter were the changes related to aging, and were so inconsistent that definite trends were not established.

Six years of age to 10.4 years of age  

Of the 17 specimens studied to represent this age span, 10 were in anestrous, 5 in metestrous, one, a week pp and one in estrous when tissues were taken. The cyclic and postparturient changes were very much as those described for younger animals. A general increase in connective tissue was observed but was not prominent.

In one cervix, from a 9 year old African Basenji (Figure 223), the
La Pr was narrow, but was composed of dense healthy collagen and numerous large stromal nuclei. It was typical of estrous. The uterus of this animal was filled with cystic endometrial hyperplasia tissue and it was felt that the appearance of the cervical tissues resulted from the abnormal endometrium and was not indicative of normal cycling. In a 9 year old Golden Retriever (Figure 231), the La Pr was cystic.

**Over 11 years of age** Eleven specimens ranging in age from 11 to 16 years of age were included in this age group. Eight of them were in anestrus, one in metestrus and one in estrus when tissues were collected. In one cervix from a 16 year old Cocker Spaniel no ovaries, oviducts or uterus were available for study so the stage of cycle was not determined. However, 5 lymph nodules of various sizes were found in the subepithelium of the La Pr of the cervix. Also in this specimen there was a significant amount of elastic tissue among the collagen fibers.

It was felt that there was an increase in collagen density in the cervical La Pr of this age group (Figures 246, 247), over the younger specimens. There was still some evidence of cycling even in the older specimens. Tissues were collected from all except one of these older age dogs between May and August. That one was collected in January. It was felt that if these older dogs were cycling they were more apt to have been in active phases during the spring and summer months than at other times and yet the cervical La Pr of the one in metestrus was no different from the others. Only the La Pr from a 11.2 year old Fox Terrier in late anestrus and from a 11.9 year old Beagle in estrous showed positive healthy cyclic changes.
**Muscularis**

The musculature of the canine cervix was found to be divisible into 4 layers, although only 2 of these were consistently complete layers. From within outward there was: (1) an incomplete internal oblique layer, (2) an inner circular layer (ICM) that varied in thickness but was usually complete, (3) an incomplete vascular layer (VL) and (4) an external longitudinal layer (ELM) that was usually complete. Found in the central and basilar portion of the cervical pillar was a large muscle mass of multidirectional fibers which appeared to be a composite of muscle bundles from both the internal oblique and the ICM. For the sake of description it was termed the cervical pillar muscle. As with other layers of the cervix, the muscularis also varied in thickness and density with cyclic changes.

**Chronological appearance**

**Birth to 2 months of age**  In the 2 day old cervix (Figure 193) there was no definite boundary discernable between the La Pr and the muscularis. There was only a gradual increased density and directional reorientation of the primordial muscle cells in a circular arrangement. No differentiation of muscle layers was observed in this specimen. By one week of age the primordial ICM was fairly well outlined but still blended into the La Pr so that no finite boundary was seen. The primordial smooth muscle fibers had begun to differentiate in the 2 week old cervix (Figure 199), which had 6 to 8 narrow strands of circularly oriented smooth muscle fibers and a few multidirectional fibers in the cervical pillar. An increase of collagen fibers in the muscularis was also observed in this specimen in which a wide band of interlacing collagen comprised the future VL and ELM areas. Muscle bundles were observed in the ELM as well as the ICM.
in the 4 week old specimen, but no other layers had developed.

Two and one half months of age to 11 months of age The ICM and ELM of the 2.75 month old specimen was composed of loosely arranged muscle bundles embedded in collagen. In the 3 month old cervices (Figure 200), all of the layers of the muscularis were well delineated. They were more dense structures in which the muscle tissue per se comprised over 60 percent of the tissue of the muscle layer area, as compared to about half that for the 2.75 month old specimen. From 3 to 11 months of age much variation of muscle development was observed. In the larger cervices, it was better developed than in the smaller specimens. It was observed that the muscle layers of the cervix (Figures 203, 206) contained a much higher percentage of collagenous tissue than was found in the muscularis of the uterus or oviduct. Thus, even though all 4 muscle layers as well as the cervical pillar muscle were usually discernable in specimens over 6 months of age they were prominently embedded in collagen. In the 10 month old specimens that were in estrous, the whole cervix was greatly enlarged. Much of the enlargement was due to connective tissue change rather than muscle change. Hypertrophy of the muscle cells was evident, however, in the cervix that was taken from an 11 month old animal in mid-pregnancy (Figure 217).

One year of age to 4.1 years of age Although the effects of cyclic changes were not as pronounced on the muscularis as on other parts of the cervix they were still discernable. The most prominent changes though were those of pregnancy and involution following parturition. In the latter case there was a loss of cytoplasm resulting in condensed masses of nuclei forming the muscle bundles and much collagenous tissue separating
the bundles (Figure 222). In the inner layers up to 20 percent collagen infiltration of the muscle bundles themselves was observed.

Fourteen specimens between one year of age and 1.7 years of age were studied. Tissues were collected from 4 of these in various stages of postparturient involution which was described above. In one of these taken 4 months pp, involution had been completed and the animal was in estrous again. No collagen infiltration of the muscle bundles was observed in this specimen as was found up to one month pp.

Infiltration of the muscle bundles by collagen was a very prominent feature of the cervical pillar muscle from 3 of these specimens. The multidirectional, often whorled appearing bundles, at the base of the pillar near the ICM, were only mildly infiltrated. Near the apex of the cervical pillar near the epithelium of the cervix, the muscle bundles were 80 to 90 percent replaced by collagen. The outline of the muscle bundles were still evident, in spite of heavy infiltration.

The other 7 specimens between one and 1.7 years of age generally had shrunken or atrophied musculature with only minor collagen infiltration of the bundles, but surrounded by much connective tissue (Figure 227). Up to 20 percent collagen infiltration of the cervical pillar muscle was observed in the cervix from one of these specimens.

In only 2 of the 12 specimens between 2.5 and 4.1 years of age was the musculature atrophied. In the rest it was well developed and healthy in appearance. It was clear of any infiltration in only one specimen, had less than 20 percent infiltration in 7. Thirty to 60 percent infiltration of collagen was observed in 4 specimens. The most severe infiltration was in the cervical pillar and ICM while the ELM was nearly always free from
collagen, except around the bundles of muscle fibers.

**Six years of age to 10.4 years of age** Of the 17 specimens studied in this group, collagen infiltration of the muscle bundles was less than 40 percent in 9 and between 30 and 90 percent in 8 of them. In general the muscles were well developed and with increased age, appeared to have more cells per unit area. This was due to an increased number of nuclei, or a decrease in cytoplasm or both. Often in conjunction with the apparent increased density of musculature there was an increase in infiltration of the muscle bundles by collagen fibers (Figure 239). This phenomenon was much more prominent when comparing specimens from this age group to younger specimens than it was within the 6 to 10 year age group itself.

Two of the cervices from this age group, from 9 year old Golden Retrievers were much enlarged mainly due to an increase in collagen but in part due to hypertrophied musculature which was 80 to 90 percent infiltrated.

**Over 11 years of age** All of the muscles except the ELM of the 11 specimens from this age group were nearly or over 50 percent infiltrated with or in some cases replaced by collagen fibers. The muscle bundles appeared as masses of nuclei. Only a small amount of cytoplasm remained in the cells. (Figures 241, 242). Often these masses of nuclei had a swirled appearance even in the ICM. Even the ELM was affected by the infiltration of collagen (Figure 243) in this age group. VL musculature although sparse in all age groups was infiltrated along with that of the ICM as were the fibers of the internal oblique muscles. Elastic connective tissue was prominent in the muscularis of the older speci-
mens (Figure 248).

Vasculature

Vasculature of the canine cervix consisted of capillaries in the La Pr and various sizes of arterioles and venules in the La Pr muscularis and VL. The larger arteries and veins supplying the cervix were found adjacent to it in the mesentery in what was termed the vascular area (VA) in this study, as different from the VL. Also found in the VL were large nerve bundles and ganglia. As with other portions of the female reproductive tract, the vascular changes varied with growth, stage of estral cycle and age.

Chronological appearance

Birth to 2 months of age  Vasculature of the 2 day old specimen consisted of a few capillaries in the outer half of the La Pr, a few arterioles with one muscle layer and a narrow but definite internal elastic membrane (IEM) in the area of the future VL. Larger arterioles with 2 layers of smooth muscle cells comprising the wall were found in the bilateral mesenteries adjacent to the cervix. Capillaries were observed in the subepithelial portion of the La Pr in the week old cervix and the other vessels had increased significantly in size (Figure 198). By 5 weeks of age although the vascularization was still minimal, a definite VL was observed and all arterioles with walls of more than one muscle layer in thickness had a prominent IEM.

Two and one half months of age to 11 months of age  Numerous capillaries were observed extending across the La Pr to the subepithelial area of the 2.75 month old specimen which had a wide VL filled with vessels. The walls of the larger veins of the VL were composed of
collagen and elastic fibers, with no smooth muscle cells. The only change observed in the 3 month cervix, was that the walls of the larger adjacent arteries had up to 6 layers of muscle cells. By 5.5 months of age numerous arterioles and capillaries were found in the La Pr but still the muscularis was devoid of vessels. The VL contained only small vessels while the larger arteries of the VA had 8 to 10 muscle layers, in which were incomplete concentric rings of elastic fibers. Early mild collagen infiltration of the tunica media of this artery was observed. IEM splitting was found in some arteries from a 6 month old specimen. From 6 months on the degree of vascularization in any particular cervix was highly variable. In some of the moderately vascular 7.1 month old and 8 month old specimens, only the arteries with over 3 muscle layers had an IEM. Even in some of the larger arteries it was incomplete. In the large arteries of a 9 month old cervix, the IEM was prominent as indicated for younger specimens. It was incomplete or missing in those arteries with less than 3 to 5 muscle layers. Arterioles with 2 muscle layers were seen in the ICM of this specimen. In the 10 and 11 month old series more splitting of the IEM and early infiltration of the tunica media with collagen was observed. Also there was much variation in the stainability of the IEM. Even when the IEM did not take the elastic tissue stain its outline was usually discernable. Numerous vessels with thick, edematous-like collagenous appearing walls were found throughout the La Pr of an 11 month old specimen (Figures 219, 221).

One year of age to 4.1 years of age

Thick collagen walled vessels (Figure 225) were observed in the La Pr of 4 of the one year old specimens, some healthy in appearance and others vacuolated and shrunken.
Usually they were somewhat oblong in shape, resembling veins or lymph vessels rather than arteries. Vasculature of the La Pr was variable in the one year age group. The IEM of the larger arteries of the VL did not stain in some specimens, but stained darkly in others (Figure 226) of the same age, diet and stage of estrous. In those where it stained well it was often hypertrophied, some elastoid was formed and splitting the IEM was observed. An increased collagen infiltration of the tunica media was observed in many of the larger arteries.

By 2 years of age many of the larger arteries had 10 to 30 percent infiltration with collagen (Figures 228, 229) which often formed a ring of collagen beneath the IEM. Splitting of the IEM and elastoid formation were observed with increasing frequency. Walls of the veins contained dense bands of elastic tissue. The variably staining IEM of some of the large arteries of the 2 year age group stained partly yellow and partly black with Verhoeff's and Van Giesen's stain (Figures 229, 230), and blue and pink with hematoxylin and eosin. The large collagen walled vessels were observed more frequently in these specimens, especially in the La Pr of the cervical pillar region. The first intimal plaque-like structures of this series were observed in the cervix from a 2.9 year old Beagle. In some of the arteries of this specimen there was also a reorientation of the subintimal circular muscle fibers to a longitudinal orientation.

Similar changes but with increased frequency and severity were observed in the vessels of the 3 year old cervices. In the cervix from a 3.6 year old Beagle, numerous, large, extremely thick walled arteries were found in the VL and VA embedded or encircled with thick collars of circularly arranged collagen fibers. An increasingly prominent aggregation of
small thick walled arteries were seen in the lateral cervical pillar area, especially numerous at the junction of the cervical pillar and the ICM. Often connections between these arteries and the larger arteries of the VL were observed.

**Six years of age to 10.4 years of age** An increasing number of vessels were involved with age changes in the 17 specimens studied in this age group. In 15 the IEM either did not stain with elastic stain at all (Figure 234) or only partly stained (Figure 238). In 2 cases it appeared to have been partially replaced with collagen (Figures 236, 238). In specimens over 9 years of age the vessels of the VL and La Pr had a 10 to 30 percent complex of intimal thickening, collagen infiltration and elastoid formation (Figure 238). In 4 specimens the tunica media of the arteries contained from 40 to 60 percent collagen. Intimal thickening was the only change observed in 3 specimens. With the 12 just mentioned, 14 out of 17 that had up to 30 percent intimal thickening. Generally the degree of intimal thickening was about 10 percent greater in the arteries of the La Pr than it was in the arteries of the VL.

**Over 11 years of age** Although there were 11 specimens studied for this age group (11 to 16 years of age), the VL was missing from the oldest one so for discussion of the larger vessels, only 10 were included. In 8 of these the IEM of the larger vessels was missing, did not stain at all with elastic tissue stain or was only partially stained by elastic tissue stain. Portions of the IEM of large arteries in 3 specimens stained positive for collagen.

In these older cervix, most of the vessels in all layers of the cervix had some morphological changes. Combined intimal thickening, collagen
infiltration and elastoid formation that involved 10 to 40 percent of the arterial wall was observed in 6 of the cervixes of this age group. The thick walled veins of one specimen, from an 11.5 year old Wirehaired Terrier were nearly hyalinized, collagenous, elastoid structures (Figures 243, 244). Collagen-walled vessels were also observed in some (Figure 247).

In this age group there was an apparent decrease in total number of vessels, but an increase in the number of vessels in which vascular changes were observed. There was also an increase in the severity of the changes.

Vagina

Epithelium

Growth and cyclic changes were the predominant features of the vaginal epithelium. Variations in stainability and size of cells, as well as in number of cell layers that composed the epithelium, were used to categorize especially the phase of the sexual cycle. Most consistent in the layers was the bz. This cell layer was usually composed of uniform cells which were in most cases aligned perpendicular to the epithelial surface. The pbs composed of an extremely variable number of cell layers, contained round to polygonal cells with prominent nuclei and abundant cytoplasm in all stages of the cycle except late metestrus and anestrus. The imz and sz were often missing. When present they were highly variable in appearance and stainability as they represented numerous stages of flattening, nuclear alterations and nuclear disappearance associated with cornification.

Chronological appearance

Birth to 2 months of age

In the 12 hour and in the 2 day old
vagina the epithelium varied from pseudostratified columnar to stratified cuboidal in appearance and was 2 to 3 cells in depth (Figures 249, 250, 251). The bz cells formed a uniform layer of cells under which was a definite basement membrane (Figure 251). The free surface of the pbz was extremely uneven. By one week of age the epithelial cells were more of a columnar type (Figure 253) and were crowded together. In many areas cytoplasm was extruded from the free surface as if in a secretory state. The lumen surface was uneven but all of the cells were oriented in a perpendicular plane to the free surface. A change from columnar to cuboidal for most of the epithelial layers was the only additional alteration observed up to 2 months of age.

Two and one half months of age to 11 months of age By 2.5 months of age the cells composing the epithelium, although still cuboidal in type, were larger. There was very little observable difference between the single layer of bz cells and the double layer of cells that formed the pbz. The free border was uneven as it followed the cell contour and no flattening of the cells was observed. In the 3 month old specimens studied (Figure 255) the bz cells were again smaller but more active mitotically and epithelial papillae had begun to develop. The only change observed in the pbz was an apparent extruded cytoplasm from the free surface cells. No changes were seen in the bz in the 5.5 month old vagina (Figure 256). The pbz was now composed of 3 to 4 layers of large light staining polygonal cells and the imz, not observed in specimens under 5.5 months of age was composed of a few cells which were oriented parallel to and covering the pbz cells. The lumen surface was still uneven. All except one of the 9 specimens studied in the 6 to 7 month age group were similar
to the specimen just described. That one had considerable evidence of
mitosis in the bz in which the large round nuclei were somewhat darker
staining than those of the other cell layers. The large polygonal cells
of the 3 to 4 cell thick pbz contained light staining nuclei and cyto-
plasm. The imz was one to 2 cells in thickness and the cells were flat-
tened, contained shrunken nearly pyknotic nuclei and formed an even free
surface. Up through the 7 month old specimens, mononuclear leuco-
cytes were rare but in one of the 9 month old specimens they were a prom-
inent feature. In all of the 8 and 9 month old specimens the epithelium
appeared to be in a degenerating state. Many of the pbz cells were shrun-
ken, had pyknotic or lysed nuclei, vacuolated cytoplasm and the imz cells
formed an uneven surface layer.

Extensive cellular growth and regression changes were the predominant
features of the 10 and 11 month old specimens. The first one of this
series showed much mitotic activity of the bz cells such that numerous epi-
thalial papillae had developed. Even in areas between the papillae the bz
cells had a crowded, nearly columnar appearance. Three to 4 layers of
large cells with oval nuclei comprised the pbz and one to 2 layers of flat-
tened, elongate cells formed the imz which had a flat free surface. A few
neutrophils were observed in the various cell layers of the epithelium.
The animal from which this section was taken was known to be in early met-
estrous when tissues were collected (Figure 257). In an early phase of
estrous was the next 10 month old specimen (Figures 259, 260). The
bz and pbz were as described for the previous specimen while the imz was
composed of 3 to 4 layers of flattened cells with faded cell outlines and
light staining round nuclei. Above this zone was the sz, composed of a
layer of cornified cells 4 to 7 cells in thickness. Most of these cells contained flattened nuclei and dark staining keratohyaline granules. In this specimen there were no leucocytes.

The third 10 month old specimen studied (Figure 261) was in early metestrous when tissues were collected. Superficial zone cells and most of the imz cells had sloughed off leaving an undulating free surface. Numerous neutrophils were observed throughout the epithelium. The next 2 specimens studied were both 11 months of age and were in middle and late metestrous respectively when tissues were collected. In the first the epithelium was composed of only 2 layers of cells; the deeper bz cells were darker staining and the superficial pbz cells, many of which had vacuolated cytoplasm and pyknotic nuclei formed a very uneven free surface. The only change in a 2 week pp specimen (Figure 264, 267, 268) was the presence of numerous neutrophils and mononuclear cells in the lumen contents of the vagina. Only one other 11 month old specimen was observed and she was in mid-pregnancy when tissues were collected (Figure 263). In this vagina the epithelial papillae were numerous and well developed. All of the bz cells were darker staining than those of the other cell layers. The pbz was one to 2 cell layers thick. It was composed of columnar cells with basal nuclei, soapy cytoplasm and often an extruded mucous secretion was observed to extend from the cell surface. Neutrophils were numerous.

One year of age to 4.1 years of age To represent the younger animals of this age group 14 specimens were studied that were one to 1.7 years of age. Three of these had whelped prior to tissue collection. At 3 weeks pp the dark staining vaginal epithelium was 2 cell layers thick. The bz cells were small and contained pyknotic nuclei, while the pbz cells
were tall columnar with basal nuclei and were in a secretory state. The epithelium of other specimens at 4 weeks pp were ragged, dark staining and narrow. In fact there were some areas devoid of epithelium.

Seven specimens of this group were in various stages of anestrous when tissues were collected. Although it varied in stainability, the epithelium was generally dark staining, narrow (one to 3 cell layers thick) and composed mostly of shrunken cells with pyknotic nuclei. In two cases possibly late anestrous, the bz layer of cells were large cuboidal structures with moderately dark staining nuclei. The 2 layers of cells that composed the pbz were hypertrophied and contained basal nuclei. A soapy cytoplasm was in some cases partially extruded from the cell. Another of this series was in an early proestrous; the next phase from that just described. In this specimen the bz cells although not large, were columnar rather than cuboidal and the pbz which was 2 cells in thickness was composed of large light staining cells with round nuclei. Still another had gone through proestrous and was in late estrous when tissues were collected (Figure 273). This epithelium was very similar to that described earlier for a 10 month old specimen in a similar stage of estrous and was up to 29 cells in thickness. The outer 10 to 12 cell layers were cornified. No leucocytes were observed so it had not yet reached the last stages of estrous.

Two specimens of this series had gone through estrous and were in middle or late metestrous. The bz cells were small while the pbz cells were tall columnar in form and were extruding cytoplasm. Both neutrophils and mononuclear cells were abundant in the epithelium of these 2 specimens.

Twelve of the specimens studied had been taken from animals that were 2.3 to 4.1 years of age. The chief changes observed were cyclic changes.
Variations in stainability, secretion, number and sizes of cells were observed in these specimens, but no epithelial changes associated with aging per se were pinpointed.

Six years of age to 10.4 years of age All phases of the cycle were represented by the 18 specimens studied for this age period. There was one in proestrous (Figure 280), 2 in estrous (Figures 281, 282, 283), 2 in various stages of involution after parturition, 8 in metestrous (Figures 284, 287, 288) and 5 in anestrous when tissues were taken. There were no visible difference in the epithelium of the various stages in younger animals.

Over 11 years of age Only 13 specimens were available covering the age span from 11 to 16 years. Although the times of the year when tissues were collected were comparable for both the 6 to 10 year and over 11 year age groups, there were only 3 of the latter specimens in metestrous (Figure 296) and 10 in anestrous, with none of any of the other stages of estrous. Histologically the epithelium from a dog in metestrous that was under 6 years of age was no different from one in metestrous that was 12 years of age. It was interesting to note that in the older age group 80 percent of the specimens were in anestrous and the other 20 in metestrous with none in proestrous or estrous. In the 6 to 10 year age group on the other hand only 27 percent were in anestrous, 50 percent in metestrous and the remaining 23 percent in the other phases of the cycle. It appeared from this data that although cycling still took place in the older animals it was much reduced.

In all specimens studied from 2 days of age (Figure 251) to 16 years (Figures 299, 300, 302, 308), there was a PAS positive basement membrane
present. It was more complete and more prominent in the older specimens.

**Lamina propria**

This structure which composed the major part of the vaginal wall consisted mainly of dense collagen, reticulin, elastic fibers and blood vessels. It formed a varying number of papillae which indented the epithelium. Their size, number and degree of development depended upon cyclic activity. They were most prominent in proestrous and estrous.

Subepithelial cellularity was also a prominent feature of this portion of the vaginal wall. The longitudinal rugae, formed chiefly by La Pr tissue, increased in numbers during the growth period and increased or decreased in size depending upon cyclic state.

**Chronological appearance**

**Birth to 2 months of age** The La Pr of the 12 hour and the 2 day old canine vagina (Figure 249) composed two thirds of the vaginal wall and consisted of a loose embryonic type of tissue. It contained large stromal nuclei and very few fine collagen fibers. No changes were observed in the one week old specimen (Figure 253). By 2 weeks of age, however, (Figure 254) there was a dense zone of increased cellularity and an increase of collagen fibers in the subepithelial area. The rest of the La Pr was increased in density of tissue as compared with the 2 day old specimen but was not as dense as in the subepithelial zone. By one month of age the collagen of the La Pr was of a more mature appearance.

Up through one month of age the lumen was simply oval or elongate on cross-section. In the 5 week old specimen it had 4 definite primary longitudinal rugae in which the moderately dense collagen and reticular fibers were oriented perpendicular to the epithelial surface.
Two and one half months of age to 11 months of age

In the 2.5 month old specimen the vaginal La Pr was essentially as that described for the 5 week old, except that there were now 6 to 7 major rugae. The large perpendicularly oriented stromal nuclei were a prominent feature of this specimen. Three month old specimens had 10 to 16 primary rugae, some of which had secondary infolding (Figure 255). Except for a lighter staining subepithelial zone the rest was dense collagen fibers with numerous fine elastic fibers interlaced. Nineteen primary rugae were found in the 5.5 month old specimen which was unchanged in other aspects (Figure 256). The maximum number of rugae had apparently been established by this age as from 6 to 11 months they varied from 9 to 16 in number with no pattern in relation to increasing age. The next major increase in size and density was observed in the 10 and 11 month old specimens. Two of these, which had been in proestrous and estrous (Figure 259) when tissues were collected, had dense collagenous subepithelial zones which took up about one quarter of the La Pr depth. The other three fourths of the La Pr were composed of loose edematous appearing collagen in which was interspersed numerous fibroblast-like stromal nuclei. Specimens in metestrous had healthy normal appearing subepithelial zones and darker, coarser, stringy, deeper zones of collagen (Figure 261). The La Pr from the tract taken at midpregnancy (Figure 263) was moderately dense, had evidence of edema but was not diagnostically different from those in early metestrous.

One year of age to 4.1 years of age

The La Pr from the pp specimens of the one year age group had a ragged appearance. The collagen fibers had a swirled orientation (Figure 269). Some of these contained subepithelial lymph nodules and were densely cellular. The specimens in
anestrous had uniform, dense collagen fibers that comprised most of the La Pr between which were many elastic tissue fibers. In the specimens that were in a late anestral state when tissues were taken the La Pr was edematous and the rugae distended. Fewer elastic fibers were found in these specimens. This appearance persisted in proestrous with an increase in subepithelial cellularity.

The outstanding change observed in the metestrous specimens was the massive infiltration of the La Pr by neutrophils, especially in late estrous and early metestrous. With increased age from 2 to 4.1 years of age, there was also an increase in overall density and maturation of the collagen of the La Pr. The 3 year old proestrous specimens had a uniformly staining La Pr in which the collagen fibers appeared to be uniformly swollen instead of separated by edema. In another 3 year old specimen that was 7 weeks pp when tissues were taken the collagen fibers were dense but ragged in appearance and prominent stromal cellularity was observed beneath the epithelium. The 4.1 year old specimen was 8 weeks pp. In addition to the above features, this La Pr had numerous pyknotic nuclei among the ragged, disarranged collagen fibers.

Six years of age to 10.4 years of age All phases of the cycle were represented by the 18 specimens of this age group. The one in proestrous (Figure 280), from a 9 year old Golden Retriever, had a wide La Pr composed of dense mature appearing collagen with multidirectional fiber orientation. Large oval stromal nuclei were scattered throughout the La Pr. In general the La Pr was typical of the proestral vagina observed in younger specimens. Estrous in a 9.7 year old Dachshund (Figures 281, 282, 283) was similar to that of younger specimens. The collagen was made up of
uniformly swollen fibers which gave a smooth even appearance to the enlarged vagina. In another specimen, a 9 year old Golden Retriever in late estrous or early metestrous when tissues were taken, the dense mature collagen of the La Pr was filled with multiple focal subepithelially located areas of plasma cells and mononuclear cells (Figure 284). Prominent perivascular cuffing by these cells was observed. Eight of the 18 specimens of this age group were in some stage of metestrous when tissues were taken. In all of these the dense collagen which composed most of the La Pr had lost the uniform swollen appearance of proestrous and estrous. The fibers had become more individually discernable. They were circularly oriented subepithelially and perpendicularly oriented in the rugae proper. Stromal cellularity throughout the La Pr was a prominent feature of all specimens in metestrous, except those in the later stages. The stromal cells of those were found mainly in the subepithelial areas, as was usual for the anestral phase of the cycle as well as for pp. In one 6.6 year old specimen, a Beagle, from which tissues were collected one week pp, the subepithelial cellularity consisted chiefly of plasma cells and mononuclear cells in addition to the stromal cells usually found there. Subepithelial stromal cellularity was especially prominent in the anestral specimens (Figures 285, 287, 288). In addition there was a decrease in total structure of the vagina and thence of the La Pr as found in a 9.1 year old Beagle and a 9.3 year old Irish Setter (Figure 285). In these specimens the rugae had become narrow, leaflike structures that had lost the plumpness typical of younger specimens of a similar cyclic stage. Numerous elastic components were found among the collagen fibers of these (Figure 285).
Over 11 years of age Out of the 13 specimens studied for this age group, 3 were in metestrous (Figure 29G) and the remainder in anestrous (Figure 29J) when tissues were taken. Of those in metestrous, the La Pr was a very prominent part of the vagina. In the younger ones of this series, from an 11.2 year old Fox Terrier, and a 11.9 year old Beagle many areas in the rugae appeared hyalinized. Throughout the La Pr there was a high content of elastic fibers. The third specimen that was in metestrous was from a 12 year old spayed Cardigan Welsh Corgi in which the thyroids were hypertrophied. The La Pr was extremely hypercellular, elastosis was prominent and muscle bundles had invaded about 80 percent of each of the rugae.

The 10 remaining specimens, all in anestrous when tissues were collected were not uniform in appearance. In 3 of the 10 the La Pr was wide and composed of dense wide collagen fibers. The vaginal La Pr from a 16 year old Cocker Spaniel was similar to the latter 3 but in addition had multiple foci of plasma cells, lymphocytes and other mononuclear cells (Figures 301, 306, 307, 308). Hypertrophy of the collagen was also found in this specimen (Figure 303). There were hyalinized areas of collagen in some specimens and in the 4 oldest specimens an abundance of intermixed elastic fibers (Figure 304). An embryological remnant was observed in the La Pr of the oldest specimen studied (Figure 305). It consisted of a group of epithelial cell filled tubules located in the outer one third of the La Pr and surrounded by a dense band of collagen.

Muscularis

The muscle layers of the canine vagina were not well developed but were composed of an ICM, a VL and a more sparse ELM. Although continuous
with the uterine muscularis these layers of smooth muscle fibers were not as prominent. As in this layer of the more cranial parts of the genitalia, any changes observed were seen first in the ICM and then later, and to a lesser degree in the ELM.

Chronological appearance

Birth to 2 months of age  At 12 hours of age and at 2 days of age (Figure 249) the ICM consisted of an ill-defined layer of embryonic cells with a circular orientation. Out from this was a wide future VL composed of connective tissue. There was only sparse evidence of the ELM at this age. By one week of age (Figure 252) the ICM precursor cells made a more prominent circular band. The VL was narrower and more condensed than at 2 days. In the 2 week old specimen (Figure 254) the ICM was a thick circular band while less development was noted in the VL or ELM. A few slender inner longitudinal and oblique strands of smooth muscle were found in the outer La Pr. The ICM was by this time segregated into prominent muscle bundles. Out from this were a few oblique to longitudinal strands, then a definite VL, with a few muscle bundles and an easily definable ELM which consisted of comparatively widely separated but well developed muscle bundles.

Two and one half months of age to 11 months of age  In this series, by 3 months of age (Figure 255) the layers were all easily defined and the muscle bundles were mature in appearance. Numerous oblique fibers were found associated with the inner portions of the internal circular muscle. The only change in the 5 month old specimen (Figure 256) was an increase in development of the ELM up to about one half the width of the ICM. All of the muscle bundles, however, were well delineated and often
semi-isolated one from another by collagen. Interspersed throughout the collagen were many elastic fibers. At 6 months of age many of the oblique fibers were extending up into the vaginal rugae. They extended about one third of the way up in 7 month old specimens, and over half way up in 9 month old animals. The muscle bundles of all layers were very mature morphologically at this age. By comparison with other parts of the genital tract, however, the muscle layers of the vagina were oriented in many directions and didn't show the definite layering seen in the cervix, uterus and oviduct. There was much more collagen in the muscularis of the vagina, even at this young age.

By 10 months of age evidence of cycling had become a prominent part of the muscularis. In proestrous, estrous and early metestrous the bundles appeared hypertrophied, and in some cases the collagen was edematous, especially near estrous (Figure 258). At mid-metestrous the collagen portions of the muscularis were ragged in appearance and by late metestrous even the muscle bundles appeared to be shrunken and fewer in number.

One year of age to 4.1 years of age Cyclic changes were a prominent feature of the muscle morphology in this series. In one specimen, one year of age, that was 3 weeks pp when tissues were taken the muscularis was composed of atrophied widely separated muscle bundles with much collagen infiltration. During anestrous, the atrophy was not as severe but there was a higher percent of collagen in the muscularis. As the collagen became more ragged in appearance in late anestrous, the elastic tissue was decreased. Then at proestrus the muscle cells hypertrophied and the muscularis made up the major portion of the vagina.

Throughout this age span the cyclic effects were prominent. The only
observable morphological change was a total overall increase in mature type dense collagen between the muscle bundles. The highest percentage of infiltration was found in the ICM and the oblique fibers that extended from it into the vaginal rugae.

**Six years of age to 10.4 years of age** Although still a part of the picture, cycling was not as prominent as in the earlier age groups. In addition to the general morphological appearance at estrous, metestrous and anestrous as described for younger animals, there was in this series an increase of collagen infiltration of the muscle bundles themselves. It was more severe in the inner layers but increased to include the outer muscles in the older specimens of this series (Figures 286, 287). Vacuolization of muscle cells was observed with increasing frequency in this age group.

**Over 11 years of age** There were specimens representing all stages of the estrous cycle in this age group, but the collagen infiltration continued. Vaginal muscle cells from an 11.2 year old Fox Terrier in metestrous (Figure 292) and a 11.9 year old Beagle in late estrous showed hypertrophy, typical of the cyclic stage but there was much more mature, dense collagen between muscle bundles. More infiltration of muscle bundles was found than had been observed in the younger specimens at the same phase of the cycle. Most of the specimens in this age group were in some phase of anestrous (Figures 297). In these there was a decrease in cytoplasm in the muscle cells and a resultant apparent increase in prominence of the muscle cell nuclei. Dense collagen separated the muscle bundles and often muscle cells. In some cases it appeared to replace some of the muscle bundles. A general increase in elastic fibers
was noted in the muscularis of the older specimens and in some, elastosis was very severe.

**Vascularization**

Arterial blood reached the vagina via bilateral vaginal arteries. In many of the specimens they had been lost in tissue collection and preparation and so the next largest arteries were those found in the VL. Thus for this study, when the large bilateral vaginal arteries were referred to they were designated as being located in the vascular area (VA) whereas the others were located in the VL of the vagina proper. The remaining vessels were described as to location in the muscularis or La Pr. Large nerves and nerve ganglia were observed in most specimens.

**Chronological appearance**

**Birth to 2 months of age** In this age group there were numerous capillaries in the La Pr and future muscularis. A few arterioles with one muscle layer were found in the VL (Figure 249). Satellite veins were also observed. Often larger arterioles with prominent IEM's and 2 to 3 muscle layers were found in the VA (Figure 252). Thus only minimal vascularization was observed.

**Two and one half months of age to 11 months of age** An increase in capillaries and the appearance of arterioles with one to 2 muscle layers were observed in the La Pr of the younger specimens of this age group. Larger arteries and veins were seen in the VL and VA. The veins contained much elastic tissue. By 5.5 months of age there were numerous capillaries and arterioles in the La Pr, numerous arteries in the VL and arteries and veins in the VA. A prominent, well stained IEM was found in all arteries up to 6 months of age. In a 6.5 month old specimen, with es-
especially well developed vascularization, the arteries of the La Pr with 2 to 3 muscle layers had no discernable IEM, whereas a slightly larger artery in the VL had a prominent IEM. The later artery, however, also showed splitting of the IEM and minor collagen infiltration of the tunica media.

Increased vascularization continued in the 7 month old specimens in which the IEM of arteries in the VL was not apparent. Numerous thick, collagen walled vessels, which were lymph vessels or veins were observed in the La Pr. These structures as well as the variably stained IEM's of arteries, were prominent features of the old specimens from this age group. In many of the arteries in which the IEM was stained there was evidence of minor splitting. A prominent collar of collagen was observed around all of the arteries and veins of those specimens in or near estrous (Figure 262). A gradual increase in vascularization was observed up to 10 months of age after which it varied with changes in the estral cycle.

**One year of age to 4.1 years of age** Cyclic changes were prominent features of this age group. In those specimens in estrous, the vascularization was well developed, especially the collagen walled lymphatics (Figure 274), whereas in those in anestrous the vessel development was minimal. During early postparturient involution the vessels were numerous and changes such as splitting of the IEM, plaque-like formations and collagen infiltration of the tunica media were noted (Figure 270). The most prominent morphological feature of this age group was the thick collagen collars observed around the arteries and veins of the La Pr and VL as well as variability in the staining of the IEM (Figures 271, 272). Breakdown of the IEM, as well as some of the other elastic elements of the artery, and elastoid formation were observed in a 3.6 year old specimen.
This was the youngest specimen with elastoid in the vaginal arteries.

**Six years of age to 10.4 years of age** The younger specimens of this group (Figures 275, 276, 277) were in various stages of involution after whelping, so there was considerable evidence of vascular changes identical with those described as due to cyclic sclerosis. These alterations, however, were superimposed on an overall increase in the size of the VL as such and an increase in the numbers of arteries, veins and lymphatics of the VL. The veins and lymph vessels were often thin walled and engorged in the postparturient specimen. Thickened intima (Figure 291) collagen filtration into the intima (Figure 290) elastoid formation (Figures 289, 291) and collagen staining portions of the IEM were noted in this group (Figures 276, 278, 279). By 10 years a collagen ring (Figure 290) was found in from the IEM in the thickened intima of some arteries from animals in anestrous. By 9 years of age the tunica media of most of the arteries of the VL were over 60 percent infiltrated with collagen (Figure 286). Reorientation of the inner tunica media cells was more extensive in the older specimens of this group. Also of prominence in this group were venous sinuses of the La Pr.

**Over 11 years of age** Morphological changes observed in this age group were of the same nature as observed in the 6 to 11 year old animals, except that the changes were more severe. Collagen infiltration of the tunica media, and especially of the thickened intima (Figure 298) as well as elastoid formation and staining variability of the IEM were some of the more pronounced changes. Plaque-like structures were found in the arteries from a 12.8 year old specimen in which most of the vessels were quite severely effected. In an 11 year old specimen and a 16 year old
specimen (Figures 293, 294, 301) there were only minimal arterial changes. There were, however, numerous engorged capillaries in the subepithelial areas and venous sinuses throughout the outer La Pr and in the VL.
DISCUSSION

Ovary

**Statistical evaluation of weight changes with age**

The linear relationship between ovarian weight or body weight with age showed that body weight increased much more rapidly in the Prematuration Age Group, up to 11.2 months of age. In the Postmaturation Age Group both ovarian weights and body weights were observed to increase but at essentially the same rate. A curvilinear relationship between relative ovarian weight and age was found in the Prematuration Age Group, due to normal growth and development, but was found nonsignificant in the Postmaturation Age Group after normal growth ceased. It was noted (Table 3) that the heaviest ovaries did not always come from the heaviest dogs. For example the combined weight of the ovaries from a 31 kg., 10 year old dog was 9.42 gm., while those from a 88 kg., 11 year old dog weighed only 1.06 gm. The former animal was in metestrous when tissues were taken and so had large corpora lutea, while the latter specimen was in anestrous and lacked a large corpus luteum. Generally, however, there was an increase in ovarian weight with age. Variations in ovarian weight due to cyclic activity made it undesirable to place any further emphasis on this means of evaluating age changes. The gradual increase in weight was due to an increase in connective tissue components.

**Germinal epithelium**

The changes observed in the germinal epithelium of the canine ovary from birth to 19 years of age were minimal. According to the study by Barton (1945) on 32 pairs of canine ovaries which came from dogs ranging in age from puberty to senility, the germinal epithelium varied from a thin
layer of cuboidal or squamous cells to an epithelium several cells in thickness, without regard for age, breed, or stage of estrous.

**Chronological appearance**

**Birth to 2 months of age**  
Sauramo (1954) reported that in the newborn human, the germinal epithelial cells of the ovary were already clearly joined to the tunica albuginea and that they remained somewhat static until puberty except for slight variations in height. The shape of the cells in the ovary of the newborn human varied from cuboidal to low columnar (Ringrose, 1963). Valdes-Dapena (1967) agreed that this was the case at birth and reported that during childhood they tended to decrease in height and apparently in numbers in a very irregular and gradual fashion. The ovary from a 2 day old dog was covered with a single layer of flattened to cuboidal cells containing large prominent round to oval nuclei, also described by Raps (1946) for this age dog. A stratification of the germinal epithelium to 2 layers in the hilus region of the one day old porcine ovary (Bal, 1966) was not seen in the canine ovary until 7 days of age. Raps observed that by 10 days in the dog, the epithelium had become flattened and by 13 days squamous. It remained in this state until the 63rd day of life, when it became cuboidal only to become squamous again at 15 weeks of age. In the present investigation the cells were quite uniformly cuboidal at 4 weeks of age and although low cuboidal in places they were not squamous.

**Two and one half months of age to 11 months of age**  
By 2.5 months of age the epithelium was up to 3 cells in depth in some places (Figure 8). Columnar cells with elongate, vertically oriented nuclei were first observed in the germinal epithelium of the 2.75 month old specimen.
The epithelium of the 2.75 and 3 month old specimen were mainly columnar with many areas of cuboidal cells. The appearance of columnar cells as well as other types were found in the porcine ovary from birth to 33 months of age (Hadek and Getty, 1959). Raps (1946) found only cuboidal epithelium in the 2.75 to 3 month age group in the dog ovary and mentioned that the epithelial cells were squamous from 3.75 to 5 months of age.

At the age of puberty in the human Valdes-Dapena (1967) found that the germinal epithelium was flattened in most areas but that islands of plump cells remained. By early puberty in the dog, 6 months of age, it was found that the germinal epithelium was composed of cuboidal cells, often 2 layers deep. A secretion-like substance was observed at the apical end of the epithelial cells. It was explained by O'Shea (1966) that this secretory material was frequently attached to or lying alongside the cells in infolded areas of the germinal epithelium. This secretory material was observed on some of the germinal epithelial cells in most ovaries over 6 months of age.

Over one year of age Very little information was found in the literature regarding the germinal epithelium of older ovaries. According to Jonckheere (1930) proliferations of the germinal epithelium were continuous from a short time before birth to old age. Barton (1945) found the germinal epithelium active in all ages of dogs from puberty to senility. The term senility with regard to the canine was not defined but the dogs used in Barton's investigation ranged from 9 months to 11 years of age.

The germinal epithelium of the 8.5 year old Beagle as described by Andersen and Simpson (1962) was pseudostratified, ciliated in appearance with an
absence of cortical indipping. No cilia were observed in the ovaries of the present study, but the ciliated appearance may have been ascribed to the secretion described by O'Shea (1966). The pseudostratified appearance was observed in this study, however. Cortical indipping of the germinal epithelium contributing to cortical elements was first definitely observed in the present study in the 2.5 month old specimen (Figure 8) and continued through the 19 year old age group (Figures 61, 62). Andersen and Simpson's (1961) report of a lack of cortical indipping in the ovaries from an 8.5 year old dog were therefore not confirmed by the present study.

Thus it was concluded that although the changes with age of the germinal epithelium of the canine ovary were minimal, their changes per se certainly were not minimal. This study verified the earlier findings of Swezy and Evans (1929), Jonckheere (1930), and Barton (1945) that proliferations by the germinal epithelium were continuous throughout the life of the dog. As the functional ovarian substance decreased with age, compensation by the germinal epithelium resulted in a microscopic wrinkling of the latter. The formation of lobes increased the surface area which compensated for the decreasing amount of functional ovarian tissue (Figures 36, 39, 58).

**Tunica albuginea**

Most investigators agreed that the tunica albuginea of the mammalian ovary was composed of connective tissue underlying the germinal epithelium. According to Brambell (1956) it also contained smooth muscle fibers. These were not observed for the canine. Neither was a layer of elastic fibers, as observed by Tóth and Gimes (1964) in the human ovary, found in the canine ovary. Areas where the thickened germinal epithelium formed folds
into which the tunica albuginea extended were observed by Barton (1945) for
the canine ovary and confirmed in this investigation. Woll, et al. (1948)
stated that the tunica albuginea was the main source for fibrosis in the
aged human ovary.

**Chronological appearance**

**Birth to 2 months of age**  In the ovaries from a 12 hour old
and a 2 day old dog a layer of fibroblasts that varied from one to 6 cells
in thickness (9 to 24 microns) plus a few collagen fibers made up the
tunica albuginea. Raps (1946) referred to a structure in the 2 day old
canine ovary as a non-continuous layer of irregular connective tissue,
one cell in thickness. He called it a basement membrane in the 6 day old
specimen. In the 8 day old ovary he felt that it was of sufficient thick­
ness to be referred to as a tunica albuginea. The findings of the present
investigation agreed with Raps (1946) regarding the development of the
tunica albuginea from one week of age to 2 months of age, except in termi­
ology. It was referred to as the tunica albuginea in all cases in this
investigation. A basement membrane (Figure 2, 6) as delineated by the PAS
stain was observed in addition to the collagen fibers of the tunica albu­
ginea.

**Two and one half months of age to 11 months of age**  It was ob­
served in the 2.5, 2.75 and 5.5 month old specimens that the tunica albu­
genia was nearly 3 times as deep at one pole of the ovary as on the
other borders. In the 5.5 month old ovary the tunica albuginea was a dense
nearly unbroken structure from 8 to 88 microns in depth. Raps (1946) re­
ferred to it in this age specimen as a thin continuous layer beneath the
germinal epithelium. The variation was probably in the definition of
tunica albuginea and cortical stroma. According to Hadek and Getty (1959) the tunica albuginea first appeared at 5 months of age in the hog ovary, and Bal (1966) added that in the 6 month old specimen it consisted of a layer of dense collagenous connective tissue composed mostly of fibrocytes. In the human infant a distinct tunica albuginea was first observed at 6 months of age (Sauramo, 1954) and was a thick structure from then on. Laishamra (1963) indicated that it was present at the end of the first year in man.

At about 6 months of age the subepithelial, parallel, fibrous portion of the canine tunica albuginea fused with the outer cortical portion which contained the septa of perpendicularly directed fibers. From this age on, there was a gradual increase in maximum thickness of this structure as it varied from 12 microns in thickness to over 200 microns. It was definitely a more prominent structure in the canine than was described for the porcine (Hadek and Getty, 1959). They reported that it never exceeded 50 microns from specimens up to 33 months of age. Epithelial nodules were often observed in the tunica albuginea of 6 to 11 month old canine ovaries (Figures 18, 22).

Hyaline degeneration and thickening of the human tunica albuginea were reported by Tóth and Gimes (1964) as being associated with senility. Only the latter was observed in this structure for the canine.

**Over one year of age** From one year to 4 years of age, epithelial nodules were nearly always observed in the tunica albuginea, especially under areas of increased germinal epithelial proliferation. In ovaries from animals 4 years of age and over there were increasingly large
areas where the germinal epithelium was pseudostratified in appearance and the underlying tunica albuginea contained no structure of germinal epithelial origin. This agreed with the findings of Andersen and Simpson (1961) for the ovary at 8.5 years of age. It was observed in the present study, however, that even though this was the case in some parts of the ovary, there were usually other areas in the same ovary where the cortical indipping and epithelial nodules were numerous, even up to 13 years of age. There was a decrease in the cortical invagination and nodules up to 19 years of age although a few were observed even at this age.

From 6 to 10 years of age a gradual infiltration of collagen fibers into the cortical stroma from the tunica albuginea was observed (Figure 47). Though follicular activity was probably not increased any more during these years it was felt that the ovary's ability to resorb the regressing follicles was decreased with age. This was in line with the thoughts of Joel and Forsaker (1959) who stated that in the human the phenomenon of corpus albicans resorption most likely halted at or near the menopause. Whatever the reason, the infiltration and incorporation of cortical tissues by the tunica albuginea was so marked, that in some areas of the ovaries from older dogs, it was difficult to demarcate the junction between them (Figures 50, 51).

In ovaries from dogs 10 years of age and older a few cyst-like structures were often seen in the tunica albuginea (Figure 51). In one ovary, the cells which composed some of the epithelial nodules were undergoing degeneration. It appeared that the resulting nodular remnants formed the cysts. The appearance of these cysts was possibly related to the waning resorptive abilities of the ovary. The cyst walls were composed of flat-
tended squamous-like cells.

In the ovaries from dogs over 12 years of age, it appeared that the germinal epithelial invagination, epithelial nodules and cord formation had been restricted more and more to the crypt areas between the lobes of the ovary. An exception to this was one ovary from a 12 year old specimen that was a mass of epithelial proliferation. It gave the appearance of an epithelial hyperplasia or perhaps cortical stromal hyperplasia (Figures 56, 58).

Cortical stroma

The term cortical stroma, as used in this investigation, referred only to the connective tissue elements of the ovarian cortex. The basis of this was made on the definition of the word stroma. Earlier authors such as Kingsbury (1914) referred to interstitial cells of the cat ovary as modified cells of connective tissue origin. Tsukaguchi and Okamoto (1928) called interstitial cells of the canine ovary active stromal cells. According to Trautmann and Fiebiger (1957) these aggregated connective tissue cells with elongated nuclei were probably not ordinary fibroblasts. Cortical stroma in this investigation referred to the connective tissue elements per se.

Chronological appearance

Birth to 2 months of age At birth, in the human ovary, there was almost no visible stroma between the oocytes (Valdes-Dapena, 1967). At 2 days of age the cortical stroma of the canine ovary consisted of embryonic fibroblasts and interlacing strands of collagen fibers. Raps (1946) explained that these strands of connective tissue in this age ovary appeared to emanate as spokes from the medullary connective tissue. This
analogy was completed by adding that these "spokes" extended through the
cortex and were joined to the "rim" which was the newly developing tunica
albuginea. When measured in cross-section the cortex of the 2 and 7 day
old ovaries comprised about 15 percent of the depth of the ovary (Figure
1). Raps (1946) did state, however that in the 9 day old canine ovary
the cortical-medullary junction was well defined and by 13 days of age
it was indistinct. The present study agreed with this. It was found
that this junction remained indistinct up to 3 months of age.

Two and one half months of age to 11 months of age

At 2.5 months of age the only specimen studied which had a distinct enough boun-
dary to be measured showed the cortex to comprise 30 percent of the
ovarian depth. It was made up mostly of connective tissue. Connective
tissue remained a prominent feature of the canine ovarian cortex until
about 5.5 months of age. This was reported by Sauramo (1954) to be true
also for the human of comparable developmental age. Hadek and Getty (1959)
reported that scanty reticular fiber strands divided the cortex of the
young porcine ovary into follicular colcles which were in turn divided by
individual reticular fibers into single follicle containing units. They
stated that in young animals the fibers were directed radially from the
medulla whereas in animals over 4 months of age the fibers were running
parallel to the ovarian surface. A similar phenomenon was described by
Valdes-Dapena (1967) for the human ovary with the added feature of a
curlique pattern as an intermediary stage during the ages of 7 to 10
years or in the pre-puberal period. This phenomenon was also observed in
the canine ovary.

In the canine ovary Raps (1946) observed that as a zone the cortex
reached its greatest size by about 13 days. It was found in the present study, however, that while in the 2 day and 2 week old ovaries the cortex formed only 15 percent of the thickness of the ovary, by 3 months it formed 30 percent. This thickness remained at about the 30 percent level up to 6 months of age.

Due to the increase in follicular development and growth that began at about 6 months of age and dependent on the breed and time of year, the cortex increased in size so that it comprised 25 to 60 percent of the thickness of the ovary. In the 15 year old human ovary which would correspond to a 6 month old Beagle as far as sexual maturity, Sauramo (1954) indicated that the cortex was composed of much stroma with a few primordial follicles interspersed. Some of the canine ovaries of this comparable age contained in addition, some growing follicles. The porcine ovary (Bal, 1966) at 6 months of age had a cortex composed mainly of dense collagen with a rich supply of vessels.

The cortico-medullary junction of the canine ovary was well delineated in the 6 to 9 month age groups with the cortical bands of connective tissue running generally parallel to the ovarian surface and the medullary connective tissue bundles running perpendicular. The only change noted from 9 to 11 months of age was a gradual increase in the density of the connective tissue septa entering the cortex from the tunica albuginea.

Ovaries taken from dogs that were in the anestrous phase of the cycle contained a higher percentage of connective tissue than the ovaries from dogs of comparable ages in proestrous, estrous or metestrous. The cortices of these ovaries, however, made up only 20 to 30 percent of the thickness of the ovary. Due to the wide variation in cortical thickness caused by the
presence of follicles and corpora lutea of various sizes found in the
cycling ovary it was difficult to accurately measure cortical thickness.
This thickness was always much greater during proestrous, estrous, met-
estrous and pregnancy.

Over one year of age From the age of one year on, increasing
numbers of epithelial type cells, degenerating thecal cells and other cells
were found in the cortex of the canine ovary. The appearance and gradual
increase in these cells resulted in a dilution of the cortical stromal
connective tissues which had up to this time been increasing in density
and amount. Therefore, the bands of collagen fibers and septa were no
longer as prominent. In ovaries from the 39 year old human Sauramo (1954)
found that the cortex had a large amount of dense fibrous connective
tissue, in which elastoid hyaline degeneration was beginning. The com­
parable age of the dog 6 and 7 years showed the above described cellular
structures to be more prominent than the connective tissue. At about 8
years the canine ovary began to change from a bean shaped structure to a
semi-lobed structure. Sauramo stated that in man the total ovarian size
decreased from the age of 30 upwards and that at 38 years of age the
fibrous connective tissue was dense and gyri of the cortex were present.
The process of aging in the human ovarian cortex was described by Lea­
them (1958) as a progressive thinning and wrinkling of the cortex. Thus
the human ovary also underwent lobation. No significant size decrease
occurred in the canine ovary up through the 13 year age period. Woll, et
al. (1948) indicated that in senility the fibrous tissue of the human
ovary penetrated the cortical stroma from the tunica albuginea and medulla
and that reticular fibers became hyalinized. According to Woll, et al, the
ovarian stroma had the ability to differentiate into cells of the granulosa, theca interna, corpora lutea, corpora albicans and that in senility this ability sometimes led to stromal hyperplasia, thecoma, cortical granuloma formation, granulosa or theca cell tumors. In the canine ovaries over 8 years of age stromal hyperplasia and thecomas appeared to be the rule rather than the exception. Twenty-two out of the 25 specimens which were from 8 to 19 years of age when tissues were taken showed definite evidence of hyperplasia. In aged cows (17, 21, 28 years of age) Yamauchi (1963) found that oocytes disappeared from the ovaries and the ovaries became a solid stromal mass. The ovaries from the 19 year old dog (the oldest in this study) showed a limited amount of oocyte activity, cysts and the remaining tissue was dense connective tissue. In the 43 year old human Sauramo (1954) reported intense elastoid hyaline degeneration of the cortical connective tissue. According to Tóth and Gimes (1964) a layer composed of elastic fibers formed a network around the cortex of the human ovary and with advancing years and increasing ovarian atrophy this ring of elastic fibers underwent decomposition. Such was not found to be the case in the canine ovary as no layer of elastic fibers was observed, although with Verhoeff's elastic tissue stain the dark whorls of stroma at lower powers gave the general appearance of elastoid tissue.

In the aging ovary of the dog with the appearance of hyperplasia, the cortical stroma was infiltrated and appeared to be decreased in density. In many ovaries this infiltration by cellular elements extended out to the germinal epithelium through the tunica albuginea. There was an increase in density and amount of connective tissue in those ovaries where hyperplasia was not a factor.
Follicles and related structures

The terms, cell nests, ovigerous cords (Pflüger's tubes) and oogonia were used according to the definitions given by Patten (1958). Primary oocyte referred to all oocytes found in the follicles of the canine ovary because according to Evans and Cole (1931) the first polar body was not shed until after ovulation. Primordial follicle and primary follicle were used in accordance with the definitions given by Hadek (1965).

Definitions:

- Ovigerous cords (Pflüger's tubes), cords of sex cells invading the ovarian stroma.
- Cell nests, groups of cells formed by breaking up of the ovigerous cords.
- Oogonia, primordial germ or sex cells, cells of the ovigerous cords.
- Primordial follicle, a primary oocyte surrounded by a layer of cuboidal granulosa type cells.
- Growing follicle, any follicle with more than one layer of granulosa cells including follicles after antrum formation and up to the mature Graafian follicle.

Other terms that referred to atretic follicles or corpora lutea were self explanatory.

Chronological appearance

Birth to 2 months of age The findings of the present investigation agreed with Raps (1946) that the cortex of the 2 day canine ovary was composed of many germ cell nests or clusters which were in turn composed of oogonia. Raps called them precursors of follicular cells and ova,
however, and indicated that the groups appeared to contain 5 to 20 cells in a mass. It was found that even at this early age cell death was taking place among the oogonia as 2 or 3 in each cluster were in various stages of degeneration. Valdes-Dapena (1967) reported that especially during the first year of life in the human there was a tremendous reduction in the number of oocytes, a phenomenon known as "post-natal slaughter". It was probably also true in the dog. Only tentative primordial follicles were found in the 2 day ovary in this study, Raps (1946) was able to definitely discern these structures in the 4 day old ovary. According to Hadek and Getty (1959) the cortex of the sow ovary at birth was densely packed with small primary follicles. Bal (1966) agreed and added that some secondary follicles were found centrally. So it would appear that shortly after birth the oogonia and follicular development of the ovary in man and swine were more advanced than in the dog.

Numerous ovigerous cords appeared to replace many of the germ cell nests in the one week old ovary. It was found that these cords extended varying distances through the cortex, also observed by Raps (1946). Raps described them as prominent in the 11 day old ovary but observed that by the 13th day they were not as prominent. Ball-like laterally dispersed cell formations whose long axis paralleled the germinal epithelium filled the area. The 2 week old ovary of the present study also showed disappearance of many of the ovigerous cords. Germ cell nests formed a narrow cortex around the periphery of the ovary.

In the 15 day old canine ovary Raps (1946) and Tanaka (1962) observed the first primary follicle. Andersen and Simpson (1961) reported that the first evidence of primary follicles in the Beagle ovary was at 103 days or
about 5 months. In the present study they were observed in the 4 month old specimen. Raps (1946) stated that there were no changes in the primary follicles between 15 days and 4 weeks. Thus the first ovarian age separation as described by Hadek and Getty (1955) was similar in both the dog and the hog inasmuch as up to 8 weeks of age there were large numbers of primary follicles found in the ovarian cortices of both species.

Two and one half months of age to 11 months of age. In the 2.5 month old canine ovary a follicle with over one layer of granulosa cells was found. Multilaminar follicles were first observed by Tanaka (1962) in the 3 month old canine ovary and by Raps (1946) in the 3.75 month old canine ovary. Bal (1966) found them in the 2 week old sow ovary. The growing follicles of the 2.5 month canine ovary were located in the outer cortex. By 3 months of age they were found mostly in the medulla. Anderson and Simpson (1961) described the cortex of the 3 month old Beagle ovary as an extremely narrow outer rim that contained various stages of follicles.

Short ovigerous cords were again prominent features of the canine ovary at 2.75 months of age according to the present study which verified similar findings of Raps. These cords differed from those found in younger specimens in that in the 2.75 month old ovary they were composed of oogonia and primordial follicles or entirely primordial follicles (Figures 9, 10, 11). Also appearing in the 2.5 to 3 month series were structures that appeared to be the result of invagination from the germinal epithelium. Swezy and Evans (1929) and Jonckheere (1930) indicated that these proliferations were present in the canine from a short time before birth to old age. Barton (1945) described them in the adult dog with re-
ference to cyclic changes. O'Shea (1966) referred to them as subsurface epithelial structures (SES) and indicated that both solid and hollow forms regularly showed evidence of mucin secretion. The ovaries O'Shea studied were from dogs one month to 2 years of age. The SES were darker staining than the ovigerous cords previously described.

Atresia was observed by Raps (1946) in a 3.75 month old canine ovary. Bal (1966) described atretic follicles in the 2.5 month old sow ovary.

In the 5 month old canine ovary Raps observed that many of the growing follicles showed evidence of disintegration. This was not a prominent feature of the 5.5 month old specimens in this study and was thought to have been indicative of individual variation.

In the 5.5 month old ovary a growing follicle with an antrum was found. A dense membrane, the zona pellucida was formed around the oocyte just prior to antrum formation. Antrum formation was first observed by Raps in the 6 month old ovary, by Andersen and Simpson (1961) in the 6.5 month old ovary and by Tanaka (1962) in the 6 to 7 month old canine ovary. Follicles with an antrum formed were found in the sow ovary at 7 weeks of age (Hadek and Getty, 1959). Observed in the 5.5 month old canine ovary were numerous germ cell nests and SES with and without lumens. Just beneath the tunica albuginea clusters of primordial follicles were found.

Granulosa cells of the growing follicles just prior to antrum formation appeared under the light microscope much like the granulosa cells of the ovary described by Lipner and Cross (1968) for the rabbit follicle. Lipner and Cross found with electron microscope studies that the membrane granulosa of the rabbit follicle was a pseudostatified epithelium. Further work should be done to see if this is the case in the
In ovaries that contained follicles with beginning antrum formation the zona pellucida was first distinguished as a prominent structure (Figure 15). With Verhoeff's and Van Gieson's stain it was a greyish brown homogeneous amyloid-like structure about 2 microns in thickness. According to Tanaka (1962) it became hyalinized as was found to be true in the present study though it stained differently than hyaline usually did. Evans and Cole (1931) reported that the zona pellucida was not yet formed in the mature Graafian follicle that was ready to rupture. It was felt that this was a problem of definition as the structure described above was too thick to have been the vitelline membrane (Figures 30, 51, 35). According to Trautmann and Fiebiger (1957) the membrane that surrounded the primary oocyte of any follicle beyond the primary follicle was a homogeneous envelope, the zona pellucida. They labelled this membrane as oolema in their diagrams. Copenhaver and Johnson (1958) and Bloom and Fawcett (1962) indicated that oolema and zona pellucida were synonymous terms. The latter authors stated that in the human, the zona pellucida appeared when the ovum reached 60 to 80 microns in diameter. In the dog it was about 2 microns in thickness on oocytes that were 60 to 70 microns in diameter and increased to over 4 microns around oocytes that were 90 to 95 microns in diameter. Kladetsky and Rosenbauer (1965) described and depicted an oolema in a secondary follicle from a lioness ovary. A structure not observed as such in this study was the vitelline membrane. Arey (1965) stated that surrounding the eggs of most mammals was a vitelline membrane and zona pellucida. A diagrammatic sketch of the vitelline membrane beneath the zona pellucida in the primary oocyte of a hedgehog was given by Trautmann and
Fiebiger (1957). Nalbandov (1958) stated, however, that the mammalian ova have no vitelline membrane. Further investigation is needed in this area. Call-Exner bodies or vacuoles as described by Hadek and Getty (1959) for the hog and described and depicted by Bloom and Fawcett (1962) and by Valdes-Dapena (1967) for the human follicles were not observed among the granulosa cells of the canine ovarian follicle.

It appeared as soon as follicles began to grow to the antrum stage that follicular atresia became a prominent feature. Kovacs (1933) described in detail physiological atresia of the canine follicle. He found as was verified in this investigation that atresia did not progress according to a predetermined scheme. Its progress varied depending on the age of the follicle when atresia began, thus it was different for the primary and growing follicle. According to Kovacs atresia was the result of obliteration or cystic degeneration, with the former generally occurring in primary and smaller growing follicles and the latter in larger Graafian follicles. Seen in the 6 to 11 month old canine ovaries were all of these various types of follicular atresia as described by Kovacs (1933). During atresia of larger follicles the theca interna cells often hypertrophied. The increased cytoplasm was fine structured in appearance and the cells contained lutein granules and enlarged nuclei. These, according to Kovacs were interstitial cells which formed islands. He observed that similar interstitial cells formed by individual dispersion in the cortical substance, as clusters or cords. In the sow's ovary Trautmann and Fiebiger (1957) found that the cortical stroma was a highly reactive tissue and that the stromal fibroblasts were capable of further differentiation, as for dye storage and proliferation, or differentiation into wandering macrophages to
round up and store lipids. Stromal cells assumed an epithelial
character for performance of nutritive and secretory functions in the fol­
llicles, and that these cells occurred singly or in clusters in the inter­
stitial cells in the bitch and cat. Kovacs (1933) felt that these inter­
stitial cells originated from the invaginated epithelial cells of the germ­
inal epithelium, while Trautmann and Fiebig (1957) indicated them to be
from cortical stromal cells. Mossman, et al. (1964) did not state direct­
ly but gave the impression that these cells came from differentiated
stromal cells. The latter authors designated the differentiated thecal
cells on the basis of follicular maturity at the time atresia set in. They
stated that luteinization of the theca interna of a ripening follicle re­
sulted in thecal gland formation, whereas luteinization of a atretic fol­
llicle resulted in interstitial gland formation. Histochemical means were
used by Mossman, et al, to distinguish these two types and indicate possible
function.

It was reported by Hadek and Getty (1959) for the hog ovary that at­
resia of oocytes and primary follicles were present even from the young­
est ages studied, especially under 5 weeks of age and that a decrease in
this phenomenon was observed so that in sexually mature ovaries only low
numbers of degenerating oocytes were observed. Such was the case in the
dog. In early sexual maturity, between 7 and 11 months of age an increase
in the numbers of larger atretic follicles were observed in varying stages
of atresia. It was observed that many of these follicles contained from
one to 5 oocytes and that follicles with more than one oocyte were seen
in all age groups of this investigation. Hadek and Getty found that these
abnormal structures were rarely present in the hog ovary after 5 weeks of
Another feature especially of the larger atretic follicles of this age range was the hyalinization of the theca interna cells and the formation of a hyaline ring around the granulosa cells. Some of these hyaline structures, in various stages of collapse and often containing a few degenerating granulosa cells, were referred to as the remnants of once large follicles (Figures 17, 20, 21, 35, 37). Valdes-Dapena (1967) in describing atresia of large follicles in the human ovary, found that at the time of follicular collapse the center of the structure was converted into a solid mass of fibrous tissue which she referred to as a corpus fibrosum. She observed that the next phase was the deposition of a hyaline ring between the atrophied granulosa cells and the peripherally arranged lutein cells. Upon shrinkage of the whole structure the hyaline band became more prominent and was referred to as the corpus atreticum in which there remained only a central core of connective tissue surrounded by an undulating pink ribbon. In the dog, at least in some cases, the hyalinization was not associated with the granulosa cells thus did not penetrate the basement membrane. In other cases structures similar to the corpus fibrosum and corpus atreticum were observed. The final stages of follicular atresia as described by Valdes-Dapena (1967) for the human were also seen in the dog. They consisted of only the convoluted pink staining band folded upon itself. She called this structure the corpus restiforme or corpus candidans. Inasmuch as this process was continuous, all in between phases were also observed. Valdes-Dapena indicated that even the corpus restiforme, however, eventually disappeared leaving no mark of its prior existence.
Another change noted in this age group, in ovaries from dogs as young as 6 months of age was mild thecal hyperplasia, stromal thecosis and in some cases hypertrophy and apparent luteinization. These columns or packets of theca-like cells were observed in many areas of the cortical stroma lying between collagen fibers. They were often closely associated with the theca interna of follicles undergoing atresia.

Associated with atresia of large follicles, although not always observed near a follicle were structures that were referred to as granulosa cell islands (GCI) in this study. These were first apparent in a 6.5 month old ovary and were observed in varying numbers in succeeding age groups (Figures 21, 24). They consisted of various sized islands of cells that pinched off and became isolated from the central portions of the degenerating follicle in the process of collapse and wrinkling of the follicle. GCI were usually elongate, slightly curved structures lined with a layer of granulosa cells which contained pyknotic nuclei and vacuolated cytoplasm.

It was observed that in the 5 to 11 month age group there was a wide individual variation in stages of ovarian maturity with regard to follicular development. Generally, however, there was an increase in size and numbers of all types of follicle, especially growing follicles, as well as in atresia and structures associated with atresia. There was an increase in collagen fibers, and also in the thecal elements in the cortical stroma.

In 10 month old ovaries corpora lutea were observed for the first time in this study. They were large functional appearing structures that comprised most of the ovary. Bal (1966) observed them first in the one year old sow ovary. Large mature follicles with the typical, complexly
folded, granulosa and highly vascular theca interna, as described by Evans and Cole (1931) and Bloom (1954) were also observed. GCI were prominent structures in these 10 and 11 month old ovaries.

One year of age to 4.1 years of age  The only change noted during this age span over the appearance of the 11 month ovaries described above, with a gradual increase of interna thecosis, combined interna and stromal thecosis and stromal thecosis. Designations as to the type of thecosis were made in accordance with the findings of Fienberg (1963) for the human ovary. In one ovary the thecal elements were well infiltrated with collagen fibers giving the appearance of a fibrous combined thecosis.

Found in ovaries from this age group were various stages of regressing corpora lutea, very similar to those described by Bal (1966) in the 2 year old sow ovary. Corpora lutea in advanced stages of regression were of two general types in the canine. In the first type the vacuolated luteal cells were light staining and loosely connected while in the second type they were more dense and perhaps even more pigmented than observed in the functional corpora lutea. The collagen infiltration was greater in the second type.

Follicular cysts were seen for the first time in this investigation in both ovaries from a 3.3 year old Beagle. Three of the larger cysts in one ovary measured 250 by 420 microns, 170 by 670 microns and 420 by 500 microns respectively. They were of a similar size in the other ovary. In general they were lined by one layer of flattened but otherwise normally appearing granulosa cells, although in some areas these cells were 2 to 3 layers in depth. A netlike mass filled the cysts. The animal from which these ovaries were taken had whelped 2 litters, totalling 17 pups. Tissues
were collected 10 days following the second whelping. No such cysts were found in a 3 year old Bitch that had whelped 3 litters, although tissues were not collected from this bitch until nearly 2 months pp.

**Six years of age to 10.3 years of age**

Cysts of a similar appearance to those described above were also observed in ovaries from a 6.6 year old Beagle with a whelping history of 4 litters totalling 27 pups and tissues taken 7 days post whelping. Cysts were observed in a number of ovaries from dogs over the age span under discussion, but their whelping histories were not available. The above observations were simply 2 observations and no conclusions could be made without further study.

The follicular activity observed in the ovaries of this age span was as one would expect for a mature female. No age changes were observed in the follicular structures per se, that would characterize the follicle as being from a 6 year old dog or a 10 year old dog. Bal (1966) did not report any major age changes during this age span in the sow. He did observe many nests of oogonia in a 6.75 year old sow ovary and in those specimens near 8 years he reported that many cells with eosinophilic granules in their cytoplasm were observed in the theca interna. Eosinophilic granulated cells were described by Brown and Nellor (1967) in the ovarian tissues of a sow from day 11 to 15 of the cycle, between 24 and 44 days and after the 86th day of pregnancy. They suggested that these cells originated from undifferentiated mesenchymal cells due to a direct effect of ovarian estrogens.

All of the 24 dogs in this age group that included 9 breeds, had combined internal and stromal thecosis. This increased in density and amount with age.
During the comparable age span of man which would be from about 40 to 57 years of age, Sauramo (1954) observed a steadily declining number of primordial, growing and Graafian follicles. He reported that in the last third of the fertile period large Graafian follicles were exceedingly rare and that in the ovaries from a 38 year old woman some corpora albicantia showed pigment and elastoidhyaline degeneration. These latter phenomenon were not observed in canine ovaries under 8 years of age comparable to a 48 year old woman. Along with the reduction in primordial follicles in the 44 to 53 year old women Bonfirraro and Subrizi (1966) observed an increase of the involutive follicular apparatus. This was found to be true for the dog. Bonfirraro and Subrizi indicated, as was found in the canine, that although these general trends were observed there were still ovaries from older individuals with many actively growing follicles.

Over 11 years of age The most prominent feature observed in the follicular and more especially follicular related structures of older ovaries was the hyperplasia and/or in some cases hypertrophy of various cell types or combinations of cell types. Most commonly involved was the theca-interna cell type. Others were cortical stromal cells, granulosa cells and subsurface epithelial cells. There was no apparent pattern with regard to these structures. From 0.5 years of age to 10 years of age in the canine ovary there was a general overall increase of cells of the various types listed above that occurred in the cortical stroma which were not necessarily associated with a follicular structure. After 11 years of age this gradual increase changed to one of variability. Thus the lack of uniformity in morphology made this phase of the investigation
more difficult.

As was indicated earlier, all 15 dogs in this age group representing 8 different breeds, showed some degree of combined interna and stromal thecosis as defined by Fienberg (1965). In 12 of these the thecosis was very dense and in some cases showed a whorled appearance. Varying amounts of collagen were present. In some cases the appearance was one of fibrosis. Fienberg indicated that in humans androgenic or estrogenic effects were often seen as a result of some of these thecomas. In studying the ovaries from three aged cows 17, 21, and 28 years of age, Yamauchi (1963) observed bilateral folliculoid structures in each case. They were larger in the ovaries from the older of the 3 cows. These were classified into 2 types according to the developmental pattern of the trabeculae; one type had granulosa cell tumors and the other contained a cystadenoma. Colloid bodies of various sizes were found in the first type located in the trabecular tissue. Rosette-like structures were commonly found in both types of folliculoids. The only structures resembling these in the canine were in two dogs. One a 11.5 year old Wirehaired Terrier, in which they were found in the medulla. They were described further in the section dealing with the medulla. The other dog was a 9 year old African Basenji of the previous age group which had similar structures in the medulla plus a structure very similar to type one described by Yamauchi (1963) for the bovine, but without the colloid. It was 750 by 920 microns in cross-section and was attached to the ovary with a broad neck of dense collagenous connective tissue and was either a GCI tumor or an SES tumor. Two similar but smaller structures were observed in the other ovary and were located in the tunica albuginea. Bloom (1954) indicated that granulosa
cell tumors were usually unilateral which was not the case in this instance. These were of a folliculoid nature but with no Call-Exner type bodies as Bloom (1954) described. Minimal follicular activity was observed in these ovaries which showed dense combined thecosis.

No references were found regarding SES tumors. These structures were found in the ovaries from 3 dogs, 2 Fox Terriers and one Golden Retriever of 11.2, 12, and 13.1 years of age respectively. The first of these specimens had numerous variably sized proliferations of SES. They retained their hypertrophic appearance and acinar or tubule-like arrangement. Varying amounts of collagen were observed infiltrating them and hyalinization was evident in some. One, large enough to fill a low power field was part of an area of interna thecosis and contained a cyst at one end (Figure 55).

In this particular set of ovaries dense combined interna and stromal thecosis was a prominent feature and there were also numerous GCI. In the second specimen, the 12 year old, the SES tumors were more discrete structures which were randomly located and contained more hyalinized material. Combined thecosis was also a prominent feature of these ovaries. The ovarian cortex of the 13.1 year old Golden Retriever contained numerous sheets of SES cells which lacked the acinar-like appearance. Large SES tumors, typical of the previous two sets of ovaries as well as all stages in between were observed. Large areas of hypertrophied, luteinized cells were also observed as part of the SES. In one ovary from the Golden Retriever, a 500 micron in diameter follicle was found in early stages of atresia. More advanced stages of atretic follicles were also observed. In the other ovary of this pair no follicular activity was found. The other two sets of ovaries contained primary follicles in the outer cortex.
and a few growing follicles under 170 microns. Atretic follicles were also observed as well as a few GCI.

In summary, numerous nonproliferating SES, GCI, cysts and an increase of hyaline tissue were observed in varying amounts of these ovaries. The amount of hyaline tissue observed was increased in the older ovaries. Only 2 cases of small cystic degeneration and one case of a follicular cyst were reported by Tanaka (1962) out of 113 sets of ovaries from dogs one day to 15 years of age. In this study a total of 13 cases of cystic ovaries were found out of 114 sets of ovaries studied. Follicular cysts were occasionally observed in old mouse ovaries (Rolle and Charipper, 1949). Tóth and Gimes (1964) indicated that in the post-menopausal human ovary some cases of germinal inclusion cysts, focal and diffuse stromal hyperplasia occurred. In the dog the first stromal hyperplasia was found in a 6 month old ovary. Although it was not prominent in most younger animals it was present much earlier than in man and was a part of the apparently "normal" functioning ovary.

There was a general decrease in the numbers of growing follicles observed in the older ovaries, especially from dogs over 11 years of age. In a sterile dingo and an aged mouse ovary, Tamura (1927) found anular follicles. He did not say how old the dingo was or give any other history. Talbert and Hamilton (1965) found that the average number of corpora lutea in the ovaries and the average number of ova recovered from the uterus and tubes of old mice were less than those found in young mice. He reported that a significantly higher percentage of abnormal ova was recovered from the old mice. In the human post-menopausal ovary Tóth and Gimes (1964) observed that there were no primary follicles, mature follicles or corpora
lutea. Ringrose (1963) reported that in 28 out of 48 post-menopausal ovaries no primordial follicle were evident. Some follicular structures and/or luteal remnants were found in nearly all of the canine ovaries studied. The general increase with age of connective tissue amount and density in the canine ovary was answered in part by Joel and Foraker's (1959) statement that the ovary's ability to resorb corpora albicantia and corpora fibrosa decreased with age. Ringrose (1963) emphasized this and reported that in one woman 32 years of age who had previously had one ovary removed, the accumulation of corpora albicantia in the other ovary was marked. He felt that this was an indication that unilateral oophorectomy possibly accelerated the aging phenomenon of the remaining gonad. It was possible that this accelerated aging was the result of the effect of the higher concentration of pituitary hormones on the remaining ovary. Perhaps the remaining ovary was not able to produce sufficient hormones to properly control the positive feedback mechanism of the pituitary. Further investigation is needed here to determine the factor that in young ovaries allows or promotes complete resorption of certain connective tissue.

Medulla

In the growing, cycling and aging ovary the medulla was observed to undergo many changes. Contents of the medulla had a bearing on its relative size and appearance. Some of the contents varied depending on stage of maturity, stage of estrous cycle and age, while others were constant. In all of the ovaries studied collagenous connective tissue and vessels formed a prominent part of the ovarian medulla. Reticular fibers as described by Hadek and Getty (1959) for the hog were studied with special stains and were found to be similar in the canine. Elastic tissue
elements were observed in the canine ovarian medulla as described by Bloom (1954) for the canine and Tóth and Gimes (1965) for the human. Harkness (1964) stated that elastic tissues were present only in the vessels of ovaries. Other structures, such as rete ovarii were often found depending upon where the section was taken. Smooth muscle fibers and nerve elements were often seen in the hilar region. Embryonic migrations of oogonia were seen in the medulla of young ovaries. Other follicular elements were observed in the medulla from time to time such as primary and growing follicles. Various stages of corpora lutea were found depending upon the phase of the estrous cycle. Migrated SES remnants were also observed in some ovaries. Sauramo (1952, 1954) described all of these structures except the follicules and corpora lutea for the medulla of the human ovary. According to Harrison (1962) "hilus cells" were chromaffin cells which were found in well developed clusters near prominent rete ovarii. These cells were almost never encountered in the human ovaries in infants or children before puberty, but were found in adults. Neither hilus cells nor any other type of accessory adrenal cortical cells were observed in the canine mesovarium.

**Chronological appearance**

**Birth to 2 months of age**  
In the 12 hour and in the 2 day old canine ovary the large medulla was composed of much collagenous connective tissue which contained tubular strands of oogonia, embryonic tubules and blood vessels. Raps (1946) in describing the 2 day canine ovary indicated that the tubular or cord-like structures appeared to be remnants of earlier cortical ingrowths or primary sex cords. Trautmann and Fiebiger (1957) de-
scribed embryonic structures, epoophoron, as blind-ended tortuous tubules in the ovaries of domestic animals. According to Sauramo (1954) it was composed of greatly enlarged branched tubules in the 37 cm. human fetus and had decreased at least near the hilus in the 2 month old human. Sauramo stated that between 6 months of age and 15 years of age there were few if any of these tubules. Similar structures were found in the present investigation in the majority of the specimens studied, but were thought to have been rete ovarii. It was stated by Trautmann and Fiebiger (1957) regarding the mammalian ovary in general that the epoophoron was lined with ciliated columnar epithelium. No cilia were observed in otherwise similar appearing structures in the dog.

In a 6 day old ovary, Raps (1946) observed that the cords of cells were narrower than they were in younger ovaries but indicated that they were not degenerative forms. A large well organized rete ovarii was observed in the 7 day old ovary typical of one described in an ovary from a 15 year old human (Sauramo, 1954). The only difference was that in the canine some of the cells were hypertrophied. In the 13 day old canine ovary Raps observed that deeply infiltrated cellular cords from the cortex appeared similar to the cortical stromal cells. From this study of the 14 day old ovary similar cells were found, but usually they existed as individual cells and were typical of theca interna cells described in an earlier section. Increased overall density of collagen fibers was observed in the one month old ovary among which nests of oogonia were observed.

Two and one half months of age to 11 months of age

By 2.5 months a migration of epithelial structures was observed. Raps indicated
that at this age a number of inactive primary sex cords were observed in the dog ovary. The cords or nests of cells found in this investigation contained granular cytoplasm, oval to round nuclei and were not degenerating. These epithelial cords persisted in the 2, 75 and 3 month old ovaries.

The rete ovarii of the dog which occupied over half the width of the medulla of the week old ovary had become smaller and was found only in the hilus region in the older ovary. Sauramo (1954) indicated that while it was also large in the human infant, the rete ovarii from then to puberty was found only at the hilus. Infiltration of this structure with increasing amounts of collagen fibers was observed up to puberty in the dog ovary. Found extracellularly in some of these rete ovarii were hyalinelike structures. No information was found in the literature regarding such structures in the canine although Yamauchi (1963) described some more advanced forms in ovaries from aged cows. From 3 to 6 months of age in the canine ovary, changes consisted of a minor increase in density of the collagen fibers. No changes were observed in the rete ovarii up to this time.

Varying density of the medulla and the appearance in many ovaries of localized areas that appeared to have been edematous were observed during this age span (Figures 52, 53, 54). Pyknotic nuclei were often found between the collagen fibers of these ovaries. In addition to these the 7 month specimens had varying numbers of SES in the medulla.

Over one year of age No changes of the SES were observed up to about a year of age after which they were often varied in appearance.

The columnar cells were sometimes replaced by cuboidal cells. Often vacu-
olated cytoplasm and pyknotic nuclei were observed while an increase of connective tissue infiltrated in between the cells. Sauramo (1954) stated that during the fertile period in humans the rete ovarii varied greatly, to the extent that it was nearly lacking in some and very large in others. He also described similar epithelial changes to those observed in the dog ovary and added that macroscopically normal ovaries, often had rete ovarii in which metaplasia of the epithelium and solid or cystic rete had occurred.

As in the previous age period a subtle increase in density and amount of collagen tissues was observed. Elastic fibers and elastic elements were seen in increasing amounts in the medulla of older ovaries. They were first observed as dense focal masses in the ovaries from a 6.1 year old Dalmation. This finding agreed with that of Bloom (1954) for the canine ovary, and Tóth and Gimes (1964) for the human ovary. Harkness' (1964) statement that elastic tissues were present only in the vessels in ovaries was not verified by this study, but Tóth and Gimes findings were. The latter investigators included in their article an excellent photomicrograph of elastic fibers found beneath the tunica albuginea of a human ovary and added that with advancing years and increasing ovarian atrophy this ring of elastic fibers disappeared. In the dog the presence of elastic fibers beneath the tunica albuginea was not observed, but increasing amounts of elastoid with age were observed in the medulla of most ovaries (Figure 81). The majority of canine ovaries from dogs over 10 years of age showed prominent foci of elastic fibers. According to Sauramo (1952) elastoid or elacin, the breakdown product of elastic tissue was one of the typical structural constituents of the senile ovary.
He indicated that the fibers were broader, less tortuous and less branched.

The other outstanding features of the ovarian medulla in this age span were related to changes in the rete ovarii. Hypertrophy of some of the cells in the acinar-like structures of the rete ovarii was quite common. Cysts as described by Sauramo (1954) in the human were also observed in the canine. The appearance of granulosa-like cells in the center of the fibrous body in the rete as found by Sauramo was observed in the canine ovary (Figures 93, 94). Most outstanding, in the canine rete ovarii was the increasingly frequent appearance of variously staining extracellular hyaline structures. These were of different sizes. In the 38 sets of ovaries studied from dogs over 6 years of age portions of the rete ovarii were observed in 24. Out of these 22 had hyalinized substance. Yamauchi (1963) described similar structures in folliculoid structures from ovaries of aged cows. He found them to be colloid bodies composed of polysaccharides which contained lipoidal substance. Two types of colloid structures were described in his study, a small type, regular in shape and of hemogenous structure and a larger irregularly shaped heterogenous structure. Both types were observed in the canine ovary. Some of the larger heterogenous type had a laminated appearance. Some, which were brown-orange staining with Verhoeff's and Van Gieson's stain and PAS positive were thought to be amyloid.

The rete ovarii of older dogs varied from healthy appearing structures to atrophic ones. According to Sauramo this was the case in the senile human ovary although he observed some ovaries in which it was a distinct branching network. Short and indistinct lumens were often observed in the dog rete ovarii.
Vasculature

According to Bunce (1964, 1965) there was a wide variation in the appearance of a distended and a collapsed artery. It was felt that by knowing this one could still evaluate vessels that were not distended when fixed. Inasmuch as many of the vascular changes involved the intima it was desirable to have it clearly defined. Bunce (1964) indicated that it was a layer of tissue lying between the endothelial sheet and the internal elastic lamina. The changes with age resulting from a breakdown of this elastic membrane into elastoid. The reorientation and migration of smooth muscle cells to the intima and the infiltration of collagen into the intima took place with advancing age. Changes of the tunica media and tunica adventitia were also discussed as well as venous changes. Delson, et al. (1948) reported that striking alterations of vascular pattern resulted in sclerotic ovaries. In 1966 Bal reviewed the sequence of microscopic changes observed in aging vessels of the pig ovary.

Chronological appearance

Birth to 2 months of age The hilar arteries of the 12 hour and 2 day old canine ovary consisted of a tunica intima composed of cells with large prominent round to oval nuclei and a very fine IEM. The tunica media had one to 2 layers of smooth muscle cells and the tunica adventitia a few collagen strands. According to Bal (1966) the one day old pig ovarian arteries were similar to that described above except for a fine external elastic limiting membrane. Only a few scattered elastic fibers were observed between the tunica media and adventitia in the dog. The fibroblast nuclei and sparse collagen fibers of the tunica adventitia were similar in both. No migration of endothelial cells from the intima into
the media was seen in the dog as Bal described for the hog. The endothelial cells were in various states of orientation due to the collapsed state of the vessel. Raps (1946) indicated that deep to the cortico-medullary junction of the 4 day old dog ovary were numerous large blood vessels.

The only change noted up to 2 weeks of age in the dog ovarian vessels was an increase in prominence and waviness of the IEM. Prominent elastic fibers were found in between the smooth muscle cells of the 5 week old ovarian arteries. Elastic fibers were observed in both the media and adventitia of the arteries from an 8 month old dog and so the elastic contents of the arterial walls of this early age were closely comparable in the dog and human.

An increase in vascularity was observed in the medulla of ovaries from 2.5 and 2.75 month old dogs. The large arteries had larger lumens, although the wall thickness was similar to earlier ages, while medium sized arteries had much thickened walls. Large veins, venous and lymphatic sinuses were observed. Bal (1966) found large venous sinuses in the 1.75 month old porcine ovary which were even larger in the 2.5 month old hog ovary. These also were observed in the dog.

Two and one half months of age to 11 months of age In some of the smaller arteries the IEM appeared as a series of dots. This was probably due to the waviness of the fibers that had been severed at high points, so that only dots of elastic fiber appeared in the section. In this age group and many later ones, empty arteries were found near others that were filled with blood. Whether some of these arteries were not as functional as others or discharged their blood through arterio-venous
shunts and appeared empty was not known. Functional significance was attached to this though. Added features of the 3 month old canine ovarian arteries was a continuous external elastic membrane and early infiltration of the tunica media by collagen fibers from the tunica adventitia and elastic fibers from the external elastic membrane. Bal (1966) reported the presence of collagen and elastic fibers in the walls of the 6 month old hog ovary. In both the dog and the hog large veins and venous sinus were prominent in the 3 to 4 month old ovaries.

Splitting of the intima was observed in the arteries of the 6 month old dog ovary in this study and also in the 6 month old hog ovary (Bal, 1966). Smooth muscle fibers filled the split area. Collagen and elastic fibers were observed in the tunica media of both species. Elastic fibers found in the collagenous walls of the veins from the dog ovary were more numerous than observed in younger ovaries studied. Bal described the prominent veins and their lack of smooth muscle fibers but did not indicate whether elastic fibers were found.

Intimal splitting became more prominent in the 6.5 and 7 month old age groups. It was observed that the migrated smooth muscle fibers found in the split areas were reoriented in the longitudinal direction rather than circular as they previously had been. This phenomenon was also observed by Bal (1966) in the hog ovary of one year of age. Mild general and localized intimal thickening was observed in arteries from 7 month old canine ovaries and according to Bal this thickening included up to one third of the vessel wall thickness in the 1.6 year old hog ovary.

In these younger age groups not all arteries of any particular ovary were affected by intimal splitting and/or thickening. It was more prev-
alent in some of the 7 month old specimens than in some of those 8 months of age. So at this age intimal changes were sporadic in appearance.

With the appearance of large corpora lutea in the dog ovary a separate vascular system was observed which served these structures. The arteries of this system had thick walls and underwent much more severe splitting of the IEM, intimal thickening, collagen infiltration and elastic breakdown than the vessels of the medulla. Those vessels undergoing cyclic sclerosis were probably analogous to those referred to by Sauramo (1954) in the 17 year old human. The more regressed corpora lutea were supplied by arteries with greater vascular changes. Changes observed in the medullary arteries of these same ovaries were much less severe.

One year of age to 4.1 years of age Up to 1.7 years of age most of the thickening was uniform around the artery. The first intimal plaque-like structure was observed in an artery from the 1.7 year old specimen. It nearly filled the lumen and was composed of smooth muscle cells, elastic tissue and collagen as Getty (1966) described for plaques found in the aortas of the dog and the hog.

Twelve of the 13 pairs of ovaries studied from the 2 to 4 year age span showed some degree of intimal changes from localized to generalized splitting of the IEM, to varying degrees of intimal thickening and collagen infiltration and elastoid formation. Up to half of the vessels were involved on any one ovary while the other vessels showed no intimal changes. Bal (1966) described a gradual increase in the intima of hog vessels during this age span and the appearance of intimal plaques but did not mention elastoid as being a component. Elastoid was most prominent in the arteries that supplied regressing corpora lutea.
In the ovaries of women that were over 28 years of age Sauramo (1954) found atherosclerotic changes in the hilar vessels. Twenty-eight years in man would be comparable to about a 3 year old dog. Sauramo observed elastoïd degeneration in the arteries of corpora albicans from the ovaries of a 3¼ year old subject (comparable to a dog just over 4 years of age). Sporadically occurring hyaline changes were observed by Kladetzky and Rosenbauer (1965) in the ovaries from a 3 year old lioness.

The only other change noted in the ovarian arteries from 2 to 4 year old dogs was a pronounced thickening of the collagenous tunica adventitia, which in most cases was thicker than the tunica media and intima combined. It blended in with the medullary stroma. The ovarian veins from this age group had thick collagenous walls infiltrated with elastic elements.

Six years of age to 10.4 years of age It was found in accordance with the pattern observed in other vessels of the dog and hog, (Getty, 1966) that an increase in intimal thickening and collagen infiltration took place during this age span. Only 2 sets of ovaries out of the 24 pair studied were free of intimal change. Most of the vessels in each ovary were involved to some extent. In over 75 percent of the ovaries from this group collagen infiltration of the tunica media was a prominent feature. In some it appeared to form a condensed ring of collagen next to the IEM. Severe arteriosclerotic changes were observed in the cortical vessels, especially those near regressing corpora lutea. In some cases near occlusion of the lumen due to intimal thickening, collagen infiltration and elastoid dispersion was found. Elastoid intercellular substances were described by Bal (1966) in the thickened arteries of similar location in the 6 year old hog ovary. Bal found an intimal plaque in a muscular vein
of an ovary from an 8 year old sow. A similar structure was observed in a
large vein from an 8.6 year old Beagle. The veins from the older ovaries
had thickened walls, almost hyaline in appearance in some cases. In such
cases elastic fibers and elastoid were also prominent components of the
wall.

Over 11 years of age In all of the ovaries from the 15 dogs
representing 8 different breeds some degree of vascular change was observed.
Only rarely were vessels found with an intact IEM and with no collagen in-
filtration. In many arteries that appeared at first to be in this category,
upon closer examination fenestrations or splitting of some part of the
IEM was found. No difference in vascular changes were seen with regard to
breed. Severe intimal thickening and collagen infiltration were observed
in some vessels of all ovaries from this age span. Nearly half of them
had intimal plaques-like structures. Elastoid was observed in many and
was a prominent feature of 3 pairs of ovaries. The smaller vessels of the
inner medullary and cortical regions had even more prominent vascular
changes than the larger hilar vessels. Hyalinization of the tunica intima
of ovarian arteries from 13 and 14 year old dogs was found by Tanaka (1962).
Bloom (1954) did not refer specifically to the intima but indicated the
arteries of ovaries from aged dogs and cats were many times thickened and
could be hyalinized. Toth and Gimes (1964) also referred to hyalinized
vessels with thick walls around corpora fibrosa. Hyalinization of art-
eries was not observed in this study.

Changes in the walls of the cortical vessels according to Trautmann
and Fiebiger (1957) were due to changing demands on the vascular system
during the development and regression of the follicle and corpus luteum.
This he called ovulation sclerosis. Sauramo (1952) divided the vascular changes of aging ovaries into 3 categories, ovulation sclerosis as described above, senile sclerosis and arteriosclerosis. The ovulation sclerosis or cyclic sclerosis as described in this investigation referred to those arteries that supplied corpora lutea. Senile sclerosis as defined by Sauramo was sclerosis of arteries of various other locations in the ovary, due to aging. He indicated that arteriosclerosis took place especially in the large arteries of the hilar area. All 3 types were observed and described in this study although they were not named as such. In the human Sauramo (1952) found that elastoid occurred even in the tissues between the vessels. This was also observed in the dog. It was especially prominent in the ovaries of the older age groups studied and around the larger veins. Whether or not all of these changes were brought about by a multifunctional arterial medial cell as described by Wissler (1967) was not known. He explained that the fibroplasia in the thickened intima may have been due to increased collagen formation by these multifunctional cells at the expense of elastin formation or myosin formation. Whatever the cause of the vascular changes, the results of the decreased lumen size were explained very well by Ringrose (1963) who stated that a reduction of one fifth in the diameter of a vessel reduced its flow capacity down to 57 percent. The effect of this gradual decrease in blood supply to the ovary was evidenced by the decrease in follicular activity in older ovaries.

Oviduct

Epithelium

The fact that cyclic changes occurred in the oviducal epithelium as well as in other areas of the reproductive tract was well documented in
the literature. It was described for the bitch by Eckstein and Zuckerman (1956), for the sow by Snyder (1923), Zupp (1924) and Palmer (1965), for the ewe by Casida and McKenzie (1932), for the bovine by Zupp (1924) and Weeth and Herman (1952) and for man by Novak and Everett (1928) and Velardo (1958).

Bloom (1954) stated that in the dog the changes were not especially prominent. Most investigators considered only the phase of the cycle and did not make any correlations with age. Inasmuch as this was primarily a study on age changes, the stage of cycle was mentioned, but the specimens were grouped into age groups rather than groups of similar cyclic phases.

Well developed primary and secondary folds as found in the fimbrial and ampullary portions of the canine oviduct (Figure 109), were similar to those described by Abdalla (1968) for the ovine oviduct. It was also found that the rugae of the isthmus were simple, unbranched structures in the canine as the latter investigator described for the sheep. The variations in the epithelium that covered these rugae in the different areas of the oviduct were discussed with regard to age groups.

**Chronological appearance**

**Birth to 2 months of age** During this age span the canine oviducal cells varied from a rather uniform height of 17 to 22 microns in height in the isthmus of the 2 day old specimen to 4 to 13 microns in this region of the week old specimen. It was 4 to 6 microns in the ampulla and 9 to 13 microns in the fimbria of the one week old specimen. Cells perpendicular to the epithelial cells and in various stages of mitosis were observed near the basement membrane in the 2 day isthmus. They were in-
creased in numbers in the fimbrial region of the 4 to 5 week old specimens. These cells were thought to be similar to the "indifferent" cell of the human tubal epithelium as described by Pauerstein and Woodruff (1967). They indicated that the near absence of mitotic figures was a striking histologic feature of the tubal epithelium. Such was also found to be true in older canine specimens but in the early age groups cells in various stages of mitosis were not rare.

In the 2 day old specimen only one type of simple columnar cell was observed, whereas in the week old specimen 2 types were observed in the isthmus. On the tips of the rugae cuboidal cells with dark staining nuclei were located and along the sides. In the crypts taller, lighter staining columnar cells were observed. Abdalla (1968) found that in the ovine oviducal isthmus the epithelium was always columnar even in the adult. In the bottoms of the crypts, invagination of the epithelium was observed and in some areas there appeared to be epithelial lined rings in the La Pr. This invagination was very prominent in the ampulla and fimbria regions of the 4 and 5 week old oviducts and in some areas the epithelium had become pseudostratified in appearance. Some of the precursor cells described above appeared to be in various stages of migration from the basement membrane into a parallel relationship with the epithelial cells. A light staining cytoplasmic secretion in various degrees of extrusion from the apical end of the cell was observed in the 4 week old specimens. Cilia were first observed on fimbrial cells of a 5 week old oviduct. Many of the non-ciliated cells showed large droplets of secreted material on their free border. Thus the epithelium of the 5 week old canine oviduct was similar to that described for the mature ovine oviduct in the prestral phase.
of the estrous cycle.

Two and one half months of age to 11 months of age  

Greater activity of the epithelium was noted in the 2.5 month old specimens in which 4 types of epithelial cells were described: (1) large, round cells with light staining cytoplasm and round basal nuclei, (2) as number one but with cilia, (3) similar to number one in size and shape but darker staining and in various stages of secretion and (4) tall columnar peg cells with long dark staining nuclei. Cell type one and 3 were both probably secretory cells with the former being in a non-secretory state. The epithelium varied from 5 to 15 microns in height. The shorter cells were on the tips and sides of the rugae and the taller cells were in the crypt areas. Cilia were found only in the fimbria region. Pseudostratification was found in some portions of the 3 month old oviduct, as described by Restall (1966) for the ovine oviduct in general and by Abdalla (1968) for the ovine oviduct in early proestrus. Lining the oviduct of the prepuberal bovine, Zupp (1924) found a ciliated columnar epithelium, complete and uniform, with elongated basal nuclei. Some of these stained deeply but most stained lightly and were granular. Zupp observed an occasional blue granular body on the epithelial surface which looked like a degenerating nucleus and some secretion that matted the cilia together. Specific age was not noted, only that the specimen was prepuberal. It appeared that the canine oviduct was a little more advanced at 3 months of age than the prepuberal bovine oviduct. Some neutrophils were observed in the oviducal lumen of a 5.5 month old canine specimen. In the 6 month age group the epithelium was generally tall columnar and up to 19 microns in height, even in the isthmus. Mitosis, cytolyses, neutrophilic
phagocytosis were noted in the basement membrane area of a 5.5 month old specimen (Figure 112). The epithelium lining the oviducts of two 7 month old Springer Spaniels and one 8 month old Beagle was very short (5 to 9 microns), showed only scanty secretion and very little evidence of cellular activity (Figure 113). This was in direct contrast to the more advanced activity and taller epithelium observed in the 6 month old specimens.

In the 8 specimens from the 9 to 11 month age groups, increased cellular activity was a uniform feature. Much invagination and complex infolding of the 10 to 16 micron high epithelium was observed. These sections were from the ampulla regions and showed either cilia or some secretory material. The rings of epithelial cells in the La Pr that resembled glands were probably just cross-sections of the highly complex infolding of the secondary and tertiary rugae. The cells lining these rings were identical to those of the crypt areas. Pseudostratification of the epithelium was noted in the oviduct from a 10 month old Beagle in which the epithelium reached a height of 39 microns. It was light staining, appeared hypertrophied and in one of the specimens nearly occluded the lumen (Figures 114, 115) while in another Beagle of the same age it was only 20 microns high, was low columnar and ciliated. Complex infolding was observed in both specimens. The first of these according to ovarian development was in late proestrous or early estrous while the latter one was in metestrous or pseudopregnancy. Thus it appeared that variations of the mucosa in different stages of the estrous cycle were more prominent than indicated by Mulligan (1942) and Bloom (1954). These authors mentioned that during proestrous and estrous the canine oviducal epithelium
was tall columnar. This was found to be partially true in the present investigation but in some areas the epithelium of estrous was definitely pseudostratified.

Of the 11 month old specimens studied, one was similar to the 10 month old oviduct described earlier which, according to ovarian studies was in late anestrum, even though tissues had been taken in April. Tissues were taken from the other 3 in April, May and July respectively. The first 2 were in late anestrous and the last one was in midpregnancy (Figure 117). The epithelial height was 6 to 15, 8 to 15 and 12 to 20 microns in height respectively in the ampulla. In the former 2 the epithelial cells were shrunken, dark staining and had a palisade appearance while in the latter specimen they were pseudostratified, ciliated and from some cells nuclear extrusion was noted as described by Abdalla (1968) for the oviducts from pregnant ewes.

One year of age to 4.1 years of age Tissues were taken between April and August from the 9 dogs that were one to 1.2 years of age. They were in anestrous or metestrous at the time of death. Epithelial height was generally 7 to 11 microns. Only limited secretion was found and no cilia were observed. Abdalla (1968) indicated that the characteristic feature of the ovine oviduct during late diestrus, (comparable to anestrous of the canine cycle) was the irregular free surface of the tubal epithelium. This was caused by the cytoplasmic projections and migrating nuclei, which he felt probably indicated degenerative changes. This was also similar to Zupp's (1924) description for the bovine in the 14th and 17th day of the cycle.

In the 1.7 year old specimen, the section was taken from the anterior
portion of the ampulla. The epithelium was tall (60 to 80 microns) pseudo-stratified ciliated columnar and indicative of estrous (as in Figure 115). Snyder (1923) indicated that the oviducal epithelium of the sow reached its peak height during estrous (25 microns). Similar findings were reported by Casida and McKenzie (1932) for the ovine (22 to 56 microns) and for the bovine by Weeth and Herman (1952) (3½ microns).

Of the 9 oviducts studied from dogs that were 2.7 to 4.1 years of age only one showed secretion (tissues taken in August), 4 others had pseudo-stratified ciliated columnar epithelium without secretion (tissues taken in May, June and August) and the remainder showed low simple columnar epithelium. The epithelium of the former 5 specimens varied from 8 to 28 microns in height, and of the latter from 4 to 15 microns in height. According to the ovaries, these animals were in metestrous and anestrous respectively at the time tissues were taken. Only cyclic changes were observed in these specimens.

Six years of age to 10.4 years of age As in the previous age group mainly cyclical changes were observed. Eleven oviducts were studied and the epithelium varied from pseudostratified ciliated columnar (in 3 specimens) to low ciliated cuboidal (in 7 specimens) and no cilia on the remaining one. The trend appeared to be one of a decrease in epithelial cell height (10 to 14 microns) in all except one which contained a 40 micron high epithelium that was in a secretory phase (Figures 125, 126, 127). There was also a general decrease in the number of epithelial rings observed in the La Pr from specimens over 9 years of age. This was possibly explained by the fact that most of the dogs were in anestrous when tissues were taken, resulting in a decreased complexity of primary
and secondary rugae, or it may have been due to aging.

**Over 11 years of age** In the 8 specimens studied in this age span the smooth trend described above was not observed. A 10 to 14 micron secretory ciliated columnar epithelium lined the oviduct of one 11.2 year old dog while in 5 other dogs 11.2 to 12.8 years of age the epithelium was 5 to 15 microns high, usually devoid of cilia and was cuboidal in nature. Of these latter specimens only the epithelial cells lining the rings had cilia. Epithelial rings were few in number in 8 of the 11 specimens. In one of the 3 remaining specimens they were not only numerous but were cystic and lined by a 4 micron high epithelium as compared to a 10 micron epithelium for the non-cystic rings and contained a PAS positive secretion (Figures 134, 135, 136). Epithelial rings were described as small epithelially lined pockets by Weeth and Herman (1952) for the bovine oviduct but they did not indicate if any cyclical changes occurred in these structures. In the dog, the epithelium that lined these structures usually appeared similar to the cells that lined the crypt areas and changed in appearance and height as the crypt cells changed. Yamauchi (1964) reported that the mean height of epithelium from 3 aged bovine oviducts was 20.8, 18.5 and 22.4 microns respectively in the isthmus and 19.4 and 13.5 microns for the ampulla. Contrasting this with the maximum height of 34 microns recorded by Weeth and Herman (1952) for the bovine oviduct during estrous and a minimum height of 18 microns during diestrous it would appear that the epithelium of the aged bovine oviduct simply regressed to the normal low height of diestrous. Secretory granules and nuclear protrusion were observed by Yamauchi (1964) in all 3 of the specimens that he studied. Similar evidence of activity was found in the older canine specimens also
(Figures 131, 132). Novak and Everett (1928) reported that there was but limited evidence of atrophic change in the human oviducal epithelium; that the cells remained high, many with cilia and that the non-ciliated cells showed no evidence of secretory change. They did, however, indicate that in some specimens from subjects over 60 years of age the epithelium had become cuboidal or even flattened. According to Kretzschmar and Stoddard (1964) the epithelium of aged human oviducts flattened and lost its cilia. Similar inconsistencies, or variations to those seen in man were very evident in the present investigation of the canine. Whenever it appeared that a trend was being established, one or 2 specimens of the age group would show extreme opposite development. The appearance of many mitotic figures in the epithelium from the oviducts of a 12.8 and a 13.1 year old dog was evidence of this even though Pauerstein and Woodruff (1967) felt that the near absence of such activity was typical of tubal epithelium.

Lamina propria

There was only limited information available in the literature on the La Pr of the mammalian oviduct. Schilling (1962) reported that longitudinal mucosal folds were more marked in the bovine, transverse ones more marked in the ovine and that during estrous the mucosal folds were markedly hyperemic. Directional orientation of the rugae was not determined in this study. Cyclic variations were observed in the mature dog, however, similar to those described by Abdalla (1968) for the ewe, in which the stromal cells became hypertrophied during estrous (Figures 114, 115).

Chronological appearance

Birth to 2 months of age    The La Pr of the 2 day old canine oviduct consisted of loose mesenchymal tissue with a few collagen strands
criss-crossing it. Mitosis was a prominent feature of the younger specimens. Cellular organization was first observed in a 2 week old oviduct in which mature fibroblasts and elastic fibers were observed. In the 5 week old specimen the La Pr appeared edematous indicating even at this early age some endocrinological response (Figure 108). The ovary from this animal showed development of primordial follicles.

Two and one half months of age to 11 months of age. A definite increase in density of the La Pr from the fimbria to the isthmus was noted in the 2.5 month old oviduct (Figures 109, 110). Copenhaver and Johnson (1958) stated that this arrangement was typical of the mature oviduct. According to Clyman (1966) the La Pr of the human oviduct consisted of many bundles of decussating collagen fibrils with interspersed fibroblasts. In addition to these structures some elastic fibers as well as other cellular elements were found in the canine La Pr (Figures 111, 113). Scattered smooth muscle cells were reported by Leeson and Leeson (1966) as being part of the human oviducal La Pr. Such was the case in the canine only near the circular muscle layer where fibers appeared to angle from the La Pr and blend into the muscularis proper (Figure 113). In the bovine Weeth and Herman (1952) found reticular fibers with the collagen fibers in some of the wider folds. Reticular and collagen fibers were prominent structures in the canine La Pr.

With the maturation of the La Pr a more circular orientation of the outer fibers and a septal orientation of the inner fibers up into the various rugae was found. Some of the other cellular elements observed in this structure even in younger specimens were neutrophils, plasma cells and lymphocytes. Inasmuch as this was mainly a growth and aging study,
particular attention was not paid to cellular elements as they were described well by Nellor (1965). No other morphological changes were noted in the La Pr up to 11 months of age other than cyclic changes.

One year of age to 4.1 years of age A gradual increase in density was the only change observed in this age span along with the cyclic changes. The amount of tissue of which the La Pr was comprised in any particular section was related to the amount of edema present and/or increased size of the stromal cells as described by Snyder (1923) for swine or the functional stage and size of the epithelium. This was in contrast to Sani's (1950) finding that the connective tissue of the ovine and bovine did not become edematous during estrous (Figures 112, 114, 115).

Six years of age to 10.4 years of age A further increase in the density of the connective tissue elements was observed. The report that the tubal rugae of some human subjects over 60 years of age became rounded and fibrous (Novak and Everett, 1926) was observed in comparable aged canine oviducts of the age group under consideration. Pinero and Foraker (1963) described increased amounts of plical tissue after the menopause and indicated that collagen fibers were more pronounced in older specimens. This was also true in comparable aged dogs (comparing a 7 year old canine to a 44 year old human). The increase in elastic elements up to 35 to 40 years of age in the human oviduct followed by regressive changes in elastic fibers as observed by Sani (1950) was also observed in the canine. A few elastic fibers were observed in younger specimens and as early as 3.6 years of age (comparable to 30 years of age in man) elastoid was observed. Pyknotic nuclei and vacuolization of La Pr cells was observed in some of the older specimens of this age group.
Over 11 years of age In contrast to the last age span studied
the La Pr was decreased in amount in most of the specimens (Figures 131,
132) although it was still dense except in the oldest specimen studied. In
none of this group were pigment cells found as were described in the ovine
oviduct La Pr (Hadek, 1965), and in that of aged bovine (Yamauchi, 1964).
No other age changes were observed in the La Pr of the canine oviduct ex­
cept some elastoid interspersed among the collagen fibers.

Muscularis

It was found that the growth changes of the oviducal muscularis were
much more easily recognizable than the age changes. The general morpho­
logical pattern was as described in the textbooks (Trautmann and Fiebiger,
1957; Copenhaver and Johnson, 1958; Bloom and Fawcett, 1962; Leeson and
Leeson, 1966; and Arey, 1968) except they only mentioned 2 muscle layers
and often 3 were found. In the dog oviduct the wide ICM and scattered ELM
were as described in the textbooks, but often longitudinal and oblique bun­
dles of muscle fibers were found scattered in the VL. Although there was
no definite line of demarcation between this layer and the outer layer
these bundles were definitely associated with the VL and embedded in the
connective tissue of that structure. (Figure 117). A similar third muscle
layer was described in the human oviduct by Kiper (1950) and Horstmann
(1952). Horstmann described the inner layer as being arranged in spirals,
that originated from different directions and intersected at regular in­
tervals. Evidence of differential fiber direction was observed in the
 canine. The VL muscle was definitely observed in canine specimens. Toni
and Maccaferri (1951a, 1951b) also described 3 muscle layers from the human
oviduct, but they found that there were inner and outer longitudinal layers
with a middle spiral muscle layer. In the dog oviduct (Figures 117, 119, 120) at the junction between the La Pr and the ICM bundles of oblique fibers were often observed but were so sparse and inconsistent that they were thought to be origins of some of the more obliquely described fibers of the ICM as described by Horstmann (1952). In one oviduct from a 11.9 year old Beagle they were very discernable but did not extend all the way around. In the older specimens fibers were observed to extend from the ICM part way into some of the folds of the La Pr. These also may have been what Toni and Maccaferri referred to as the inner longitudinal layer, if such was the case then there were 4 muscle layers instead of 3. Schilling (1962) also described an internal self-contained longitudinal muscle which he found in the caudal ampulla of the ruminant oviduct.

The outer muscle layers were often not observed as they were easily disrupted in the taking of tissues at the time the oviduct was removed from the ovarian bursa. It was found that the ELM layer of the oviduct and its surrounding connective tissue were almost inseparably connected to bursal tissue (Figures 113, 117).

According to Trautmann and Fiebiger (1957) the tunica muscularis of the mammalian oviduct was rich in elastic tissue which gave off numerous radial strands into the neighboring submucosa. Elastic fibers were found in the canine oviducal muscularis, but were not prominent. Also, according to definition there was no submucosa in the canine oviduct because the La Pr was adjacent to the inner muscle layers.

**Chronological appearance**

*Birth to 2 months of age* The future areas of muscle differentiation were seen but were comprised of embryological type cells with a
few scattered collagen and elastic fibers. During the first 2 months of life the only change noted was a slight increase in density of the pre-smooth muscle cell nuclei, collagen and elastic fibers. No information was found in the literature on the early growth changes of the oviducal muscularis of the canine or of any other species.

Two and one half months of age to 11 months of age An increase in collagenous connective tissue was observed in all layers of the oviducal muscularis up to 5.5 months of age at which time a few circular smooth muscle cells were observed near the La Pr. They had formed a narrow band 2 to 3 cells in depth in the 6 month old specimen. By this time a very dense wide layer of circularly disposed connective tissue was observed between the circular muscle band and the vascular layer which was also composed of dense connective tissue of variable direction and an outer connective tissue layer which contained a few longitudinally directed smooth muscle fibers. The circular muscle developed more rapidly and became much wider in the isthmus than in the more cranial portions. A corresponding increase in connective tissue was observed in the cranial portions of the oviduct. By 11 months of age the musculature of the canine oviduct was mature in appearance. A prominent well defined circular muscle was observed as far cranial as the cranial ampulla. The ELM of this specimen was composed of a band, one to 2 cell layers wide in the ampulla with some muscle bundles also observed in the vascular region. No information was found in the literature regarding this growth phase.

One year of age to 4.1 years of age With the exception of degenerative changes observed in the musculature of some of the one year old oviducts studied, the remainder of the specimens showed a trend of in-
creased density of the muscularis. This was the result of collagen infiltration as well as by smooth muscle cell growth and development. The increase of collagen was much more prominent in the fimbrial region.

**Six years of age to 10.4 years of age**

It was again observed that there was no intrinsic musculature in the cranial fimbria portion of the oviduct. In the older specimens of this age group smooth muscle fibers were often seen extending part way into the rugae of the more caudal sections. A general increase in numbers of nuclei observed in the microscopic field was found in the older oviducts as a result of an apparent decrease in cytoplasm (Figure 129). According to Toni and Maccaferri (1951a) there was a gradual increase in oviducal musculature in the human from 20 to 50 years of age, compared to 2 to 9 years of age for the dog.

**Over 11 years of age**

Lack of consistency was the main observed feature for this age span. The musculature varied from low density to high density (Figures 130, 131). There appeared to be a greater infiltration of the ICM by thick collagen bands from the La Pr. The musculature of 2 of the older oviducts studied appeared to be especially lacking in cytoplasm as the large dark staining nuclei were very prominent in the vacuolated cytoplasm. Toni and Maccaferri (1951a, 1951b) reported that muscle layers and muscle cells decreased with age from 50 to 80 years of age in the human or comparably 9 to 16 years of age in the dog. The decrease in muscle layer thickness and cell degeneration observed in some of the oviducts from older dogs was indicative of a trend but the small number of specimens used and inconsistencies found decreased its significance.
Vasculature

The vascular pattern found in the dog was essentially as described by Copenhaver and Johnson (1958) for man. Subepithelial capillaries, capillaries and arterioles of the ICM, vessels of the VL and the adjacent vessels were constant features of the canine oviduct. They varied greatly in number and size. During proestrous, estrous, early metestrous and pregnancy they were more numerous and usually engorged with blood, especially in the ampulla and fimbria regions. Whereas during anestrus the vascular development was minimal. Trautmann and Fiebiger (1957) mentioned that during pregnancy the oviducal vessels were especially numerous, followed a longitudinal course and formed a dense subepithelial plexus.

No other reference was found regarding cyclic vascular variations as related to the oviduct. Very little information was available on any aspect of oviducal vascularization except by Gillet and Pietri (1967) who divided the human oviducal vascularization into 4 parts: (1) la vascularisation arterielle de la mugueuse et le territoiri capilloire, (2) les arterioles musculaires, (3) le reseau arteriel soussereux, and (4) l'arcade arterelle juxta-tubulaire at ses collaterales. They indicated that the latter arteries varied in size with hormonal influence, especially during pregnancy. With regard to the first arteries of this series, which corresponded to arterioles of the La Pr described in the present investigation, they found spiral or helicine arteries comparable to the spiral arteries of the endometrium, and which were similarly influenced by hormones.

Chronological appearance

Birth to 2 months of age The capillaries found in the La Pr and the capillaries and arterioles found in the other layers of the oviduct
increased in size and number in the first week of postnatal life. Some of the arterioles of the VL had gained another muscle layer. In the week old specimen smooth muscle cells were observed in the walls of the veins. By 4 weeks of age arterioles were observed in the La Pr, probably corresponding to the spiral arteries described by Gillet and Pietri (1967) for man. These arteries were especially prominent in the fimbria region of the 5 week old specimen.

Even in these early age groups it was apparent that the vasculature was poorly developed in the isthmus where the intrinsic musculature was well developed. The vasculature became progressively better developed in the ampulla and reached its peak of development and complexity in the infundibular portion where intrinsic musculature was nearly non-existent (Figures 106, 107). The following may have been reasons for this morphological phenomenon: (1) the infundibulum was much more active than the isthmus, thus required a greater blood supply, (2) the epithelial surface area of the cranial portions was increased many fold over the caudal areas, thus needed an increased blood supply for nourishment and secretion, (3) the increased action of the fimbria and infundibular musculature during normal physiological function indicated a need for greater blood supply and (4) the necessity of getting positive rapid reactions by the infundibulum (as at ovulation) from changes of hormonal blood levels intimated need for more complex vascularization in the cranial portions of the oviduct.

Two and one half months of age to 11 months of age It was confirmed more prominently in this age group than in the younger one that the blood supply was relatively poor for the isthmus and much more highly
developed in the ampulla and infundibulum. The main changes in vasculature in this age group were the appearance of arterioles and venules in both the primary and secondary rugae of the La Pr of the 2.5 month old oviduct, and in the dense part of the La Pr proper in the 3 month old specimen. Thus it appeared that the greater vascular development had taken place in the La Pr. Although the arterioles of the VL had increased in size and development correspondingly as they now had up to 4 muscle layers and a complete IEM.

Veins with both collagen and elastic fibers were also found in the 3 month old oviduct. Up to 11 months of age the vessels developed in size and number and were especially numerous and even engorged in the oviducts from animals that were in proestrous, estrous, metestrous or were pregnant when the tissues were collected.

Some of the larger arteries found in the bursal tissues adjacent to the oviduct had age changes as early as 6.5 months. In the latter specimen a split IEM was observed. Plaque-like formations were found in a similar artery from a 10 month old oviduct and the elastic tissue content in the walls of adjacent veins had increased noticeably.

One year of age to 4.1 years of age Vascular changes that occurred in vessels adjacent to the oviducts, other than enlargement and engorgement, related to hormonal influences. They mainly affected the larger arteries and veins in the adjacent bursal tissues. No intimal change was observed in these arteries in the one to 1.2 year age group, although some collagen infiltration of the tunica media was observed. In the oviduct from a 1.3 year old dog, thickened collagenous rings had formed around the tunica media. An increase of elastic fibers was observed in the veins of a 1.4 year old specimen. No other visible changes were found up
to 3 years of age at which time intimal changes involving 10 to 30 percent of the intima were seen (Figure 123), and in one specimen splitting of the intima, collagen and elastic infiltration of the tunica media were observed (Figures 121, 122). Elastoid was first observed in the adjacent oviducal arteries of a 3.3 year old specimen in which some of the arteries also had 30 percent intimal involvement. In a 4.1 year old oviduct an increased amount of elastoid was found not only in the tunica media but in the adventitia and surrounding tissues. The arterial and venous changes observed up to this point were not necessarily gradually increased in severity as the vessels from many of the specimens showed no change. Even when changes were observed they often involved only one or 2 vessels with the remainder showing intact vascular walls. Thus of the 23 oviducts studied in this age span; only one plaque-like structure was found (in a vein from a 3.6 year old specimen), elastoid was observed in 2, collagen and/or elastic infiltration of the tunica media in 3, intimal splitting in 3 and varying degrees of intimal thickening in 6. In some oviducts one, 2 or more changes were observed in the same artery so that actually only 12 of the 23, or just over 50 percent of these specimens showed vascular changes. If one picked out only the oviducts with changes it would appear that an increasing severity of involvement was found with age.

Six years of age to 10.4 years of age A similar phenomenon was found in this age span as was observed in the one to 4 year old specimens. Fewer numbers were involved in this group but changes were seen in only 4 out of the 11 studied. Of these the degrees of collagen infiltration, elastic fiber breakdown, intimal splitting and thickening were more prominent than in the younger age groups. No changes were observed in the
oviducal vessels from 7 dogs. The reason for this may have been that most of these animals were in anestrous when tissues were taken. The stage of estrous was known to effect changes in the vascular walls even on a cyclic basis. It was recognized that the vessels in which changes occurred served not only the oviduct the the ovarian bursa as well; so if their primary purpose was the latter their need for cyclic involvement was less than if they served only the oviduct.

**Over 11 years of age** The only changes observed in the 7 specimens studied for this age group were in the arteries of the vascular layer from a 11.9 year old Beagle. This animal was in metestrous when tissues were collected and the arteries were engorged with blood. Thirty to 90 percent intimal thickening was found, elastoid was abundant and the tunica media was highly infiltrated with collagen. It was observed that some of the collagen infiltrating bands were near hyaline in appearance (Figure 133).

In the other 6 specimens, only the vessels adjacent to the oviduct had changes and none were observed in the intrinsic vasculature. The degree of involvement varied from 10 to 30 percent of intimal thickening, varied amounts of collagen infiltration and elastoid formation. At least one or more of the larger arteries of each specimen was affected. Other arteries were free of change. Five of the animals were in anestrous and one was in metestrous when tissues were taken. The latter one showed only engorgement of the intrinsic vessels but no vascular changes.

Disruptive changes in the walls of arteries and veins were rarely found in the intrinsic vessels of the canine oviduct irregardless of age or stage of cycle. Changes of the extrinsic vessels were highly variable
in their occurrence, but when they did occur they seemed to be of greater severity in the older specimens. The small numbers involved and the variability of the locations on the oviduct from which the sections were taken precluded more positive statements in this regard. Also, a greater variety from all ages of different stages of the estrous cycle and pregnancy would have to be evaluated in order to see if cyclic changes such as intimal thickening and collagen infiltration occurred and resolved themselves each cycle. The fact that such a phenomenon was observed in the follicular-corpora luteal vascular system of the canine ovary, and that the only vascular wall changes of intrinsic oviducal vessels of this study were observed in an oviduct from an older animal in metestrous, indicated the possibility of the same thing occurring in the oviduct. Further studies are needed in this area.

Uterus

Epithelium

The most prominent feature of the uterine epithelium was its variable appearance at different stages of the estrous cycle. Associated with nearly a 7 fold change in cell height (9 to 60 microns), variations in cytoplasm and nuclear stainability, variations in nuclear location, variations regarding the presence or absence of cilia were also observed. Due to the fact that the tissues were collected when the animals had reached a certain age rather than with regard to stage of estrous cycle it was difficult to find specimens of different ages that were at exactly the same phase of the cycle when tissues were collected. Zupp (1924) stated with reference to the bovine that there was no part of the female genital tract which lay dormant during any part of the estral cycle and that microscopic
changes were going on continuously. With this fact in mind the difficulty of trying to discern age changes of such a dynamic tissue was doubly difficult. It appeared, however, at least histologically that the uterine epithelium of the old or young cycling animal was similar. Perhaps there were more subtle changes, for it was reported by Talbert and Kroun (1966) that the uteri of old mice were less able to support pregnancy than were the uteri of young adult mice. It was recognized that although there were many other factors affecting the maintenance of pregnancy, the uterine epithelium, especially with regard to the implantation phase, was very important.

The appearance of the canine uterine epithelium at different stages of the estral cycle, as described by Evans and Cole (1931), Mulligan (1942) and Eckstein and Zuckerman (1956) were confirmed by this study. The complex epithelial proliferation observed in the canine uterus during the post-estral period was not found by Zupp (1924) in the bovine or Green (1950) in the porcine, so this was thought to be a species difference.

No free blood was found to have escaped through the epithelium in the proestrous period in the specimens studied during that phase of the cycle. Evans and Cole were not able to find any either although it was still thought that the uterus was the source of this blood (Venzke, 1967). Donovan (1968) felt that it came from the vagina.

**Chronological appearance**

**Birth to 2 months of age** The tall (23 to 28 micron) pseudo-stratified columnar epithelium of the 2 day old specimen was reduced in height (10 to 14 microns) in the 2 week old specimen (Figures 138, 141). This reduction in height may have been due to the loss of maternal hormonal
influence after parturition and was sufficiently significant that it showed up in statistical analysis (Graph 3, Table 4). Mean epithelial height for this group was 15 microns. The nuclei which had been located in various parts of the cells at 2 days of age had become more basally located. More numerous areas of infolding of the epithelium were observed which indicated that changes other than growth per se took place even at this early age (Figure 141).

Two and one half months of age to 11 months of age A decrease in mean cell height (11 microns) was observed in these specimens. In the 2.5 month old specimen the epithelium was cuboidal. The cells had large central nuclei and light staining cytoplasm. Large mononuclear cells were found in the lumen of the 2.5 month old uterus and many were observed in various stages of migration through the epithelium. These cells were observed in different concentrations in all of the uteri examined. It appeared that the epithelial activity had slowed down almost to a standstill by 2 months of age, except for areas of invagination and new gland formation. This infolding was increasingly more prominent up to 6 months of age at which age secretory material was first observed on the epithelial cells. Recalling the fact that ovarian activity had reached its first real follicular growth phase at this age perhaps explained the secretion in the 5.5 and 6 month old specimens. A PAS positive bm was also well defined at this age (Figure 147).

The next change was an increase in height of the epithelium up to 23 microns and the appearance of cilia. A linear increase in gland epithelial height was observed as the ovarian weight increased (Graph 7) which was due to normal growth and development. These cells contained basal nuclei
and moderately dark staining apical cytoplasm. By this age, depending upon the time of year the animals were cycling (Figures 151, 152), thus the appearance of shorter 9 to 12 micron cells in most of the 11 month old uteri was explained. In an 11 month old pp specimen the epithelium was tall (12 to 16 microns) pseudostratified, columnar, and was highly proliferated as Mulligan (1942) had also observed in the pp uterus. Thus, this particular animal had been through a cycle and pregnancy by the time tissues were collected in July. Except for the pyometria this uterus was in the state of involution expected. Neutrophils and mononuclear cells appeared in great numbers in the complexly folded epithelium of this specimen, presumably as a part of the inflammatory process.

One year of age to 4.1 years of age The uterus of the first one year old specimen studied was also pp (about 3 weeks) when tissues were taken. The lumen of this uterus contained large collagenous masses, leucocytes, cell debris, and sloughed groups of epithelial cells. The complex infolding of the epithelium as described for the 11 month old pp specimen was not observed as most of it had presumably already been sloughed off. The remaining epithelium was 23 to 32 microns in height and light stained. Mulligan (1942) described partially hyalinized fibrous connective tissue at 10 days pp in the bitch. In some cases these were small isolated islands surrounded by leucocytes and cellular debris, while in others they were semi-intact and it appeared as if large areas of the epithelium and underlying La Pr had become collagenous.

The only changes observed in the remainder of the uteri studied for this age span were cyclic changes previously described in the literature and without additional significance.
In the previous age span 62 percent of 26 specimens were in proliferative stages while in this span 39 percent of 18 specimens were. Tissues were taken from 78 percent of the dogs from April through September during which period one would normally expect cyclic activity. Thus it appeared significant that such a decrease in cyclic activity was observed in these older animals. Mulligan (1942) indicated that as far as it could be estimated the estral cycle continued regularly up through the longest survival period which was put at 14 years of age, with an average of 8 or 9 years of age. The results of the present study showed that cycling occurred up through 10 years of age but not necessarily regular cycling.

Over 11 years of age As was observed in the oviduct, the trend set in the 6 to 10 year old age span was reversed by the older age group. Although only 10 specimens were studied for this span, over half of them were at least in early proliferative states while the rest were in regression in which the height of the epithelium was decreased. No age changes per se were observed in the epithelia of the older uteri as compared with similar stages from younger specimens other than perhaps fewer areas of invagination resulting in less undulation of the uterine lumen contour. According to Speert (1949) the epithelium from the aged human uterus consisted of a single layer of cuboidal cells devoid of cilia and that only rarely did surface invaginations make connection with the underlying glands. This was true for the older canine uteri which happened to be in anestrous. In the uteri from 3 aged cows Yamauchi (1964) also found that the superficial epithelium seemed to be normal.

Although there was a trend of decrease in epithelial height noted in
the older specimens studied (Table 4) the regression coefficient was not significant because of the limited number of available specimens. There was, however, a significant linear increase of the various uterine muscula ris layers with measured increase in epithelial cell height (Graph 12). In this case the regression coefficient was significant. This showed the close synchronous relationship between the various parts of the uterus to hormonal influence.

**Lamina propria and glands**

The canine uterine La Pr was composed of a network of collagen fibers, some elastic fibers, stromal cells and lymphoid cells similar to the components given by Arey (1968) in reference to the human uterus. Arey, however indicated that it was a reticulin network rather than collagenous and elastic. Collagen fibers were found to be part of the La Pr by Harkness (1964). The conclusion by Fainstat (1964) that the extracellular fibers which had classically been called collagen and reticulin appeared to have been interconvertible or reversible in the endometrium in various reproductive states. He explained that collagen and reticulin were essentially 2 histological names used for many years to describe what was apparently the same extracellular fiber. In the present study, most of the extracellular fibers stained positive for collagen with Verhoeff's and Van Giesen's stain although it was realized that this observation was based on the old definition and it was admitted that Fainstat could have been very correct in his statements. Reticulin stains showed that these fibers were also dense in the La Pr (Figure 145).

The four basic layers of the canine La Pr as described by Bloom (1954) were: (1) the outer or crypt layer, (2) the connective tissue stromal layer,
the tubule layer and (4) the innermost or basal gland layer. They varied in proportion in different stages of the cycle. Large mononuclear cells, referred to as lymphoid cells by Arey (1968) for the human, were found in varying numbers in different specimens, but were found in all sections studied. The lymphocytes described by Nellor (1963) in the bovine uterine La Pr only during the luteal phase may have been different cells. Turnbull's blue, iron stain was used by Mulligan (1942) to observe pigment containing macrophages. These were not present in the immature La Pr but were present in older animals and were especially noticeable in proestrus and in the post-parturient period. Cyclic changes were described only as they related to long term changes or as they indicated continued function. Cyclic alterations that related to thickness of the La Pr were recorded to determine if there was any change with age as was found in the sow in the study by Hadek and Getty (1959).

Chronological appearance

Birth to 2 months of age In the 2 day old canine uterus 51 percent of the 240 micron thick uterine wall was La Pr as compared with 55 percent for the new born porcine uterus (Hadek and Getty, 1959). As Hadek and Getty found in the pig, there were a few indentations in the epithelium but no glands as such in the 2 day old specimen. The framework of the La Pr in the 2 day uterus consisted of 2 concentrations of collagen fibers, subepithelially and near the muscularis respectively, joined by a few perpendicularly oriented collagen fibers. In this was embedded the stromal cells, lymphoid cells and capillaries of the La Pr.

The uterine wall thickness had increased in both the one and 2 week old specimens, but the La Pr which made up 52 percent of the former uterus
comprised 62 percent of the latter. Infolding of the epithelium to form uterine glands was observed for the first time in the enlarged La Pr of the 2 week old specimen. Subepithelial glands and some gland tubules that extended half the depth of the La Pr, similar to those described by Kadek and Getty (1959) for the 2 week old porcine were found. An increase in connective tissue elements was observed in the 2 week old specimen in a swirl-like perpendicular orientation. This wavy, swirl appearance may have been early helicine formation of collagen fibers which Fainstat (1964) indicated may have been the manner in which collagen fibers existed rather than as 2 dimensional waves which was the typical concept. Fainstat's explanation of interwoven branching spirals appeared to be very feasible.

Two and one half months of age to 11 months of age The endometrium of the 2.5 month old canine uterus made up only 57 percent of the 630 micron uterine wall as compared with 62 percent for the 2 week old specimen. More important, however, the uterine wall had nearly doubled in thickness. In the two porcine specimens of 2.5 months of age measured by Kadek and Getty (1959) it was shown that the La Pr which had formed up to 39 percent of the uterine wall in the 5 week old uterus, made up only 80 percent of it in the 6 week old specimen. Thus the trend of an early high proportion and a later decrease was similar in both species but not as prominent in the canine. Closely related to the La Pr fluctuations was the glandular development, which extended nearly to the muscularis in the 2.5 month old canine uterus. Only 14 glands or sections of glands were observed in a 2.5 month old and a 3 month old uterus compared with 40 in another 3 month specimen. This was an indication of individual variation. A similar degree of gland development was observed by Kadek and Getty in the
3 month old porcine uterus. The endometrium of the 5.5 to 6.5 month canine specimens comprised about 60 percent of the uterine wall compared with a constant finding of 69 percent for the porcine La Pr at both 3 and 4 months of age. It was interesting that in the canine the uterine wall increased in thickness by one third (630 to 980 microns) while according to Hadek and Getty (1959) the porcine uterus during the same period had increased only slightly (5696 to 3822 microns). It was assumed then that the porcine uterus reached maturity earlier than the canine uterus. Up through 11 months of age there was a linear increase in La Pr with age in the canine (Graph 3). The regression coefficient was significant in this analysis of measurements from 30 animals (Table 3A).

A breed difference was observed in the 7 month old canine age group. The endometrium of the 2 Beagle uteri comprised 61 percent of the mean uterine wall thickness of 970 microns compared with 55 percent for the mean wall thickness of 670 microns for the uteri from 4 Springer Spaniels. Glandular development was also more advanced in the Beagles. The fact that the Beagle tissues were collected in March and the Springer Spaniel tissues collected in August was thought to have some bearing on this, as well as the fact that larger breeds of dogs were slower to mature.

Cyclic changes as observed in the 9 uteri which had been collected when the dogs were 8 to 11 months of age made it difficult to evaluate growth or age changes as the La Pr fluctuated in thickness from 500 to 1600 microns and comprised from 24 to 67 percent of the uterine wall. It was found that the uterine La Pr from 2 bitches that were in heat when tissues were taken had an increased thickness (1000 microns) and that the La Pr comprised 50 percent of the wall thickness (Figure 151). While on the
other hand the La Pr from a 10 day pp uterus in an interzonary region made up only 24 percent of the uterine wall and another from a 2 week pp uterus was 1600 microns thick or 60 percent of the wall. Thus, instead of growth changes, cyclic changes had become the major factor of change in the width, glandular and connective tissue components of the La Pr. Inasmuch as cyclic changes were described by Evans and Cole (1931) and Mulligan (1942) they were described in the present work only for verification in some areas and as they related to continued function in other specimens.

One year of age to 4.1 years of age As in the 8 to 11 month age group, cyclic variations were the most prominent feature of this group. The La Pr of 13 specimens (from one to 1.7 years of age) made up 30 to 60 percent of the uterine wall and in 12 specimens 2.3 to 4.1 years of age it was 25 to 60 percent. Total mean uterine wall thickness for all the uteri in the one to 4.1 year age span was 700 microns with a mean wall thickness of 2000 microns. It would appear from this that the La Pr and total uterine wall thickness was slightly less than found in the 8 to 11 month age group. These figures, however, simply indicated a high fluctuation of La Pr thickness (300 to 1200 microns) and total uterine wall thickness (1100 to 4000 microns) in the canine uterus over 6 months of age as compared with the finding by Hadek and Getty (1959) that from 6 months on these structures in the porcine were more or less constant and showed only small variations. From 25 to 38 years of age in the human, (comparable to 2 to 5 year old dog) Sauramo (1954) indicated that the endometrium comprised 16 to 20 percent of the uterine wall.

The glands varied in size and number with the stage of the cycle and were especially numerous in metestrous or pregnancy. Collagen bands,
although generally of increasing density were loose and appeared edematous in the uterine La Pr from a Beagle that was in late anestrous when tissues were collected. Mulligan (1942) indicated that the La Pr became edematous in proestrous so the above could have been approaching an early proestrous state.

The only abnormal structures found in this series were cysts in a 1.1 year old Beagle uterus. Some were round on cross-section and were 1400 microns in diameter, others were oval and were 500 by 1200 microns in size. They were lined with a layer of flattened cells and contained cell debris.

Large blood filled sinuses were found in the La Pr of a 3 year old Beagle in which large collagen structures, masses of fluffy, light stained epithelium and leucocytes were also seen. This uterus was typical of one described by Mulligan (1942) for a 2 month pp specimen.

Six years of age to 10.4 years of age  The La Pr of the 16 uteri studied for this group comprised 25 to 60 percent of the uterine wall, very similar to the younger adults. However, the total thickness of the wall was greater (1000 to 2900 microns) with a mean of 1800 microns. The figures were deleted for the one week pp uterus that was still in early stages of involution. The range otherwise was 1000 to 3600 microns, and the mean uterine wall thickness 1950 microns which was still greater than in the younger specimens. The mean La Pr thickness after such a deletion was 580 microns, or just about 100 microns less than it was for the one to 4.1 year-age group.

Connective tissue orientation of the La Pr depended on the gland development as was found to be the case in younger groups. It was perpendicular to the muscularis during anestrous and early proestrous, when gland
development was minimal, and either disoriented or somewhat circularly oriented during the other phases of the cycle when gland development was greater.

The La Pr of the uterus from a 9 year old African Basenji was nearly filled with various sized cystic glands (endometrial hyperplasia according to Bloom, 1954). Some of the cysts were large enough to fill a low power field and were lined with simple columnar to tall ciliated pseudostratified columnar epithelium. The interglandular and inter-cyst stroma were composed of dense collagenous tissue. A ring of smaller but thin walled glands, similar to the uterine glands of pregnancy was observed in the basal portions of the La Pr. Between the superficial cysts and the deep glands were some smaller glands lined with tall, ciliated, pseudostratified columnar epithelium. Yamauchi (1964) found cystic distension of uterine glands in the uteri from three aged cows, 17, 21 and 28 years of age respectively. These glands frequently contained secreted material and sometimes hyaline substance was present in the epithelium of the cystic glands. Cystic glands were also found in postmenopausal human uteri, (Noer, 1961). Whether the cysts found in the African Basenji were related to aging or other endocrinological disturbances was not known. Probably a combination of both as the condition was found in increased frequency in older dogs (Bloom, 1954).

Over 11 years of age Uterine sections from 10 dogs between the ages of 11 to 13 years, represented this age group. The mean uterine wall thickness was 1600 microns, less than the 1800 microns for the 6 to 10 year age group, while the mean La Pr thickness was 500 microns or slightly lower than in the former group. Thus it appeared that the overall changes
in the La Pr for this age were minimal. A thinning of the La Pr noted by Bigelow (1958) and Kretzchmar and Stoddard (1964) in the human was observed in this study although the regressing coefficient was not significant with the small numbers of animals involved.

Cysts were found in the La Pr of 6 of these specimens. They were of all sizes and lined with flattened or pseudostratified columnar cells. Due to the high proportion of dogs of this age group that had cysts in the La Pr it was thought to be an aging phenomenon. Cystic glands were reported in the bovine by Foley and Reece (1953), Yamauchi (1964) and Mochow and Olds (1966) and in the human by Bigelow (1958), Noer (1961) Kretzschmar and Stoddard (1964) and Mansour and Baradi (1965). There was no reorientation of the glands from perpendicular to the muscularis to vertical as found by Bigelow for the human.

Inasmuch as there was evidence of cycling, even in the oldest specimens studied it was difficult to discern if there was increasing fibrosis of the La Pr stroma as was observed by Bigelow in the human. Wolfe, et al. (1942) indicated that in the uteri from old rats (180, 360 days of age) the zone of collagen made up 80 to 95 percent of the total width of the endometrium, with the thickness of the collagen fibers being much greater in the older specimens. This was not so pronounced in the canine. Neither atrophy of the uterus nor lymph nodules in the La Pr were found in the canine specimens, as Yamauchi (1964) found in one of the 3 aged bovine uteri that he studied.

**Myometrium**

The ICM, VL and ELM comprised the 3 distinct layers of the canine myometrium. Although bundles of fibers, especially in the ICM and the ELM
were often found directed obliquely rather than circularly or longitudinally, no other distinct, consistent layers of muscle were observed. This verified the findings of Rudolph and Ivy (1930) for the canine uterus. Weeth and Herman (1952) found that the ICM of the bovine uterus contained no collagen fibers. In the present study these fibers were found in varying numbers, especially in the uteri from older dogs. Although the ICM interdigitated with the La Pr to some extent in the younger specimens it was well delineated in the younger as well as all older uteri and was not at all difficult to demarcate as Bird and Willis (1965) indicated was the case in the human uterus. A possible reason for this was the fact that according to Leeson and Leeson (1966) and Arey (1968) the inner muscular layer of the human uterus was longitudinal and therefore more difficult to delineate in cross-section.

The fact that the muscle bundles of the VL and ELM were well delineated by, and in some cases almost embedded in, dense collagen was not found in the literature regarding the canine uterus. The variations in collagen, elastic tissue, elastoid products and muscle content as they related to cyclic and age changes were described.

Involvement of the VL and ELM in fiber direction adjustments and incorporation into the mesometrium as found in this investigation were mentioned by Trautmann and Flebiger (1957) as being present in all animals. Nothing was found in the literature regarding the knot of tissue as it appeared in cross-section (longitudinal ridge in the intact uterus) opposite the mesometrial attachment. It was present in varying sizes in all specimens studied. This was probably a remnant of the embryonic mesentery that originally joined the Mullerian ducts, analogous to the ventral
oviducal ligament found in the avian species. Especially the ELM and often the VL were incorporated in the composition of the above grossly visible remnant structure. Often there was an aggregation of vessels in the VL at this point.

**Chronological appearance**

**Birth to 2 months of age** The ICM of the 2 day old canine consisted of circularly directed embryonic smooth muscle cells with light-staining oval nuclei. Coiled among these cells were fine collagen fibers. The VL that surrounded this was composed of a narrow band of collagen fibers, stromal cells, a few blood vessels and a few nerve bundles. By 2 weeks of age all 3 layers were delineated but the ELM was sparse. The overall appearance was one of embryonic type tissue. This same pattern of early differentiation and development of the muscularis was described by Wolfe, et al. (1942) in the rat uteri.

**Two and one half months of age to 11 months of age** In this age span the ICM developed from a well defined but dense structure that comprised about 33 percent of the muscularis, less than 300 microns in depth, to a mature muscle layer that comprised 30 to 50 percent of a muscularis that was 700 to 1000 microns in depth. An increased infiltration of the ICM with collagen was also noted. In one case, of a 2 week pp uterus from an 11 month old Beagle the ICM comprised over 50 percent of the 2250 micron uterine wall. It appeared in general that both cyclic and growth changes were manifest to a greater degree in the ICM than in the other layers of the muscularis. Pyometra had delayed the normal process of involution. Regression of the muscle walls was less advanced than was found in other specimens that were 2 weeks pp. Delayed involution was possibly due to the
fact that injury, in this case the pyometra, caused a decreased rate of resorption of collagen as indicated by Morrione and Zai Ru (1964).

During the 2.5 to 11 month age span the VL more than doubled in thickness and from 5.5 months of age on definite obliquely oriented muscle bundles were observed in this layer. In some of the 9.25 month old specimens these bundles had formed a narrow ring of muscle on each side of the vessels. An increase in elastic tissue was found in both the VL and the ELM during this period of time.

The development of the ELM as to thickness of layer, paralleled that of the VL. Longitudinal bundles were well separated by the abundant collagen of the layer. Although the uterus of the 6 month old Beagle was capable of maintaining pregnancy, the uterus and especially the myometrium continued to develop in size and content up to nearly 11 months of age.

The increase in thickness of the VL with age and with ovarian weight in this Prematuration Age Group was due to the increased need for blood by the rapidly developing uterus. It was felt that to some extent the increase in circulating hormones as well added to the VL development. The linear relationship found between the MT and WT with age was indicative of the prominent part played by the VL in the uterine development, because the regression coefficient for the ICM and ELM was not significant, whereas it was for the VL and MT. It was felt that for similar reasons the linear relationship existed between the ICM, VL and ELM with ovarian weight but with the hormonal effect being of primary importance in this case.

The increase of the WT with an increase in body weight was thought to have been due simply to the necessity of a larger uterus in a larger dog. Maximum uterine growth had taken place by 11 months of age. Cyclic effects
had begun to play a major roll on the thickness of the various portions of the uterine wall in the older specimens of the Prematuration Age Group.

One year of age to 4.1 years of age An increase of collagen infiltration was observed, especially in the ICM in specimens of this age group. Wolfe, et al. (1942) indicated that this collagen may have resulted from a conversion of the reticulin, around the muscle cells and the blood vessels, into collagen. Some 2.9 year old Beagles showed 80 to 90 percent infiltration of the ICM. The circular orientation of the ICM of one of these had been disrupted because of the overwhelming infiltration. It was found by ovarian evaluation that these two animals had been in early metestrous when tissues were collected. This may have had some bearing on the increased collagen content of the muscularis. Although collagen infiltration appeared to be a more prominent feature of the uterus in estrous, metestrous and pregnancy, it did not usually exceed 50 percent infiltration. The ELM from the most severely affected of the above specimens was not infiltrated at all, and the VL was absent. The VL of other specimens from this age span, in similar cyclic states but with lesser collagen infiltration were well developed.

In some of the 3 year old Beagles that had been in early metestrous when tissues were taken, minor to 50 percent collagen infiltration was observed. The muscle cells were hypertrophied as Mulligan (1942) indicated happened in pregnancy in the dog. According to Eckstein and Zuckerman (1956) for the first 2 weeks following ovulation, it was not possible to differentiate the metestral from the pregnant uterus. Strauss (1965) indicated that hyperplasia as well as hypertrophy occurred in the myometrium of the pregnant uterus. In the metestral uteri of this in-
vestigation the muscle cells were definitely hypertrophied and the muscle layers similar to aged anestral uteri. In one anestral uterus from a 4.1 year old specimen, the cells of the ICM were shrunken, disoriented and contained vacuolated cytoplasm indicative of physiological cyclic muscle atrophy.

**Six years of age to 10.4 years of age** In the myometrium from a uterus of a 6.4 year old Beagle, that had whelped 3.5 months before tissues had been taken, involution was incomplete. The muscle cells were dense but well defined and very little collagen infiltration was observed. After only one week of involution on the other hand, as observed in a 6.6 year old specimen the muscle cells were still hypertrophied from pregnancy, were vacuolated, in disarray and those of the ICM heavily infiltrated with collagen. Palmer (1965) indicated that in the pp sow uterus, reduction in the myometrium resulting from decrease in size of muscle fibers and interspersed connective tissue continued up to 21 days after parturition. It then remained static during a 62 day lactation. No canine specimens to represent the 2 month lactation period were available for the present study.

Collagen infiltration was observed in 9 of the 18 specimens studied for this age group. It was minor in 3, from 40 to 60 percent infiltration in 5 and 95 percent in one. In the uterine mucosa of a 9 year old African Basenji, that had a cystic endometrial hyperplasia, the infiltration was nearly 60 percent and disorientation of the ICM fibers was prominent. An estimated 95 percent infiltration of the ICM and 80 percent of the muscles of the VL and ELM was observed in a 9 year old Golden Retriever. According to ovarian examination both of these dogs were metestrus or estrous
when tissues were collected. In the first case the ovary showed cystic activity, amyloid and mixed thecosis so abnormalities were seen also in the ovary as well as the uterus. The second case was considered normal cycling. In the rat myometrium, ICM, Wolfe, et al. (1942) first observed narrow collagen fibers in 90 day rats. They were of increased thickness and abundance in 180 and 300 day rats. An extreme condition, similar to that described above was found in the ICM of a 793 day old rat.

Focal endometrioses (adenomyosis) was the diagnosis made of a cone shaped glandular structure, 40 microns in height and 20 microns at the base, found in the ICM of a 10.2 year Norwegian Elkhound. It contained 3 oval glands with lumens 20 by 35 microns and lined by 60 micron epithelium. Bloom (1954) indicated that endometrial hyperplasia often accompanied endometrious as was the case in this specimen, and that the ectopic endometrium was usually hyperplastic.

The VL was nearly twice as thick in the muscular layers from the uteri of this age group as it was in the younger specimens studied. Increased collagen as well as larger and more numerous vessels, especially thick walled arteries and veins caused this increase. Yamauchi (1964) attributed the variation of thickness of the myometrium of aged bovine specimens to the large number of thick walled vessels, but did not indicated if an overall myometrial thickness was observed.

Over 11 years of age A decrease in mean thickness of the myometrium was noted in the 11 to 13 year age group (1060 microns) as compared with the 6 to 10 year age group (1210 microns). The regression coefficient for this decrease was not significant. Coinciding with an overall decrease in myometrial thickness was an increase in collagen infiltration which was
found in varying degrees in 9 out of the 10 specimens studied in this age span. Wolfe, et al. (1942) felt with regard to the uteri of aged rats (450 to 823 days of age) that atrophy of the smooth muscle cells was proportional to the degree of disappearance of reticulin and the deposition of collagen. This reached such an extent in the rat uteri that the only way the ICM and the endometrium were distinguishable was by the fact that the collagen fibers of the ICM area were narrower and more circularly disposed. Rolle and Charipper (1949) found a replacement of smooth muscle of the ICM by collagen in the uteri of old hamsters. The ELM was much less affected than the ICM in the aged rat specimens. This fact was well born out in the present study. Schaub (1964, 1965) found, also in the rat uterus, that in aging, the collagen content increased and tripled in concentration. He further stated that the percentage of thermolabile collagen decreased from 10 to 1½ percent in young rats, to 5 percent in 30 to 40 month old rats, but that in the pregnant uterus the newly formed collagen was young collagen. It was possible that the collagen formed during cycling was also young collagen. According to Schaub regarding involution of the uterus after pregnancy, only the young collagen was degraded and removed while the aged cross-linked collagen resisted breakdown. In the human, according to Woessner (1963) the wet weight collagen and elastin increased to a maximum by age 30 (comparable to a 4 year old dog), remained constant until about 50 years of age (comparable to a 9 year old dog) and then declined to about half the maximum in the period of 50 to 65 years of age. From histological preparations there appeared to be an increase in collagen and elastin in the
dog uteri up to oldest specimens studied, 13.1 years of age which would be comparable to a 68 year old human. The reason for this was probably that cycling persisted, although not regularly, even in the older dogs, whereas cycling had ceased in the human. The disorientation of muscle fibers observed in canine specimens with over 80 percent collagen infiltration was not found described in the literature of the dog or other species. This may have been an early stage of a leiomyoma. A small leiomyoma 300 by 350 microns was found in the ELM in the uterus of a 11.5 year old Wirehaired Terrier. It was located in the ear of tissue located opposite the mesenteric border and consisted of whorled, hypertrophied, smooth muscle cells and very little collagen. A discontinuous band of collagen surrounded it. Of interest was the fact that all of the rest of the muscle tissue of the ICM and ELM was atrophied. Many of the cells had pyknotic nuclei. A larger leiomyoma 2200 by 2400 microns was found embedded in the ICM of a 11.9 year old Beagle. It displaced the entire ICM at one point, bulged into the La Fr of the uterus, caused a decreased uterine lumen, and protruded into the VL and ELM on the other end. The whole structure was composed of whorled and criss-crossed mass of collagen fibers and smooth muscle cells. It was surrounded by a band of collagen 50 to 150 microns thick. Two or 3 large mononuclear cells were observed per oil immersion field in this tumor. No secondary degenerative changes were observed in these specimens such as hyalinization, hemorrhage, edema, cystic degeneration, infection, suppuration or necrosis as Bloom (1954) indicated were frequently found associated with canine uterine leiomyomas.

The lack of uniformity in size of the VL especially in older specimens was also described by Yamauchi (1964) for the uteri from aged cows.
In the dog, large thick walled arteries and veins were found in abundance about half to three fourths of the way around the uterus and the VL was essentially non-existent the rest of the way. The increase in collagen, elastic tissue and especially elastoid was especially abundant near the vessels from these older specimens. Although the collagen infiltration was not as prominent in the ELM as in other areas of the myometrium, a thick band of collagen was found between the ELM and the serosa of older specimens. Wolfe, et al. (1942) also observed this in the uteri from old rats.

Although the regression coefficients between muscularis alterations and age were not significant, the decrease observed indicated a possible trend. It was felt that with more data, significance could have been established. The linear relationship between the muscle layers and epithelial height indicated a synchronous relationship in the uterus although this was not directly related to aging. It was found in the latter case as epithelial height increased, ICM and ELM increased at a greater rate than the VL. Inasmuch as the ICM and ELM were predominantly muscle fiber structures whereas the VL was mainly connective tissue, it was concluded that muscle cells and epithelial cells reacted more rapidly and in a more parallel manner than did the connective tissue structures. Further investigations should be carried out to see if there is any impairment on the responses of the ICM and ELM as they are infiltrated with collagen. Other significant correlation coefficients were found, but they related more to synchronous physiological function than to age changes.
Vasculature

Vasculature of the canine uterus varied with cyclic state. In early metestrous or pregnancy the vessels were often engorged and increased in size and numbers. The arteries of the endometrium were especially prominent in these 2 phases. During these phases new vessels had penetrated the ICM from the VL, traversed more or less directly through it and reached the endometrium. According to Trautmann and Fiebiger (1957) the VL of the canine uterus was embedded in the ICM, while in this investigation and according to Harrop (1960) the VL was a definite and complete layer, distinct from the ICM in all phases of the cycle. No instances were observed wherein a definite vascular layer was found in the ICM.

The canine uterine vascular system did not appear to be as well developed as that described by Leeson and Leeson (1966) and Arey (1968) for man, in which a definite double system of arteries, a permanent basal set and the changeable spiral artery complex supplied the endometrium. Cyclic changes in the canine uterus were not as severe as those that took place in the human. The vascular need was not as great. The vessels did form periglandular and subepithelial capillary networks in the canine as described by Trautman and Fiebiger. An increase in elastic tissue elements in and around the uterine vessels after pregnancy was observed in this study as was observed in the dog by Trautman and Fiebiger, and in man by Bloom and Fawcett (1962). This increase in elastic tissue did not include the IEM of arteries, as these structures were less visible in the arteries of early pp uteri than at any other time of the cycle. Albert (1967) indicated that in the guinea pig, the IEM began to fragment early in pregnancy and that by term it was virtually absent but formed again within 22 days after parturi-
tion. Only one pregnant specimen was available for this study. It was observed in the canine that the IEM of uterine arteries at mid-pregnancy and from a 2 week pp uterus from an 11 month old Beagle contained only incomplete IEM composed of narrow fibers. The arteries of a one week pp uterus from a 6.6 year old Beagle contained much elastoid and collagen infiltration but no definite newly developed IEM. No layering effect of rings of elastic fibers was observed in the canine to indicate numbers of or multiple pregnancies as Albert and Bhussry (1967) observed in the guinea pig. In the uterine vessels of multiple pregnancy bitches over 7 years of age there were often some incomplete elastic rings in the tunica media, under a thickened intima. The elastic tissue of the arteries and veins of the canine endometrium and myometrium was not increased in early pp but was increased later in the regression period. This was in regard to the statement of Duke (1945) and Bloom (1954) wherein they stated that elastic tissue of the blood vessels was markedly increased during proestrus and in the pp period of the dog. If they referred to late pp, the results of this investigation were in agreement.

**Chronological appearance**

**Birth to 2 months of age** The vasculature of the 12 hour old and the 2 day old canine uterus increased only slightly by one week of age. By 2 weeks the only prominent changes observed were in the VL, 2 layers of muscle cells surrounded some of the arterioles, a definite IEM was observed, and veins with walls of smooth muscle cells, collagen and much elastic tissue were seen. Inasmuch as the uterine veins lacked valves (Trautmann and Fiebiger, 1957) it was thought that the high elastic content of these veins aided in blood flow in the absence of valves.
Two and one half months of age to 11 months of age

Still no change was observed in the endometrial or ICM vasculature in the 2, 5 or 3 month old specimens as these areas had not begun to undergo any increased development. Arterioles with 3 muscle layers were observed in the VL and the larger arterioles were grouped at the mesometrial pole and in the opposite uterine wall from the mesometrium. The IEM of these arterioles was complete and in the walls of the larger veins 2 to 4 fairly constant rings of elastic fibers were found. By 5.5 months of age vascularization was needed for normal growth of the uterus as well as in preparation for the added needs of the cycling. Arterioles with one muscle layer had entered the ICM while larger arterioles with up to 4 muscle layers were found in the VL. In some of the 6 and 7 month old specimens studied, prominent subepithelial plexuses had formed in the endometrium and the VL contained more and larger vessels. One 6.5 month old specimen contained arterioles that extended from the ICM up through the endometrium to the subepithelial plexus, definitely not spiralled, at least at this stage of the development. One arteriole in the uterus from a 7.1 month old Springer Spaniel showed early localized intimal thickening. Intimal change in the form of splitting of the IEM was first observed in the ovaries of this study in a 6 month old specimen. A definite intimal splitting was first observed in the uterine arteries from 10 and 11 month old specimens. In this age group all of the layers of the uterine wall were well vascularized and arteries of the VL had up to 9 muscle layers. All phases of the cycle were found in the 9 to 11 month age group. In at least one or 2 arteries in most of these older specimens, splitting of the IEM and up to 10 percent intimal thickening was observed, as well as collagen infiltration of
the tunica media. Thus within 4 months after the first signs of cyclic activity some collagen infiltration had occurred while the first intimal changes were observed simultaneously with the beginning of cycling.

**One year of age to 4.1 years of age**  In 11 out of 14 specimens studied in the one to 1.7 year age range, up to 15 percent intimal thickening and up to 60 percent collagen infiltration of the tunica media was observed. In 2 specimens breakdown of the IEM was observed. One was in proestrus and one in metestrous when tissues were taken so these changes were attributed to cycling rather than aging. Their vasculature was more highly developed than any observed up through that age.

Intimal plaque-like structures were observed in a one year old high protein Beagle and a 2.5 year old regular protein Beagle. These may have been just isolated findings and no significance was placed on the fact that the younger of the 2 had been on a high protein diet. More high protein specimens would have to be studied.

Ten out of 12 animals that were from 2.3 to 4.1 years of age when tissues were taken had arterial wall changes that included some degree of IEM hypertrophy or disappearance, up to 20 percent intimal thickening and up to 80 percent collagen infiltration. Those with the most severe changes had been in metestrous when tissues were collected. Most of those in anestrous had 10 to 15 percent collagen infiltration. Vascular changes attributed to aging were observed most clearly in the anestrous uteri as they were not masked by the more severe cyclic or pp changes. In the 25 year old human, comparable to a dog around 2 years of age, Sauramo (1954) indicated only that the arteries of the body of the uterus were of the muscular type. By 28 years of age (comparable to a 3 year old dog)
arterioscleroses was distinct, elastoid was present and elastoid-hyaline degeneration was observed in some. The changes found regarding canine uterine vasculature were very similar to those described by Sauramo (1954) for the human in comparable aged specimens. Sauramo, however, did not describe collagen infiltration of the tunica media as one of the changes in the human.

Six years of age to 10.4 years of age Eleven specimens out of the 18 studied for this age span were in anestrous when tissues were taken. Intimal thickening from 10 to 90 percent and collagen infiltration 10 to 40 percent were observed in these specimens and the majority of the arteries were involved. As in younger groups the most severe changes were found in those vessels from metestral uteri. In a 6.6 year old specimen, from which tissues were collected one week after whelping, very little elastic tissue was noted at all, even though collagen infiltration was observed. At 3 months pp there was much elastic tissue. This agreed with Albert's (1967) finding that in the guinea pig, the IEM of uterine arteries virtually disappeared during pregnancy and was not reformed until about 20 days pp. It sometimes took longer in older specimens (Albert and Bhussry, 1967). Elastic tissue and elastoid were prominent features of most of the uterine vessels of this age span, however, especially of the veins. Intimal plaque-like structures were observed in 2 specimens, one in a 9 and one in a 10 year old.

In 51 year old human specimens, comparable to a 9 year old dog, Sauramo (1952) found hyaline degeneration of the infiltrated collagen and elastoid, chiefly around the large arteries. The hyaline degeneration was not observed in the canine uterine arteries but the elastoid was. Although
the canine uterus was not subject to the menopause, the arterial changes were similar to those found in the human.

Over 11 years of age Vascular changes in this age group (11 to 13.1 years of age), were found in all 10 specimens. Intimal thickening involved 10 to 50 percent of the arterial wall, collagen infiltration (observed in all except one specimen) infiltrated from 20 to 80 percent of the arterial wall of the rest. An increased elastoid content not only of the vessel's walls but of surrounding tissues was a prominent feature of the vasculature. Although there was evidence of cycling in some of these specimens, cyclic changes were not the prominent features as had been the case in younger age groups.

Sauramo (1952) found in the 59 year old human uterus (comparable to the 11 year old dog) that elastoid-hyaline degeneration was far advanced and that there was distinct arteriosclerosis. This same picture was described up through 74 years of age or comparable to a 14.5 year old dog. Tóth and Gimes (1954) also found that excessive hypertrophy and hyperplasia of the elastic fibers were prominent features of human myometrial vessels and that the lumen was constricted by intimal proliferation. Telangiectatic endometrial vessels as described by Noer (1961) for the aged human were not found in the canine specimens of this study.

Yamauchi (1964) indicated that the vascular walls from a 28 year old bovine with an atrophic uterus were but scarcely thickened, but that many thick-walled blood vessels were found even in the La Pr of the uteri from 2 somewhat younger specimens, 21 and 17 years of age respectively. Although ages of the bovine were not given, Mochow and Olds (1966) observed that elastosis of uterine vessels were found in only 3.9 percent of the
animals that had not calved and 56.5 percent of the animals that had calved. Whelping histories were not available for all of the specimens studied in the present investigation, but elastoid was found in the vessels of all of the older animals. Mochow and Olds (1966) finding was in agreement with the finding of Albert and Bhussry (1967) that in guinea pigs, aged animals showed more elastin in their vessels than younger ones of comparable parity.

Cervix

Epithelium

As was expected, the canine cervical epithelium varied from a low pseudostratified type to a stratified squamous type dependent upon area of the cervix, stage of growth and stage of estrous cycle. The nomenclature of the squamous epithelium was as given by Papanicolaou (1954), and was described in the observations of this study for the 5 cell zones. According to Restall (1966) the epithelium of the ovine cervix was similar in height throughout the cervix, so that in the ovine it did not matter where the sections were taken for study of the epithelium.

The lumen of the canine cervix was formed by an oblique canal as described by Christensen (1964) instead of a parallel canal as was found in other animals. Due to the oblique canal the cross-sectional appearance had its own peculiarities. Most of the sections for this study were taken from the caudal one third of the cervix where the cervical lumen was eccentrically located and generally quarter moon shaped (Figure 193). The portion out from the greater curvature of the crescent was called the cervical body and that inside the inner curvature of the lumen, the cervical pillar. It was often observed that the epithelium that lined the 2 curvatures varied
considerably in depth and stainability. That of the pillar region was usually better developed. Although more invaginations and simple gland invaginations were observed on the canine cervical pillar, no definite folds as such were observed. According to Belonoschkin (1946) the spindle shaped human cervical lumen was characteristically recognized by its "arbor vitae" folding of the well developed cervical glands. Zupp (1924) indicated that the bovine cervical mucosa was generally thrown into numerous primary and secondary folds with tertiary or quadruplicate foldings not infrequently observed and that it was lined by simple columnar epithelium, any cell of which could have become a goblet cell. The peculiarly shaped folds gave the canal a winding course and no glands were described (Belonoschkin, 1946). The cross-section of the ovine cervix which Belonoschkin said was similar to that of the bovine had 4 to 6 claw-like projections of varying sizes between which were leaf-like longitudinal folds which gave the canal a cork-screw appearance. They were lined with a single layered columnar epithelium. Restall (1966) agreed and stated that numerous goblet cells were found in the high columnar epithelium. The latter was reduced in height during diestrous. Seven to 12 horizontally arranged projections which closed together like teeth separated the porcine cervix from the vagina, and according to Belonoschkin they were lined by a single layered columnar epithelium near the uterus and a multiple layered columnar epithelium near the vagina. He noted that the entire porcine cervix was free from glands.

Thus it was found that although the cervical epithelium was similar in many aspects, the lumen shapes were highly variable in the different species. The cervical glands as described by Trautmann and Fiebiger (1957)
for carnivores were not found in the canine cervixes of this investigation although there were hollow and solid epithelial cones that extended into the underlying tissue (Figures 209, 210, 211), as Trautmann and Fiebiger (1957) found in sheep and swine. In the dog, these were found chiefly on the cervical pillar and were described as simple tubular glands or well developed epithelial papillae depending on whether they were hollow or solid. Numerous large branching glands and ciliated columnar cells as described by Leeson and Leeson (1966) for the human cervix were not observed in the canine.

**Chronological appearance**

**Birth to 2 months of age** The 4 to 7 layered, moderately dark staining epithelium of the 2 day old canine cervix became lighter staining and developed numerous shallow but abrupt invaginations into the La Pr in the one week old specimen. Mitotic figures were found in some of the basal cells of the latter specimen. The loss of cytoplasm and appearance of numerous pyknotic nuclei in the 2 week old cervical epithelium was attributed to the lack of hormonal stimulus that had affected the cervix in utero but was not able to after parturition. Forsberg (1965) indicated that in the mouse, the cervical epithelium was pseudostratified columnar at birth but underwent active proliferation 2 or 3 days after birth and developed 2 zones. A bz with small cells and a sz with tall columnar cells comprised these zones. The multicellular arrangement found in the 2 day canine specimen was indicative of an early proliferation as found in the mouse. A layered appearance was observed by one week of age in the canine. The sz was composed of cuboidal rather than columnar cells which had basal nuclei and light staining apical cytoplasm. No other changes were noted up
to 2 months of age in the canine cervix.

**Two and one half months of age to 11 months of age** Large mononuclear leucocyte-like cells were observed in the 2 to 3 cell deep epithelium of the 3 month old specimens. The nuclear chromatin of these cells varied from a uniform mass to large clumps. These cells were found in varying numbers in nearly all specimens from 3 months on. Numerous shallow invaginated gland-like areas lined with tall columnar cells were found in this age group. The first proliferative changes observed since those of the 2 day old specimens were in one that was 5-5 months of age. The 6 to 8 cell deep epithelium had a bz of columnar cells, a pbz of 2 to 3 layers of plump polygonal cells and an imz of flattening cells. Numerous mononuclear cells were found in the epithelium. This proliferated epithelium was the result of hormonal action that corresponded with the formation of large follicles in ovaries from specimens of similarly age. Although some of the specimens of the 6 and 7 month old age group were less developed than indicated above, the majority were more developed and had increasing numbers of invaginated areas in addition to the thickened epithelium.

Numerous neutrophils as well as mononuclear cells were observed in the 4 cell layer thick epithelium from an 8 month old specimen. The sz cells had basal nuclei and dark staining or in some cases extruded, apical cytoplasm.

The next significant change observed was in a 10 month old specimen known to have been in estrous when tissues were taken (Figure 23). The epithelium was over 20 cells in thickness. Numerous La Pr papillae were seen upon which lay the crowded cells of the bz, then the 4 to 6 cell pbz of large, round cells upon which lay a second 4 to 6 cell layer of flattening
cells of the imz and finally a narrow condensation iez. Next to the lumen was the 10 to 12 layers of flattened, laminated-appearing cells with elongate pyknotic nuclei of the sz. In the human, the peak of cornification occurred slightly prior to the presumed ovulation according to O'Brien, et al. (1964). This was also thought to have been the case in the dog.

No leucocytes were found in the epithelium of the specimen described above as was found by Zupp (1924) for the bovine cervix at estrous.

The bz and pbz of 2 other 10 month old specimens, which were in metestrous when tissues were taken were similar to the above specimen, except they lacked the imz and sz (Figures 209, 210, 211, 215). Many of the lining cells of the latter specimens were vacuolated and among them were numerous neutrophils and mononuclear cells.

The epithelial lining of a cervix taken at mid-pregnancy (Figures 219, 220) from an 11 month old Beagle, was 2 to 4 cells in depth. The cells were dark staining on the cervical body portion, while on the cervical pillar the epithelium was more developed, with numerous invaginated branched tubular glands. There appeared to be some secretion from the well delineated apical cytoplasm of these cells, Sauramo (1954) found in the human that the cervical glands of pregnancy were present in large numbers.

Cervical epithelium during anestrous, as observed in other 11 month old specimens was ragged in appearance, atrophic and usually only 2 cells in depth with large areas of autolysis in evidence.

Thus in the 10 and 11 month age group specimens representing estrous, metestrous, pregnancy and anestrous were observed and described.

One year of age to 4.1 years of age A 3 week pp specimen taken from a one year old Beagle had partly sloughed, tall columnar, "pop-
corn-like" epithelium with apical nuclei and light staining lacy cytoplasm (Figure 223). These popcorn-like cells were similar to those described earlier for the pp canine uterus. Dark staining, shrunken tall columnar cells with basal nuclei replaced the sloughed cells. The cervical epithelium of a 4 week pp specimen was identical to that described above except no popcorn cells were observed in the latter cervix. Sauramo (1954) indicated that the cervix from a 15 year old human (comparable to a 1.2 year old dog) was well delineated, had high squamous epithelium and branched cervical glands essentially devoid of secretion.

The 13 specimens studied for the 1.7 to 4.1 year age span showed only cyclic changes as already described. No age changes of the epithelium were observed in these cervices. Mucus secretion, observed in active epithelia from 5.5 months of age in the dog, was observed at 22 years of age in the human (Sauramo, 1954), comparable to a 2 year old dog.

Six years of age to 10.4 years of age The epithelial lining of a one week pp uterus from a 6.6 year old Beagle was much more convoluted than that of the 3 week pp specimen described earlier. It had numerous neutrophils in it, as well as in and around the sloughed popcorn-like cells. Eleven of the 18 cervices studied in this age group were in an anestrous phase of the cycle and had variations only in stainability. The epithelia were all 2 to 3 cells in height. Generally the cells were cuboidal type with scanty cytoplasm, dark staining and often pyknotic nuclei. Three of the remaining 5, which according to their ovaries had been in anestrous, were lined by an early proliferative type of epithelium in which the bz, pbz and in some cases the imz were delineated. The respective uteri of these cervices were also in proliferative states, one of them in a nearly
cystic condition. It was felt in the case of the non-cystic uteri that the animals were in late anestrous or very early proestrous. The cervix from the cystic uterus was possibly in a similar state but more likely was in a chronic proliferative state due to the condition of the uterus. The epithelium from a 9 year old Golden Retriever was thick and proliferative. It was similar to that described above except the whole cervical pillar was filled with uterine-like glands as in cystic endometrial hyperplasia (Figure 231). The cysts were lined by cuboidal epithelium and the smaller gland structures were lined with tall columnar secretory cells. The uterus from this animal was in a state of endometrial hyperplasia but no cysts were observed. It was evident that the cervical condition of the canine was closely dependent upon the condition of the uterus. A section from the mid part of the cervix showed only a healthy proliferative stratified squamous epithelium and no cysts or glands as were found in the anterior pillar region. Thus a caudal cervical biopsy would not have been sufficient to diagnose the uterine problem.

The remaining cervix, also from a 9 year old Golden Retriever had a healthy stratified squamous epithelium with all 5 layers (Figure 235). The sz consisted of 6 to 8 layers of flattened cells. Although the ovarian studies indicated this animal to be in late anestrous as far as follicular development was concerned, the uterus and cervical examinations showed it to be in a state of late estrous. Whether the condition of hyperplasia of the germinal epithelium and dense combined thecosis had anything to do with the state of the rest of the tract was not known but it was presumed that it did.
Over 11 years of age Only 2 of the 11 specimens in the 11 to 16 year age group were in metestrous. One of these was in early metestrous when tissues were collected and had a typical proliferated epithelium, many epithelial papillae, light staining cells and many infiltrated neutrophils. The other, in late metestrous had a low degenerative type epithelium with a vacuolated ragged appearance. Of the 8 cervices in anestrous, 7 were in quiescent or decreased metabolic states with dark staining low cuboidal cells and the other one was proliferative in appearance and in late estrous, probably very near proestrous.

The epithelium of the oldest cervix studied, from a 16 year old Cocker Spaniel, was dark staining, 4 to 8 cells in depth with some of the outer cells flattened, and contained neutrophils and large mononuclear cells. From the appearance of the epithelium it was felt that it was in an abnormally thickened state. In the human, over 60 years of age (comparable to an 11 year old dog) the cervical epithelium was found by Sauramo (1952) to be atrophic, except at the cervico-vaginal junction where it was still distinct. Throughout the cervical epithelium and in the deeper tissues of these older specimens Sauramo found numerous retention cysts. In the canine the cervical epithelium during anestrous, which was in its lowest phase had no apparent differences from the old or young anestrous animal. Retention cysts were not found in the cervical epithelium of the specimens from this age group.

Lamina propria

The canine La Pr was divided into 4 general portions for descriptive purposes (Figure 200). That portion from the greature curvature of the half moon shaped cervical lumen was referred to as the cervical body.
portion. That which was found in from the lesser curvature of the cervical lumen was called the cervical pillar portion. Each of these divisions was again divided into the subepithelial La Pr and the deeper La Pr. The high primary and lesser secondary cervical folds described by Trautman and Fiebiger (1957) for domestic animals in general were not observed in the canine. During pregnancy and estrous a few simple or branched tubular gland-like structures that resulted from infolding of the cervical pillar epithelium were found in the dog, but no normally existing permanent folds. A few elastic fibers were observed in the mainly collagenous La Pr of the canine cervix. Trautman and Fiebiger indicated that was also the case in other domestic animals.

An aggregation of collagen fibers was often found at the boundary between the epithelium and the La Pr. A PAS positive basement membrane was observed as described by Wolfe, et al. (1962) in the rat and Dougherty (1961), Younes, et al. (1965) and Warren, et al. (1966) in the human.

**Chronological appearance**

**Birth to 2 months of age** The uniformly wide La Pr of the 2-day old canine specimen contained large round to oval stromal nuclei, capillaries, blood elements and in the deeper regions some collagen. The less dense subepithelial areas had only a few collagen fibers. The cervix in general at this age was a nearly homogenous structure of large embryonic type nuclei and primitive connective tissue fibers. The cellular density in the 2-week old specimen was probably the result of loss of maternal hormone action that had stimulated reproductive tract tissues of the in utero female. As these effects were nullified within a few days after birth, it
was felt that the only changes observed in the younger specimens was a gradual, slow increase in cellularity of the multidirectional collagen fibers. Wolfe, et al. (1942) similarly observed that along the cervical canal of the rat was a zone of thin, nearly parallel collagen fibers. A similar collagen zone was not found in canine specimens under 3 months of age.

Two and one half months of age to 11 months of age The chief feature of the 2.75 month old specimen was the more dense outer La Pr and less dense subepithelial structure (Figure 201). Two months later, after increasing 4 times in size, the cervix had become functional although perhaps not completely mature. The La Pr was relatively narrower and moderately dense. In some of the 5, 5 and 7 month old specimens an edematous appearing subepithelial zone was observed. By this age growth changes per se had been completed, as any further changes were masked by cyclic variations. Migrating leucocyte-like mononuclear cells with large, oval, densely stained uniform or granular nuclei were observed in these and nearly all older specimens (Figure 202, 207, 220). Nellor (1963) referred to similar cells as lymphocyte-like cells, or lymphoblast-like cells (Nellor, 1965) and felt that they were of genital tissue origin in gilts and heifers.

At estrous, the cervix had doubled in size to what it was in the 5 to 9 month old specimens. This, however, was not considered to be a growth or age change but was thought to be the result of hormonal action. The prominent feature of the La Pr at estrous, in addition to size increase was the dense but non-fibrillar uniform appearance of the collagen and the generally sparcely located stromal nuclei. The latter structures were con-
centrated only in a narrow subepithelial zone. The reason for this aggre-
gation was unknown while the reason for their apparent decrease in the rest
of the La Pr was due to hypertrophy of the collagen fibers and at least
with light microscopy a near amalgamation of these fibers into a homogeneous
structure. As the cervix decreased in size during metestrum, the stromal
nuclei as previously described in the estral cervix appeared more numerous
due to the loss of collagen or collagen components. Definite bands and
fibrils were again observed in the metestrual cervix as well as tissue
spaces between them. The study of other 11 month old specimens in an an-
estrous state revealed that the atrophy, which began after estrous, con-
tinued through metestrual. Atrophy was especially prominent the latter
part of metestrual and into anestrous. In the latter state dense, stringy,
collagen fibers and a few shrunken stromal nuclei were the chief components
of the La Pr. Due to the long period of time between cycles in the dog,
atrophy was more complete than in the diestral phase of regularly cycling
species.

Tissues collected at midgestation from an 11 month old Beagle revealed
a dense metestrual appearing La Pr of the cervical body and an extremely
edematous appearing cervical pillar (Figures 217, 219). The pillar con-
tained only a sparse network of connective tissue elements filled with
ground substance. According to Danforth and Buckingham (1964) the in-
creased size of the pregnant cervix was dependent upon more than simple
edema as only a slight increase in water content was observed. They did
demonstrate significant decrease in total collagen concentration in the
human cervix as pregnancy advanced. Further investigations should be made
in the canine reproductive tract to compare the collagen content during
pregnancy in the young animal and in the old animal. Similar investigations should be made on the cervix at estrous, during metestral regression, and pp regression in the younger and old canine specimens.

One year of age to 4.1 years of age Postpartum involution of the cervix as observed in some of the one year old specimens was similar to metestral and anestral involution described above, except that it was more severe. The La Pr had a ragged appearance in these instances with the atrophied, multidirectional collagen fibers and masses of pyknotic stromal nuclei.

It was not possible to compare the cervices from one year old anestrous specimens with that from a 4 year old specimen as the latter was 2 months pp. As growth changes had been essentially completed by one year of age, only cyclic changes were observed in this age span.

Six years of age to 10.4 years of age Ten of the 17 specimens studied for this age group were in anestrous, 5 in metestrous and the remaining two in early pp and estrous respectively. Due to the stage of involution of the metestral cervices from this age group it was difficult to differentiate them from the anestrous by means of the La Pr. Comparing the La Pr of cervices from opposite ends of the age group revealed only a wide variation in density, stainability of collagen and in the condition of the edematous-like subepithelial tissues.

The cervix from one dog, a 9 year old African Basenji was defined as metestrous from the ovarian picture, had a cervical La Pr similar to that of estrous, except that it was too narrow and contained too many stromal cells. Judging from the healthy, dense collagen through it was definitely not from a quiescent tract.
Eight of the 11 specimens studied for the 11 to 16 year age group were in anestrus when tissues were collected. All except one case the tissues were taken between May and August when dogs were expected to be cycling. Although the state of the estral cycle was not a primary morphological finding it was an indication of decrease in cycling in the older animals. Whelping records of the Beagle colony at the Anatomy Department, Iowa State University indicated that Beagles over 5 or 6 years of age were much more sporadic in their whelping patterns than dogs under that age. Although consistency of cycling, and whelping of healthy litters was not compared directly when considering older animals; it was felt that a decrease in number of litters born over a 2 or 3 year period was some indication of abnormal cycling. In some cases obesity of the older females resulted in a full season passing without any external signs of heat being observed, even though these animals were in the presence of an aggressive fertile male at all times.

No definite increase in connective tissue was observed in the La Pr of the older anestrual dogs as compared to that from younger anestrual dogs. In some specimens of this age group the whole cervix was smaller than usual and the La Pr was accordingly shrunken and atrophic in appearance with an apparent increase in concentration of stromal nuclei.

In the cervix from a 16 year old Cocker Spaniel (Figure 246) 5 lymph nodules of various sizes were observed in the subepithelial La Pr. Demetrakopoulos and Green (1950) described lymph follicles in the human cervix and indicated that they were probably the result of chronic inflammation. No definite conclusions were made regarding the canine lymph nodules as they were found in only one specimen. It was interesting that
they were found in the oldest specimen of this study.

Muscularis

It was found in this investigation that the musculature of the canine cervix was divisible into 4 layers: (1) internal oblique bundles, (2) ICM, (3) VL and (4) ELM. In most of the sections studied these various layers were observed. The internal oblique muscle was incomplete and consisted of a varied number of bundles situated in the dense connective tissue between the La Pr and the ICM. The ICM on the other hand was complete but varied greatly, in both thickness and density, in the different specimens. Portions of these 2 inner muscle layers formed or were continuous with a large multidirectional muscle mass defined as the cervical pillar muscle of the canine cervix (Figure 206). In the VL scattered bundles of smooth muscle were usually found which varied in number and size with the specimen. The ELM was consistently found and was generally a complete layer of longitudinally directed fibers. Outside from the ELM was a layer of dense fibrous tissue covered by a single layer of serosal cells. With regard to observations made regarding the uterine muscularis, in the cervix large amounts of collagen formed the bulk of the muscularis layer per se rather than just serving as the binding tissue for large muscle masses, as was found in the uterus. Trautmann and Fiebiger (1957) indicated that the cervix musculature in domestic animals was rich in elastic fibers and highly developed. Although well developed in the dog, it was not prominent by comparison with uterine musculature. The well developed middle muscle layer of the uterus according to Tourris, et al. (1963) was nearly absent in the cervix of the cow, sheep, pig, horse and human. The findings of the present investigation agreed with those of Danforth (1947) that the
human cervix was composed predominantly of fibrous connective tissue.

Muscle content was usually between 10 and 15 percent of the cervix, but in some cases it went up to 40 or 45 percent. Danforth's findings of the wide variation in amount of muscle in different cervices were definitely true for the canine cervix. As to fiber direction, Korte (1965) described only 2 layers in the human cervix as observed by longitudinal section, a wide inner, interwoven, basket pattern, diagonally directed layer and narrow, outer, tubular, longitudinal muscle layer. It was felt that if the canine cervix were sectioned in a similar manner the same pattern would have been observed with the internal oblique muscle and the VL bundles being the ends of the interlacing fibers that appeared circular in orientation in cross-section. According to Korte the inner muscle was often torn or bruised during parturition and that especially in older multiparous individuals it was unable to contract properly. Thus when the outer longitudinal muscle contracted, cervical ectropion resulted. It was not determined if this was a problem in older dogs. Lierse (1960) and Krantz and Phillips (1962) mentioned an inner longitudinal muscle of the human cervix and Wolfe, et al. (1943) described a series of parallel fibers between the epithelium and muscle layers in the rat cervix. These were perhaps comparable to the internal oblique muscle bundles of this study.

Chronological appearance

Birth to 2 months of age  No definite boundary was discernable between the La Pr and muscularis in the 2 day old canine cervix (Figure 193). This was reasonable as there was no need for functional muscle in the cervix at this early age and even when fully developed the muscularis of the cervix was rather sparse when compared with that of the uterus. The
first differentiation of the primordial muscle fibers was observed in a 2 week old specimen (Figure 199) in which there were 6 to 8 narrower circular strands of smooth muscle fibers plus a few multidirectional fibers in the cervical pillar. The action and purpose of the pillar muscle was not determined although it was felt that it must have been important to warrant such early development of this muscle. Perhaps it served in a dilatory capacity at estrous or term. Tourris (1964) felt that a lamellar muscular structure that stemmed from the external muscle layer of the uterus and vagina of animals and man passed in an oblique direction to the internal muscle layer and played an active role in dilatation of the cervix.

Muscle bundles were first observed in the ELM in the 4 week old specimen in which the ICM was well differentiated. No other layers were observed in this specimen.

Two and one half months of age to 11 months of age All of the layers of the muscularis were delineated in the 3 month old specimen (Figure 200). They were well embedded in collagen, which comprised nearly half of the muscularis layer. The internal longitudinal muscle layer of the rat cervix, thought to be comparable to the oblique folds of the dog, appeared first at 30 days of age according to Wolfe, et al. (1942).

From 3 to 11 months of age much variation in musculature was observed with the more highly developed musculature found in the larger cervices. It was felt that the hormonal action on the epithelium and La Pr of the early maturing dogs also affected the growth rate of the musculature so that when the dog first cycled at about 6 months, as in the case of the Beagle, the muscles were functional. In the slower maturing breeds, such
as the Springer Spaniel the cervical musculature was rather poorly developed even by 7 months of age. A 2 fold enlargement of the cervix, due mainly to connective tissue change, was observed in 2 cervices from 10 month old animals that were in estrous when tissues were taken. Muscle hypertrophy on the other hand caused the enlargement found in the cervix from an 11 month old animal in mid-pregnancy. Sieve (1927) as quoted by Krantz and Phillips (1962) indicated that there was a decrease in cervical musculature during pregnancy, but did not indicate at which part of gestation this became evident.

These changes observed in the canine cervix due to normal cycling and pregnancy were described not as age changes but to indicate that at least in the smaller breeds of dogs full functional development of the cervix had been completed under one year of age.

**One year of age to 4.1 years of age** The pronounced change that took place in the pp cervical muscularis was opposite that observed in the pregnant cervix. This was not to be confused with atrophy and degeneration due to aging. At 3 weeks pp (Figure 222) as observed in a one year old Beagle, there was a decrease in cytoplasm and a ragged appearance to the muscle bundles probably due to the collagen breakdown more than from muscle degeneration. In aging the muscularis retained a solid dense uniform appearance. Some collagen infiltration of up to 20 percent of the muscle bundles was observed in the pp specimen, as was also seen in cervices that were fully involuted.

Collagen infiltration and/or replacement especially of the pillar muscles started at the base of the pillar with about 20 percent infiltration and increased so that at the apex 80 to 90 percent of the muscle tissue was
replaced by collagen. This was observed in 3 specimens that were one year of age. Tissues were collected from 2 of these animals in April and from the other one in August. From studies on the ovaries of these animals it was determined that all were in late anestrous or proestrous when tissues were collected. A similar picture was observed in the cervix from a 1.7 year old animal in early estrous from which tissues were taken in February. Although simple infiltration to varying degrees was observed in the apices of many of these cervical pillar muscles, replacement with retention of the muscle bundle outline was observed only in late anestral or estral specimens.

Only minor collagen infiltration was observed in the pillar/muscles, oblique muscles, ICM and VL muscles up to 2 years of age. This increase in severity was such that in 4 out of 12 specimens studied between 2 and 4 years of age 30 to 60 percent of these muscle masses were infiltrated with collagen. The ELM was not involved.

Six years of age to 10.4 years of age During this age period an apparent increase in muscle nuclei concentration, in addition to the increase in collagen infiltration, was observed (Figure 240). The former was probably the result of decrease in cytoplasm of the muscle cells without a decrease in muscle cells. All 17 of the cervices studied in this age range had some degree of infiltration. It was a major feature involving 30 to 90 percent of the muscle in 8 of them.

Increase in collagen per se and increased infiltration of the musculature were doubly prominent features in the much enlarged cervices from 2 Golden Retrievers that were 9 years old when tissues were taken.
Over 11 years of age It was easy to observe that all muscle layers of the 11 specimens from this age group (11 to 16 years of age) were nearly or over 50 percent infiltrated, or in some cases replaced by collagen (Figures 241, 242). The muscle bundles, due to the loss of cellular substance appeared as masses of nuclei, often whorled in arrangement. Even the ELM suffered loss of cytoplasm although it was not infiltrated by collagen. Wolfe, et al. (1942) referred to aging of the cervix musculature in rats as a dilution of muscle fibers that became more and more pronounced and spread throughout the musculature. In hypertrophied cervices of the rat the muscle cells were often broken up into small aggregates by wide collagen fibers. Hypertrophy of the cervix as found near estrous was observed in only one case in the dog (Figure 248). According to Wolfe, et al. nearly complete disappearance of the reticulin around the muscle cells as it was transformed into collagen was associated with partial atrophy of the smooth muscle cells. They felt that persistence of the reticulin was necessary for the survival of muscle cells. Sauromo (1952) indicated with regard to human cervices studied from 54 to 74 years of age that muscle tissue was present, or in some cases scarce but did not list any changes in the musculature itself.

Vasculature

Danforth and Buckingham (1964) stated with reference to the human cervix, that as knowledge increased in this area it was increasingly difficult to elucidate any structural alteration as to growth, aging, physiological cyclic phenomena, disease, or repair without discerning the manner and degree of influence by the connective tissue system. The cervix contained a higher percentage of connective tissue than any part of the female
genital tract thus far described. Thus the vessels of the cervix instead of being loosely embedded in a sparse connective tissue network and then ramifying in muscle and glandular tissue spend much of their courses in varying densities of tissue with high connective tissue content. They were surrounded by thick collars of collagen, reticulin and in some cases elastin.

Chronological appearance

Birth to 2 months of age Only minimal vascularization was observed in the youngest specimens studied. It was confined to the outer La Pr and future VL. Inasmuch as the cervix was still very immature at this time and its functionality was not necessary for the maintenance of the life of the animal, as was that of organs such as the bladder and ureters, it did not need an abundant blood supply. The first increase in vascularization was noted in a 5 week old specimen in which a minimal but definite VL was observed. All of the arterioles had more than one muscle layer and a prominent IEM. The stainability of this latter structure was very definite with Verhoeff's elastic tissue stain in younger specimens whereas in older specimens it was not so constant and definite. Between one and 2 months of age, the maternal hormonal effect was gone and the level of intrinsic hormones had not increased to a point where morphological changes were observable. Minimal vascularization with a slow but definite increase as warranted by growth changes were the pertinent observations of this age group.

Two and one half months of age to 11 months of age In the youngest specimens of this age group the capillaries were no longer limited to the outer La Pr, but extended to the epithelium. The vessels of the VL
were now more numerous and larger which indicated an increase in growth rate. This began at about 2.5 months of age. Even in the 5.5 month old specimens there was very little vascularization in the muscularis, while large arteries (with 8 to 10 muscle cell layers) were found in the VL. Mild collagen infiltration of these arteries was taken as a possible result of hormonal stimulation, or "normal" maturation of the arterial wall. There was, however, intimal splitting seen in some of the arteries from 6 month old specimens. This indicated an abnormality. It was recognized that the collagen infiltration of the tunica media may have been abnormal as well. From 6 to 11 months of age the vascular picture was extremely variable as the hormonal effects were made manifest. The stainability of the IEM was inconsistent in this group. In most of the smaller arteries it was not observable and in parts of the larger ones it was not seen, whereas in others it was very well defined. Although it was felt that this erratic stainability and/or absence of the IEM was due to hormonal influence, no consistent pattern was observed. It appeared to be more than a simple fragmentation and reformation as seen by Albert (1967) in the uterine arteries of the guinea pig during late pregnancy and in puerperium. In the cervical arteries of the dog, portions of the IEM that did not stain were often visible as outlines and in some instances stained positive for collagen with Van Gieson's stain. Further histochemical work needs to be done to determine the staining variability of the IEM of cervical arteries. One would expect changes in older specimens, but it was surprising to note them to this extent in animals under 11 months of age.

One year of age to 4.1 years of age The interesting feature of this group was the thick collagen-walled vessels found in the La Pr.
They varied from a healthy, near edematous appearance to a shrunken vacuolated state and were generally oblong in shape. The latter appearance was probably due to their comparatively large lumen size with respect to wall thickness. Fanger and Barker (1963) described similar structures in the human cervix and indicated that they were probably venules or lymphatic vessels. Morphologically it was difficult to tell, but there were definite veins found near these structures. So in line with the findings of Krantz and Phillips (1962) that the cervix is quite well drained by lymphatics and the fact that there were definite veins observed, it appeared that those which did not contain blood cells were lymphatics.

As in the previous age group, variable stainability of the IEM was again observed but with increased prominence. There was also an increased incidence of collagen infiltration of the tunica media, splitting of the IEM, and elastoid formation. Increasing incidence and severity of these phenomenon were observed through this age group, in addition to the appearance of wide circularly arranged collagen collars around the thick walled arteries of the VL and VA. There appeared to be an increase in small thick walled arteries in the lateral cervical pillar areas, especially at the junction of the pillar muscle and the VL. These arteries were continuous with those of the VL. A possible reason for this apparent increase was the dynamic status of the cervical pillar in cyclic morphology. All of the blood reached this enlarged structure from a comparatively narrow neck and thus as the vessels grouped in one place, they were more noticeable than when spread out as in the uterus. There may have been a real increase related to the edematous nature of the La Pr observed at certain times of the cycle, hence also the well developed venous and lymphatic drainage
Six years of age to 10.4 years of age

Stainability of the IEM of this age group varied from collagen positive staining to lack of stainability (Figures 236, 238). The VL and La Pr vessels from specimens over 9 years of age had a 10 to 30 percent complex of intimal thickening, collagen infiltration and elastoid formation. The severity of change appeared to be about 10 percent greater in the arteries of the La Pr than in those of the VL. It seemed that during this age span due to the continuous cycling, and in some instances whelping also, that the vessels suffered due to the continual lumen alterations dependent upon the changing demands of the cervix. Thus over time, the continual, and in the case of pregnancy, exaggerated demands on these vessels produced definite histomorphological alterations.

Over 11 years of age

In this age group the IEM in many cases was not observed at all, especially in the larger arteries of the VL. The collagen infiltration of many of these vessels extended into the thickened intima as well as the tunica media. Elastic fibers and elastoid along with increased density of collagen formed the tunica adventitia. In one case especially, the collagen wall of a large vein was nearly hyalinized (Figures 243, 244).

As in other portions of the genitalia, there were specimens in each age group in which the arteries were clear of collagen infiltration and the IEM was "normal" in its ability to take up elastic tissue stain. The trend though was as indicated, with an increasing percentage of specimens with vascular changes as the age increased. Thus the vessels of the canine cervix underwent similar changes to those described by Tóth and Gimes (1964).
for the human uterine vessels. They described excessive hypertrophy and hyperplasia of elastic fibers, intimal proliferation, lamellary elastohypertrophy and hyalinoses. The latter 2 features were not prominent in the dog.

Vagina

Epithelium

The nomenclature used to describe the canine vaginal epithelium was a modification of that used by Krantz and Phillips (1962) for the human cervix and Krantz (1959) for the human vagina, namely bz, pbz, imz and sz.

In the canine (Mulligan, 1942) in the sow (Done and Heard, 1968) as in the human (Krantz, 1959) the latter 2 layers, imz and sz were missing in late metestrous and anestrous (diestrous in the sow and human). Thus, the terminology used here included all but a keratohyaline granule containing layer that was found beneath the sz in the human (Papanicolaou, 1954).

Chronological appearance

Birth to 2 months of age During this age period the canine vaginal epithelium consisted of 2 to 3 layers of cells. The bz was a uniform layer that varied from cuboidal at 2 days of age to columnar at one week of age and back to cuboidal at 2 months of age. Cells that formed the pbz, were generally cuboidal, formed an uneven free surface, and many of them had extruded cytoplasm on their free border. This agreed with Mulligan's finding that the vaginal epithelium of the immature bitch consisted of 2 layers of cells. According to Wied and Keebler (1967) neither leucocytes nor vaginal flora were present in the vagina of the newborn human. Within a few days or weeks the pattern changed rapidly from a proliferative to an atrophic type. The morphological alteration as described
above for the canine was perhaps a parallel situation to the finding of Wied and Keebler (1967).

Two and one half months of age to 11 months of age  The main change observed early in this period was an increase in cell size of both the bz and the double layered pbz. The free surface was still uneven. Mitotic activity in the bz cells was a prominent feature of the 3 and 5.5 month old specimens and the pbz in the latter had increased to 4 cell layers in depth. Wied and Keebler noted that in the human during childhood, there were few if any imz cells. If present they indicated an inflammatory reaction rather than a hormonal influence. Imz cells began to appear normally as menarche approached. It was interesting to note that out of the 9 specimens studied in the 6 to 7 month age group only one showed significant evidence of mitosis of the bz cells, increased size of the bz and pbz cells and a 2 cell thick imz of flattened cells with pyknotic nuclei. This epithelium had an even free border. This group of dogs included Beagles, Dachshunds and Springer Spaniels. It was felt that the increased development of the one was due to individual variation rather than breed difference. A proliferative alteration in the atrophic type of epithelium of the young child took place at least one to 4 years prior to the menarche. Thus, the mitotic activity in the 3 month old canine was a parallel phenomenon, in preparation for cycling which began at 6 to 7 months of age. In the 8 and 9 month old canine specimens studied, an imz was a usual feature although most of the epithelial cells were atrophic in appearance. Extensive cyclic changes were observed in the 10 and 11 month old specimens. One of them was in proestrus and the other in estrous when tissues were collected. They showed much mitotic activity of the now
columnar bz cells and well developed epithelial papillae. The pbz was 4 cells deep, the imz 2 cells deep and a sz had 4 to 7 layers of cornified cells. Trautmann and Fiebiger (1957) indicated that the vaginal epithelium was cornified during estrous in the dog and rodent. According to Restall (1966) keratinization followed the desquamation in the ewe. Arey (1968) stated that the human vaginal epithelium was unkeratinized but the near ovulation keratohyaline granules accumulated in the superficial cells. The appearance of the increased mitotic activity especially in the specimen that was in proestrous agreed with the finding of Laguchev (1965) that there was a statistically significant rise at that time also in the human and that it was the first of 2 to occur in the normal cycle. The second occurred during the first day of diestrous or comparable to the first day of metestrous in the bitch. The other features observed for these phases of the cycle were as reported by Mulligan (1942) and Eckstein and Zuckerman (1956) for the bitch. The important point in this study was not the histological appearance of the cyclic phases, but that in the specimens studied cyclic activity was sporadic up to 10 months of age and then was a prominent feature of 10 and 11 month old specimens. Two of the 10 and 11 month old animals in metestrous had sloughed the outer epithelial cells. The epithelium had become according to Mulligan a basal cuboidal layer and an outer columnar layer. Numerous neutrophils and mononuclear cells were found throughout the epithelium. The lumen contents of the vagina during metestrous were leucocyte concentrations according to Schutte (1967).

One of the 11 month old specimens was in mid pregnancy when tissues were collected. The outer mucus containing cells of the vaginal epithelium were as described by Mulligan (1942) for the bitch. The appearance of the
one to 2 cell layer columnar pbz cells with basal nuclei and soapy appearing cytoplasm was not mentioned (Figure 263). This was probably related to the mucous secretion which Mulligan indicated disappeared with pp regression. In the vagina of the ewe, Restall (1966) observed that there were no secretory cells except near the cervix and then only during pregnancy. Done and Heard (1968) indicated that in the pregnant sow the regular arrangement of the 2 to 3 layered epithelium was diagnostic of pregnancy but lost its regularity the last 3 weeks of pregnancy. More specimens should be studied to determine if this happened in the canine.

One year of age to 4.1 years of age Various stages of pp involution, anestrous, proestrous, estrous and metestrous were observed in the specimens studied for this age range. Thirteen of the 26 studied were in anestrous and the other 13 were in other phases of the cycle. The photomicrographs of vaginal smears taken at various stages of estrous cycle in the canine by Schutte (1967) and Witiak (1967) compared favorably with the photomicrographs of the intact epithelium of this study. The present investigation gave a more complete picture of what happened deep to the desquamating cells. Especially prominent features were those relating to cornification and to the disappearance of leucocytes at ovulation and reappearance after. It was stated by Wied and Keebler (1967) that in the human, the vaginal epithelium was a "more sensitive end organ than the endometrium to reveal ovarian activity."

Six years of age to 10.4 years of age As in the previous group only cyclic changes were observed in the 18 specimens studied in this age group. Only 5 of these, however, were in anestrous, while the others were in various stages of cyclic activity.
Over 11 years of age. Ten of the 13 specimens of this age group were in anestrus when tissues were taken, even though the tissues were collected between March and August in all except 2 cases. Those 2 were taken in January. In conjunction with the decreased cycling was a decrease in cornification of epithelial cells. There was still cycling, however, (Figures 296, 300, 302). Kretzschmar and Stoddard (1964) indicated that in the human at ovulation, 60 percent of the epithelial cells were cornified whereas just after the menopause this was reduced to 15 percent. Thirty years after the menopause only 10 percent were cornified. In the older canine specimens studied the sz cells were rarely observed and thus cornified cells were just not observed. The surface was composed of imz cells or more frequently of pbz cells. The description given by Töth and Gímécs (1964) of the senile vaginal epithelium more closely fits that of served in the canine. They indicated that it was severely atrophic, in most cases with smaller cells, reduced glycogen in the cytoplasm and shrunken nuclei. Often in the older canine specimens the epithelium consisted of 2 rows of ragged shrunken cells with pyknotic nuclei. No second epithelial proliferation was observed in the canine as Giacomini and Lucchini (1965) described for the human at 68 to 74 years of age which was comparable to a 13 or 14 year old dog. Only one 13 year old specimen was available for study (Figure 300). It was observed, however, that the vaginal epithelium of that animal was composed of 3 to 6 layers of large healthy appearing cuboidal cells, more than double the width of other ancestral epithelia of this age group. A similar epithelium lined the vagina of a 16 year old dog (Figure 302).
Lamina propria

The La Pr of the canine vagina consisted mainly of collagen fibers but with some elastic fibers. According to Velardo (1958) elastic fibers were numerous beneath the epithelium but were rare in other parts of the vaginal wall of the human. Papillae which indented the epithelium were especially prominent in proestrous and estrous (Figure 273), but were also observed in other phases of the cycle. Krantz (1959) felt that they probably played a significant role in aiding the epithelium to stretch during parturition. The subepithelial stromal cellularity was more prominent in some specimens than others. In those from which tissues were taken during metestrous, the increased stromal cellularity was not limited to one area but was observed throughout the La Pr. It was stated by Trautmann and Fisbiger (1957) that in domestic animals the connective tissue was rich in cells near the epithelium. It was found in the canine that these cells were of differing types, stromal cells, plasma cells, lymphocytes, other mononuclear types and in some instances neutrophils (Figures 259, 264, 260, 282, 284).

Chronological appearance

Birth to 2 months of age    A loose netlike embryonic type tissue with many stellate cells and very few fine collagen fibers formed the La Pr of the 2 day old canine vagina. By 2 weeks of age an increase in cellularity as well as a definite increase in collagen fibers subepithelially was observed (Figure 254). According to Wolfe, et al. (1942) the La Pr had developed into a definite collagenous structure in 5, 6 and 7 day old rats, which was somewhat earlier than found in the canine. The loosely arranged collagen fibers in the rat La Pr extended through the stroma to areas where they were almost in contact with the epithelium. Wolfe, et al.
indicated that in some areas the fibers aggregated to form an incomplete argyrophilic basement membrane. In the human, Pundel, et al. (1967) also referred to an incomplete basement membrane as possibly being composed of scleroproteins of a collagen type. A PAS positive basement membrane was found under the canine vaginal epithelium (Figure 251). By one month of age the collagen that formed most of the canine vaginal La Pr appeared to be of a mature type, similar to that found by Wolfe, et al. (1942) for the week old rat.

Up to one month of age in the canine no rugae had developed. In this study longitudinal rugae were first observed in the 5 week old specimens, and then only 4 were found.

Two and one half months of age to 11 months of age By 2.5 months of age 6 to 7 primary rugae were found in the canine La Pr and by 3 months (Figure 255) 10 to 16 were observed. Nineteen were found in the 5.5 month old specimen. By this age the maximum number of rugae had apparently been established, as from 6 to 11 months of age they varied from 9 to 16 in number.

From 10 months of age on, cyclic changes were the predominant feature. Dense collagenous subepithelial zones of proestrous and estrous (Figure 257) were not described for other species. The loose edematous outer three fourths of the La Pr was as Cole (1930) indicated was the case for the whole La Pr of the bovine in estrous. The increased density observed in the canine in early metoestrous was similar to that described by Cole (1930) and Roark and Herman (1950) for the postestral bovine La Pr.

One year of age to 4.1 years of age In the one year age group there were some specimens in various pp states. In these the La Pr had a
ragged appearance and the dense collagen fibers were oriented in swirls between which were numerous stromal nuclei (Figures 266, 269). In the subepithelial area were lymph nodules. Roark and Herman (1950) described the pp bovine vaginal La Pr as dense and fibrous and did not mention the degree of stromal cellularity. The pp bovine description more closely fit the anestral canine La Pr which was a dense, uniform, collagen structure with interwoven elastic fibers. The number of elastic fibers decreased in number in late anestrous. As proestrus approached the La Pr became edematous again and the rugae became distended. Elastic fibers were first observed in the human vaginal La Pr at 17 years of age and then were mainly subepithelial in location. In the canine they were mainly associated with the vessels of the La Pr and varied in distinctness or numbers with the estral cycle.

The increase in overall density and maturation of the La Pr collagen from 2 to 4.1 years of age was probably parallel with the gradual increase in thickness and density of collagen fibers of the rat as described by Wolfe, et al. (1942). They indicated that the La Pr was almost entirely collagenous and that the only portions stained like reticulin were the portions of the fibers that came in contact with the b2 cells of the stratified epithelium.

**Six years of age to 10.4 years of age** Due to the prominent cyclic changes it was difficult to discern the more subtle age changes that took place during this age span. Wolfe, et al. (1942) indicated that in the rat, along with the increase in thickness and density of the collagen fibers which took place throughout life, there was a decreased cellularity. The latter was not necessarily the case in the canine, as there appeared to
be in addition to the cellular changes associated with the cycle, a gradual increase of stromal cellularity with age. Neutrophilic and various mononuclear changes were generally associated with cyclic activity. Wolfe, et al. (1942) did not designate which type of cell was associated with the decrease due to aging in the rat.

Over 11 years of age In addition to the increased density of collagen of the older specimens there were in some specimens areas in the La Pr that appeared hyalinized. There was also a general increase in elastic fiber content (Figures 285, 287, 297). In some this elastosis was extreme and was associated with a stromal hypercellularity. Tóth and Gimes (1964) found that in the aged human, intact elastic fibers of the La Pr surrounded the vagina in the form of a compact ring. La Pr papillae of the older canine specimens retained their capacity for silver impregnation although a compact ring was not found as was the case with some of the old rats. In some cases even the papillae of the rat vaginal La Pr had become entirely collagenous (Wolfe, et al., 1942). The observations by Wolfe, et al. that the stromal cell distribution of the rat La Pr was uniform, was definitely not the case in the canine (Figure 297) except during metestrous (Figure 294). At other cyclic stages, as well as in older specimens the predominance of cellularity was in the subepithelial regions, whether it was stromal or leucocytic in nature (Figure 287). There was, however, an increase in the number of flattened or pyknotic nuclei associated with the stromal cells in the older specimens as Burack, et al. (1941) reported in the rat.
The lack of definite, well delineated muscle layers in the canine vagina was a prominent morphological feature as compared to the well defined layers of the uterus. Instead of forming layers as such, the vaginal musculature was made up of bundles which were in general oriented in the typical inner circular and outer longitudinal directions (Figures 254, 255). However, there were numerous oblique fibers in the outer La Pr and inner part of the ICM area, as well as in the outer part of the ICM area and in the VL. The ELM although mainly longitudinal in orientation, often contained bundles of oblique fibers as well. These findings agreed with those of Christensen (1964) except that he called the innermost oblique bundles longitudinal, and did not mention oblique fibers in the other layers. Observations of the present study would agree more closely with those of Ricci, et al. (1949) who stated that in the human vagina there was no definite muscle layers (not found in this study), but that the muscularis was composed of circular, oblique and longitudinal fibers. Velardo (1958) and Krantz (1959), however, both stated that the muscularis of the human vagina contained an inner circular and outer longitudinal layer as Christensen observed in the canine. There were some sections studied in which the muscle fibers appeared to be as indicated by Christensen, but in most cases there was evidence of oblique fibers. Further studies of the submacroscopic nature should be done to clarify this. Age changes of the vaginal muscularis consisted of a gradual infiltration of the muscles with collagen, starting with the inner layers and extending outward. This agreed with the findings of Wolfe, et al. (1942) for the rat.
Chronological appearance

Birth to 2 months of age During this span of time only early growth changes were seen. The muscularis developed from an ill defined layer of circularly oriented and a few outer longitudinally oriented embryonic cells in the 2 day old specimen to a definite muscle layer by 2 months of age. As early as 5 weeks the oblique fibers on either side of the ICM were well defined. The VL had a few isolated muscle bundles and the ELM consisted of longitudinally oriented bundles of fibers separated by collagen. Wolfe, et al. (1942) found in the vagina of the week old rat that there was a single incomplete layer of longitudinal smooth muscle interrupted by collagen strands.

Two and one half months of age to 11 months of age Increase in growth and development of the muscularis continued so that at 3 months of age the muscle layers were quite well defined in spite of the confusing oblique fibers. The ELM which up to this time was very insignificant in development, by 5.5 months made up a muscle layer nearly half as wide as the more well developed ICM. Collagen and elastic fibers infiltrated between the muscle bundles.

In the 7 to 9 month old specimens, oblique muscle bundles were observed to extend nearly half way up in to the vaginal rugae. By this age all of the muscles appeared morphologically mature and by 10 and 11 months of age cyclic changes were the chief factor in the appearance of the vaginal muscularis.

One year of age to 4.1 years of age Cyclic effects continued to be the most prominent factor contributing to the morphological appearance of the muscularis. As estrous approached, the muscle cells hyper-
trophied and the interspersed collagen became edematous. By mid-metestrus the muscle bundles were atrophied and the collagen was ragged in appearance. Atrophic muscle bundles were a prominent feature of the muscularis in the pp vagina, in which collagen infiltration appeared to have separated the muscle bundles from each other. In addition to the cycling effects, the muscularis from the older animals of this age group had a total overall increase in dense mature appearing collagen between the muscle bundles. Whether this was the result of a developing inability to resorb the extra collagen found at each cycle and during pregnancy was not determined, but was a possible factor.

Six years of age to 10.4 years of age Cycling played a decreasing roll in the morphological appearance of the vaginal muscularis of this age group. The collagen infiltration of the muscle bundles had become more prominent in the older specimens, especially the ICM and associated oblique fibers. In some of the oldest in this group, even the ELM was involved. Increased vacuolization of the muscle cells was observed, possibly due to circulatory disturbances that resulted from the increased density of the infiltrating collagen. The hormonal effect of estrogen on the connective tissue apparently rendered sufficient change to allow for the increased vascularization necessary for the muscle hypertrophy observed at estrous even in the older specimens studied.

Over 11 years of age The trend of collagen infiltration and muscle atrophy as described in the younger age groups continued. There was still some evidence of cycling although most of the specimens from this age group were in anestrous when tissues were taken. Infiltration and in some instances replacement, of muscle bundles by collagen involved all musclete
layers. This agreed with the findings of Wolfe, et al. (1942) that in aged rats the dense collagen fibers separated the ELM into widely isolated units. In the canine all layers were affected. Elastic fibers which were found in all ages, were so numerous in some areas of the muscularis of old specimens that prominent bundles were observed. This severe elastosis was not seen in younger specimens.

Vascularization

The canine vagina received its blood supply from the vaginal artery, a branch of the urogenital artery (Christensen, 1964). In many cases the former bilateral vessels had been lost in tissue collection. When present they were located in the VA which was in the tissues around the vagina, rather than in the VL, between the muscle layers. From the larger muscular arteries of the VL, ramifying branches supplied the muscularis, La Pr and epithelium of the vagina. Although they were not studied, large nerves and nerve gnaglia were seen in most sections. In the young specimens vascularization was minimal. This was expected for a non-vital organ so soon after birth. Gradual growth changes, increase in size and number of vessels took place up to 8 or 9 months of age. After that age cyclic changes masked any further growth changes. Although it was realized that cycling began at about 6 months of age, maturation of the vagina was not completed until later.

Cyclic changes of the vasculature were its chief feature up to about 6 years of age. After that definite structural changes of the vessels were seen with increasing frequency with age.
Chronological appearance

Birth to 2 months of age Only minimal vascularization was observed in this age group. It consisted of numerous capillaries in the La Pr and muscularis, with a few arterioles and satellite veins in the VL. During this span of time there was no need for further development of the vagina than that found at birth, hence the blood was put to work in other areas of the body where it was needed for rapid growth and development of the young animal.

Two and one half months of age to 11 months of age Follicular growth had been observed at about 3 months of age, indicating some hormonal activity. An increase in numbers of capillaries and arterioles in the La Pr and of larger arteries and veins in the VL and VA was observed by 3 months of age. This indicated simultaneous increase in vasculature of the vagina and early follicular growth at the same time. By 6 months of age the various portions of the vagina were well vascularized, indicative of the beginning of cyclic activity. Also at this age the variability of staining of the IEM was first observed, as well as minor intimal splitting and early collagen infiltration. It was felt that these morphological findings were related to the estrous cycle rather than growth or aging per se.

Increased vascularization continued through the rest of this age group including the appearance of numerous, thick, collagen walled vessels in the La Pr, thought to be lymph vessels. After 10 months of age further growth changes, if present, were masked by the cyclic changes. These were increased vascularization during proestrus, estrous and early metestrous or pregnancy then a decline in vascularization in late metestrous, in pp
and in anestrous.

**One year of age to 4.1 years of age**  
Cyclic changes were the prominent features of this group. In pp specimens, the involutionary changes as they affected the arteries were exaggerated. Splitting of the IEM, the formation of plaque-like structures in the intima and collagen infiltration of the outer tunica media were noted. The most prominent morphological change was the appearance of thick collagen collars that formed around the arteries and veins as well as the increased variability in the staining of the IEM. Whether this extra collagen around the arteries was a result of hormonal influence and allowed protection to the arteries from possible stretch damage during whelping was not positively discerned.

Another alteration observed in this group was the appearance of elastoid which was found in small amounts in a 3.6 year old specimen and in greater quantities in some of the older specimens of this group. This was an indication that breakdown of elastic tissue began comparatively early in life.

**Six years of age to 10.4 years of age**  
There appeared to be in this group an increased number of vessels of the VL. It seemed to be a prominent area of the vagina. This was probably due to continuous cycling, and or whelping, but with an incomplete involution of the vessels after each phase or parturition and a resultant buildup. Changes in the arteries thickened intima, collagen infiltration through the tunica media and into the intima, elastoid formation and collagen staining portions of the IEM were all prominent features of this age group. They were seen with increasing frequency in the older specimens. In the 7.5 year old animals
some of the vaginal arteries had a collagen ring in the thickened intima. It was felt that the 60 plus percent collagen infiltration of the tunica media, reorientation of the tunica media cells to a longitudinal direction and increased prominence of venous sinuses of the La Pr were only partly related to cycle, but were generally more a result of aging. These changes have been observed in organs not undergoing changes of the estral cycle (Getty, 1966).

Over 11 years of age Increased severity of the changes already described, were found with greater frequency in the vaginal vessels of these older specimens. This appeared to be the result of a decrease in the ability of the animal body to handle connective tissue as the animal became older. The other interesting aspect was that although the trend of increased vascular changes was observed, older specimens were studied in which the majority of the vessels showed no morphological alteration. Thus, as with all biological material the exceptions to the general trend were also observed in this study.
SUMMARY

Growth and age changes of the canine ovaries and tubular genital tract, as determined by light microscopy were investigated. The youngest specimen studied was 12 hours of age when tissues were taken and the oldest specimen from which the complete tract was studied was 13.1 years of age. Some tissues were taken from 16 and 19 year old animals.

In order to systematically discuss these organs they were first categorized into basic morphological components. Areas of discussion in the ovary were: germinal epithelium, tunica albuginea, cortical stroma, follicles and related structures, medulla and vasculature. The remainder of the tubular genital tract was divided into 4 areas: epithelium, lamina propria, muscularis and vasculature.

The ovary, a 0.15 gm organ in the youngest specimen had grown and developed to a maximum mature weight of 2.22 gm. in the 11 month old animal, the relative ovarian weight increased from 0.38 to 0.49 grams per kilogram during this growth period. The increase in ovarian weight during the remaining years paralleled the increase in body weight.

In the 12 hour old specimen, the germinal epithelium was composed of flattened cuboidal cells. Growth and age changes consisted of wrinkling of the ovarian surface and layering of the epithelial cells in the invaginated areas. Variation of cell type was from flattened cuboidal to tall columnar. Secretion-like material was observed on the apical surface in specimens over 5 months of age that had begun to cycle.

From 11 months of age to 19 years of age only cyclic changes were observed. During proestrus the cells were light-staining, columnar and contained numerous clear cells. In 70 percent of the metestrus specimens
the cells, cuboidal in shape, had moderately dark staining apical cytoplasm, light staining basal cytoplasm and dark staining nuclei. The cells of the remaining 30 percent were light staining and were often columnar. Anestrus specimens, of which there were increased numbers in the older age groups had numerous epithelial cells with pyknotic nuclei and decreased cytoplasm.

Cellular activity was limited to the depressed areas of the prominent lobulated ovaries in specimens over 6 years of age. In the oldest specimens studied there were some areas of epithelial proliferation, though most of the surface was covered by low cuboidal cells.

Underlying the germinal epithelium was a PAS positive basement membrane that varied in thickness and completeness in the specimens studied, but in general increased in thickness with age. Beneath this was a tunica albuginea. It varied from a thin layer of fibroblasts and collagen, 9 to 24 microns in depth, in the younger specimens, to a dense belt of collagen fibers up to 234 microns in thickness in older specimens. In most specimens this structure was much thicker at the ovarian poles than along the other borders. Cysts lined by a single layer of epithelial cells were observed in some specimens that were over 10 years of age. Epithelial nodules were found in the tunica albuginea with decreased frequency in the older specimens.

Connecting the tunica albuginea to the deeper structures of the ovary was the cortical stroma. In the younger specimens it consisted of a few criss-crossing collagen fibers and embryonic fibroblasts between the tunica albuginea and the medulla. The cortex made up 15 percent of the thickness of the ovary in the youngest specimens. As the fibroblasts matured and the
collagen fibers thickened, the cortex increased up to 30 percent of the ovary by 3 months of age. In older cycling specimens it varied from 30 to 60 percent. An increasing number of follicle remnant cells were observed in the ovaries from animals over one year of age. These were very prominent in animals over 6 years of age but were often hypertrophied. As the ovary aged the cortex became narrower.

Oogonia were the only follicular structures found in the youngest specimens. By one month of age primordial follicles were observed, later by 2.5 months of age there were primary follicles as well. Subsurface epithelial structures that originated from the germinal epithelium were found in many ovaries over 3 months of age. Follicles in which an antrum had formed were first observed in a 5.5 month old specimen. Many of the follicles had 2 or more ova. By 11 months of age all types of growing and mature follicles were observed as well as the varied stages of atretic follicles. As atresia progressed, the follicle collapsed and pinched-off structures filled with granulosa cells resulted. These were called granulosa cell islands in this study. In some of the older specimens the cells that formed these structures became hyperplastic and often tumor-like structures resulted.

Follicular cysts, first observed in a 3.5 year old specimen were seen with increasing frequency in older ovaries.

Other abnormal phenomenon were observed in some follicles. Not only did granulosa cells become altered, but also the theca cells changed. In some specimens their hyperplasia resulted in interna thecosis of the cortex. In others it appeared as if the whole stroma had become whorls of thecal cells. The latter cases were called stromal thecosis. Still others
had a mixture of the 2 or combined thecosis.

These various structures were embedded in an increasingly dense collagen matrix in the older specimens. In some cases it was nearly hyaline in appearance. Amyloid was found in some of the latter specimens.

Some follicular structures were found in the medulla of the ovary. Also found in the medulla were blood vessels, lymphatics, paroophoron tubules, rete ovarii, migrated epithelial cord elements, smooth muscle and nerve elements. The collagen, reticulin and elastic fibers that made up most of the medulla increased in amount and density with age. In older specimens amyloid was found in the epithelial portions of the rete ovarii as well as in migrated subepithelial structures and in some granulosa cell islands. Elastoid was also found in increasing amounts in older specimens as well as hyalinized collagen. In the medullary tissues of some of the older ovaries various sizes of leiomyomas were observed.

The ovarian vessels which were largest and most numerous in the medulla also underwent growth and age changes. Those found in the cortex were of 2 types, follicular or corpora luteal. In the latter cyclic sclerosis was prominent. The other vessels whether cortical or medullary underwent more gradual changes.

A very thin internal elastic membrane was observed in the arterioles of the youngest specimen studied. Incomplete elastic rings were observed in the tunica media of arteries from older specimens. Splitting of the internal elastic membrane was observed as early as 6 months of age, as well as reorientation of some of the innermost tunica media cells to a longitudinal direction. Thickening of the intima was observed first in a 7 month old specimen. More of the arteries were involved in older specimens,
and these to a more prominent degree. Sometimes these changes were observed, only on one side of the vessel wall while in others the changes were uniform around the vessel.

Infiltration of the tunica intima with collagen fibers, from without toward the lumen was visible in year old specimens, and increased in amount with age. In some the fibers formed a dense ring of collagen immediately out from the internal elastic membrane. Increasing amounts of elastoid were found in the tunica media and adventitia of older vessels. These various changes occurred singly in some cases, but generally they were superimposed one upon another. Although the trend was one of increased vascular change in older specimens, there were individual ovaries, even in the older age groups that had only minimal changes as detected by the methods used.

Tubular genital tract changes were basically similar to those observed in the ovarian tissues. The 4 basic components were similar in all parts of the tract. Changes, including growth, cyclic and age were discussed.

In the youngest specimen studied the oviducal epithelium consisted of tightly wedged columnar cells up to 22 microns in height. They were shortened to 4 to 13 microns in height by one week of age as maternal hormonal influence waned. By 2.5 months of age, 4 types of cells were observed, some of which were ciliated. In specimens over 5 months of age, cyclic changes masked the gradual growth changes observed up to that age. The epithelial cells were up to 80 microns in height and pseudostratified in appearance in proestrous or estrous. Cytoplasm was extruded from some of these cells. In other phases of the cycle the cells varied from 5 to 20 microns in height. Cilia were found in decreased numbers in older speci-
Epithelial cysts were found in increased numbers with age. No real pattern was observed regarding other morphological aspects of the oviducal epithelium. The only constancy was individual variation.

The epithelial cells that lined the uterus varied from 9 to 60 microns in height. Some of them were ciliated. All variations of stainability were observed. Usually the epithelial cells were simple in morphology, but during metestrous and postpartum they underwent a complex proliferation. This resulted in a fluffy, popcorn appearance to the cells. During most of the cycle the epithelium varied from cuboidal to columnar and the cells were 15 to 20 microns in height except during metestrous or postpartum when they became tall and pseudostratified. After the initial growth changes, the chief changes were related to the estrous cycle. In an increasing number of animals over 6 years of age, endometrial hyperplasia or cystic endometrial hyperplasia was observed. The epithelium of most of the older specimens though was in a regressed state. A PAS positive basement membrane was found underlying the uterine epithelium as well as the epithelium that lined the other parts of the tubular genital tract. It was more prominent in older specimens.

The cervical epithelium varied from uterine-like in the anterior part of the cervix to vagina-like in the posterior part of the cervix. The latter, stratified squamous epithelium varied from 2 to 12 cells in depth and at estrous was composed of 5 zones; basal, parabasal, intermediate, intraepithelial and superficial. In the cervix, the lumen was crescent shaped with the points directed dorsad. The epithelium that lined the lesser curvature of the crescent was related to the pillar of the cervix and was typically cervical in morphology. That lining the greater curva-
ture was associated with the body of the cervix and was more closely related to the vaginal epithelium.

In the youngest specimens the cervical epithelium was composed of 4 to 7 layers of moderately dark staining cells. It was decreased by 2 weeks of age and then showed an increase again as the intrinsic hormonal effect began at about 5 months of age. Cyclic changes were the prominent feature in specimens over 11 months of age. Popcorn cells as described in the uterus were also found in the anterior part of the cervix during metestrous and postpartum. Numerous leucocyte cell types were observed in these epithelia. As in the uterus, most of the cervical epithelia of older specimens were in a regressed state, although there were some, even in the oldest specimens that were proliferative in appearance.

The vaginal epithelium was stratified squamous in type. Changes were very similar to those described for the posterior part of the cervix. Cyclic changes were predominant features. In a few of the older specimens there was evidence of cycling although most were in an anestral state.

Lamina proprial changes were somewhat similar in all parts of the canine tubular genital tract. Rugae, upon which the epithelium lay, were found to varying degrees in most parts of the tract.

In the oviduct of young specimens the lamina propria consisted of dis-oriented masses of embryonal cells, collagen, elastic and reticular fibers, plasma cells, lymphocytes and mast cells. In specimens over 6 years of age there was a general increase in density and fibrosity and increased evidence of cellular degeneration. The rugae of specimens over 11 years of age were less complex than those found in younger ones. In the oldest specimen studied a 13.1 year old, the lamina propria was sparse and loose.
in contrast to the trend of increased density.

Greater variations were observed in the lamina propria of the uterus than in the oviduct. The type of embryonal cells of the younger specimens was the same in both cases. In the youngest uterus studied the lamina propria comprised 51 percent of the uterine wall thickness. No glands were present and there were but few indentations from the epithelium into the lamina propria indicative of where glands would form. By 2 weeks of age the lamina propria had increased to 62 percent of the wall thickness and gland crypts were observed. The connective tissue components had increased in density. The glands extended across the lamina propria to the musculature by 2.5 months of age. From that age on they were increased in prominence and varied in development and numbers depending on cyclic stage. Connective tissue components also varied with the stage of estrous. From birth to maturity the lamina propria grew from 120 microns up to 1500 microns in thickness. In specimens with endometrial hyperplasia most of the lamina propria was involved as it contained hyperplastic glands, cysts, collagen islands and numerous leucocyte cell types. Over half of the animals over 11 years of age had cystic endometrial hyperplasia. In this age group the mean lamina proprial thickness was decreased from what it was in younger mature specimens.

With the exception of glands, the lamina propria of the cervix was similar to that of the uterus. By 7 months of age the connective tissue components were mature in appearance and formed a dense layer except in the subepithelial area where the tissues were edematous. This zone was most prominent at estrous. The lamina propria was thickest also at this time. In anestrous the lamina propria became shrunken. Cytoplasmic
vacuolization and pyknotic nuclei were common morphological features. Cyclic activity was observed in all age groups but with decreased frequency in the older groups. Lymph nodules were found in the cervical lamina propria of one specimen.

In the vagina, the components of the lamina propria were as they were in other portions of the tubular genital. Longitudinal rugae first appeared in the 5 week old specimen. They increased in numbers and development so that by one year of age there were 9 to 16 present. Subepithelial lymph nodules were present in some. As with other parts of the tract, cyclic state determined the lamina propria morphology. In some of the older specimens the collagen of the rugae appeared hyalinized, the lamina propria was decreased in thickness and there was an increase in elastic fibers and elastoid. An embryological remnant composed of tubules was found in the outer one third of the lamina propria of the oldest specimen studied.

Out from the lamina propria was the muscularis. Since the oviduct was embedded in the ovarian bursa, it was difficult to discern between the muscle tissue of the oviduct and that of the ovarian bursa. The muscularis was divided into an inner circular layer, a series of oblique bundles in the vascular layer and a series of longitudinal or oblique bundles of the external longitudinal layer. Out from this were a few collagen fibers and the serosa. In all layers collagen and elastic fibers were observed.

The future muscularis in the youngest specimens was easily delineated from the lamina propria by fiber direction. Definite muscles became visible first in the circular layer at about 6 months of age. By 8 months of age the external longitudinal layer consisted of a very narrow band of
cells. In all age groups the muscle layers were most sparse near the in-
fundibulum and were increased in thickness in the isthmus. No increase in
density of muscles or connective tissue components was observed up until 6
years of age. By 10 years of age, some of the infundibular muscle cells
were replaced completely by collagen. Muscle bundles extended into rugae
in these specimens. In some specimens over 11 years of age mild collagen
infiltration of the bursal musculature was found. This was not observed
in younger specimens. The infiltration was prominent in all other layers.
Remaining muscle cells of the latter were often vacuolated, had decreased
cytoplasm and pyknotic nuclei.

The basic composition of the uterine muscularis was as described
above for the oviduct. Early development was similar but more rapid. By
2 weeks of age the inner circular layer was narrow though dense and the
outer longitudinal had just began to develop. Oblique muscle fibers were
observed for the first time in 3 month old specimens. By maturity the
myometrium had developed from 400 microns in thickness to 2250 microns.
The excess thickness of the latter was due to early postpartum involution.
Infiltration by connective tissue elements which was not prominent until 6
years of age in the oviduct was observed as early as one year of age in the
uterus, and was prominent by 2-9 years of age. Cyclic alterations were de-
creased in specimens over 6 years of age. An increase in collagen infil-
tration of the inner circular muscle continued up through 10 years of age,
after which it was not as prominent. The vascular layer increased in
thickness due to increases in collagen around the vessels and increases in
numbers of vessels up through 10 years of age. In specimens over 11 years
of age, all layers of the muscularis showed some degree of collagen infil-
tration and an increasing number showed disorientation of fiber direction of the internal circular muscle. There were 2 cases of focal endometriosis and 2 uteri that contained leiomyomas in the older age groups. The vascular layer was no longer uniform in thickness in the older specimens. It was very wide and well developed for one half to three fourths of the way around the uterus and then nearly non-existent the rest of the way. An increase in elastic fibers, elastoid and collagen was observed in the vascular layer of all older specimens. There was also an apparent cytoplasmic decrease in the muscle cells of the old uteri.

In the canine cervix the muscularis consisted of 4 layers other than the 3 observed in the uterus and oviduct. The fourth layer was located from the internal circular muscle and the fibers were oblique in their orientation. In addition to this, in the cervical pillar region there was a large muscle mass with multidirectional fibers.

No definite boundary between the cervical lamina propria and muscularis was discernable in the youngest specimens. By 2 weeks of age the various muscle layers, although poorly developed were distinguishable. They were well delineated by 3 months of age. Even at this age it was discerned that the cervical musculature contained a much higher percentage of collagen than did the musculature of the uterus or oviduct. All layers were embedded in collagen. This was typical of the cervix. Cyclic effects were not as discernable in the cervix as in the uterus but were observed. Increased collagen infiltration of the cervical musculature especially prominent in postpartum specimens.

In specimens over 6 years of age there was an apparent decrease in muscle cell cytoplasm as the nuclei appeared much more prominent and were
closer together. Collagen infiltration was increased in these specimens. Most specimens over 11 years of age had over 50 percent collagen infiltration of the muscularis. Replacement of some of the muscle cells by collagen was noted. Elastic fibers were seen with increased frequency in these specimens. As in the older uteri, muscle fiber disorientation was also noted in some of the cervices.

The vaginal muscularis consisted of 3 layers of muscle. Each were poorly developed when compared with other portions of the tubular genital tract. In the younger specimens the internal circular muscle consisted of an ill-defined layer of embryonic cells with circular orientation. The vascular layer was wide and the external longitudinal layer was hardly discernable. By 2 weeks of age the vascular layer was narrower and all layers more easily definable. At 3 months of age the muscle bundles were mature in appearance and there were numerous oblique fibers associated with the inner portions of the internal circular muscle. The external longitudinal muscle layer was well developed in 5 month old specimens. As in the cervix each muscle layer was well delineated. Each was separated from the other layers by collagenous tissue interspersed with numerous elastic fibers. Over 10 months of age cycling prominently affected the vaginal muscularis. In proestrous, estrous and early metestrous the bundles appeared hypertrophied. The collagenous tissues were edematous at estrous, while at mid-metestrous even the muscle bundles appeared shrunken and decreased in number. Cyclic changes were prominent up to 6 years of age after which they gradually became less prominent. Collagen infiltration of specimens over 6 years of age was greatest in the internal circular muscle but included some of the outer musculature as well. This in-
filtration was not just between the muscle bundles as noted in earlier specimens but included infiltration of muscle cells.

All phases of the estrous cycle were observed in specimens over 11 years of age although most were in anestrus. Collagen infiltration was generally more severe in these specimens. There was a noticeable increase in replacement of muscle cells by collagen. An increase in elastic fibers was observed in the older specimens and in some elastosis was very severe.

Vascularization of necessity involved each layer of the tubular genital tract from the oviduct to the vagina. The vessels most consistently seen in all sections studied were those of the vascular muscle layer interposed between the circular and longitudinal layers. Often large bilaterally located vessels were found out from the outer muscle layer. These were called adjacent vessels.

Generally the vascularization was noted to increase through the growth period. This was especially noticeable in the vascular layer vessels in the 5.5 and 6 month old specimens. In the 10 month old specimens the adjacent arteries had thickened intima and early splitting of the internal elastic membrane. Walls of the satellite adjacent veins were composed of collagen and elastic fibers and smooth muscle cells. Although in most of the year old specimens there were but few vascular changes, in some there was a dense layer of collagen that encircled the tunica media of the larger arteries. Arteries of some of the 3 year old specimens had thickened intima, collagen and elastic fiber infiltration of the tunica media and splitting of the internal elastic membrane. Elastoid was observed in an oviducal artery from a 3.3 year old specimen. A plaque-like structure was found in a large vein from a 3.6 year old oviduct. Vascular changes as a
result of cycling were the chief alterations observed in specimens 6 to 10 years of age. Only one third of those studied showed changes. In most specimens over 11 years of age at least some of the oviducal arteries had intimal thickening or collagen infiltration.

As in the oviduct there were growth, cyclic, pregnancy, and age changes in the vessels of the canine uterus. As early as 3 months of age the arteries of the uterine vascular layer began to group at opposite poles, adjacent to and opposite the mesenteric border. Early localized intimal thickening was observed in an arteriole in a 7 month old specimen. This was seen with increased frequency in 10 and 11 month old specimens as well as splitting of the internal elastic membrane and collagen infiltration of the tunica media.

Cycling was a prominent feature from one to 4 years of age and many of the vascular alterations were associated with it. The most severe changes were in proestrous, estrous and metestrous specimens. In these degeneration of the internal elastic membranes was observed in some of the arteries. The changes observed in anestrous specimens were thought to be associated with age rather than cycling. They were increasingly prominent in animals over 6 years of age. Arteries from these specimens had up to 40 percent collagen infiltration of their musculature, up to 90 percent thickening of the intima and varying amounts of elastic fibers and elastoid. Internal elastic membrane degeneration was observed in postpartum specimens. In the uteri with the most severely affected arteries, the veins had very thick walls which were filled with elastoid.

Varying degrees of the vessel changes described above were found in all but one of the specimens that was over 11 years of age. Changes were
prominent in over half of these specimens. Cyclic changes were minor in these older animals.

The same factors affected the cervical vascularization as that of the uterus. In the cervix large bilaterally located adjacent arteries were often found. Even in the youngest specimens there was a discernable internal elastic membrane in the arterioles. It was a prominent structure in specimens over one week of age. Splitting of this membrane was first seen in a 6 month old specimen. It was incomplete in most of the arterioles and many arteries of 7 and 8 month old specimens. Variability in staining was noted in the internal elastic membrane of arteries found in 10 and 11 month old cervices. Sometimes it took the elastic stain, sometimes the collagen stain and sometimes appeared as a translucent outline. Numerous vessels with thick, edematous-like, collagenous appearing walls, were found in an 11 month old specimen. These were found with increasing frequency in older specimens especially those in or near estrous. Most of them were lymphatics, while some were veins.

Variation in stainability of the internal elastic membrane, infiltration of the tunica media with collagen, splitting of the latter structure and elastoid formation were all observed with increasing frequency in specimens over one year of age. In the cervix infiltrated collagen often formed a dense ring around the internal elastic membrane. Some plaque-like structures were found in 3 year old specimens as well as reorientation of subintimal muscle cells from a circular to a longitudinal direction. In specimens over 6 years of age the internal elastic membrane showed more prominent variability in its reaction to elastic stain. Portions of it stained positive for collagen in some of the specimens over 11 years of
age. In most of the older cervices, combined intimal thickening, collagen
infiltration and elastoid formation involved 10 to 40 percent of the arterial wall. As in the oviduct and uterus a higher percentage of vessels
were involved in the older specimens and these more severely than in the younger age groups.

In the vagina there was only minimal vascularization in the youngest specimens. Even in the animals under 5 months of age the walls of vaginal veins contained much elastic tissue. A prominent well stained internal elastic membrane was found in all arteries up to 6 months of age. In those a little older, the smaller arteries with less than 3 cells comprising the media lacked a visible internal elastic membrane. Splitting of this membrane and early infiltration of the tunica media were observed in 7 month old specimens. Variability in staining of the internal elastic membrane involved all sizes of arteries in 11 month old specimens. Cyclic changes were prominent up to 4 years of age. In specimens over 6 years of age the vaginal vascular layer was increased in thickness. Thickened intima, collagen infiltration into the intima, elastoid formation and collagen stained portions of the internal elastic membrane were features observed in specimens over 7 years of age. In a 7.5 year old animal there was a dense collagen ring incorporated in the thickened intima of some of the arteries. Venous sinuses were prominent in the lamina propria of some of the 9 and 10 year old specimens. The vessels from specimens over 11 years of age were more severely affected than those in younger groups, and a higher percentage of the arteries were involved.

Many of the growth and age changes found in the various portions of
the canine female genitalia were similar. There were, however, differentiating features distinctive of each portion.
LITERATURE CITED


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APPENDIX A

TABLES OF ABBREVIATIONS, SPECIMEN IDENTIFICATION, WEIGHTS, MEASUREMENTS AND WHELPING HISTORIES
Table 1. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Ab Ov Wt Gm</td>
<td>Absolute Ovarian Weight in grams</td>
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<tr>
<td>AB</td>
<td>African Basenji</td>
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<td>bz</td>
<td>basal zone</td>
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<td>B</td>
<td>Beagle</td>
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<tr>
<td>Bd Wt Kg</td>
<td>Body weight in Kilograms</td>
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<tr>
<td>CWC</td>
<td>Cardigan Welsh Corgi</td>
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<td>CS</td>
<td>Cocker Spaniel</td>
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<td>C</td>
<td>Control protein</td>
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<td>Dch</td>
<td>Dachshund</td>
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<td>Dlm</td>
<td>Dalmation</td>
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<tr>
<td>Ep Ht Mc</td>
<td>Epithelial height in microns (uterine lumen)</td>
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<td>ELM</td>
<td>External longitudinal muscle</td>
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<td>FT</td>
<td>Fox Terrier</td>
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<td>GSP</td>
<td>German Shorthair Pointer</td>
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<td>GI Ht Mc</td>
<td>Gland epithelial height in microns (uterine)</td>
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<td>GR</td>
<td>Golden Retriever</td>
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<td>H</td>
<td>High protein</td>
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<td>ICM</td>
<td>Internal circular muscle</td>
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<td>IBM</td>
<td>Internal elastic membrane</td>
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<td>iez</td>
<td>intraepithelial cell zone</td>
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<td>imz</td>
<td>intermediate cell zone</td>
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<td>IS</td>
<td>Irish Setter</td>
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<td>La Pr</td>
<td>Lamina propria</td>
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<td>L</td>
<td>Low protein</td>
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<td>LR</td>
<td>Labrador Retriever</td>
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<td>N</td>
<td>Medium protein</td>
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<td>MT</td>
<td>Total uterine muscle</td>
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<td>Norwegian Elkhound</td>
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<td>postpartum</td>
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<td>RI Ov Wt</td>
<td>Relative ovarian weight</td>
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<td>SES</td>
<td>Subepithelial Structures</td>
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Table 5. Whelping records from Beagle Colony, Department of Anatomy, Iowa State University
(Included only those animals from which tissues had been taken)

<table>
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<th>Age in Years</th>
<th>I.D. Number</th>
<th>Diet</th>
<th>No. of Litters</th>
<th>No. of Pups</th>
<th>Whelping Span</th>
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</table>
APPENDIX B

GRAPHS OF DIMENSIONAL CHANGE WITH AGE
Graph 1. Body weights, ovarian weights and relative ovarian weights in 30 dogs ranging in age from 0.02 months to 11.0 months.

Body weight; data for linear regression, where $Y =$ body weight in Kg and $X =$ age in months

- $N = 30$
- $Y = 1.25 + 0.68 X$; $s_y = 2.17$ kg
- $b = 0.68 \pm 0.12$ kg/month; $P < 0.01$
- $\bar{Y} = 5.13 \pm 0.40$ kg; $P < 0.01$
- $r = 0.74$; $P < 0.01$

Ovarian weight; data for linear regression, where $Y =$ ovarian weight in gm and $X =$ age in months

- $N = 30$
- $Y = 0.07 + 0.09 X$; $s_y = 0.32$ kg
- $b = 0.09 \pm 0.02$ gm/month; $P < 0.01$
- $\bar{Y} = 0.61 \pm 0.18$ gm; $P < 0.01$
- $r = 0.71$; $P < 0.01$

Relative ovarian weight; data for curvilinear regression, where $Y =$ relative ovarian weight in gm/kg and $X =$ age in months

- $N = 30$
- $Y = 0.328 - 0.073 X_1 + 0.006 X_2$, $s_y = 0.085$
- $b_{y1.2} = -0.073 \pm 0.015$ gm/kg/month, $P < 0.01$
- $b_{y2.1} = 0.006 \pm 0.001$ gm/kg/month, $P < 0.01$
- $\bar{Y} = 0.160 \pm 0.020$ gm; $P < 0.01$
- $r = 0.68$; $P < 0.01$
OVARIAN WEIGHT IN GRAMS

RELATIVE OVARIAN WEIGHT IN GRAMS PER KILOGRAM

BODY WEIGHT (•)
RELATIVE OVARIAN WEIGHT (△)

AGE IN MONTHS

BODY WEIGHT IN KILOGRAMS
Graph 2. Body weights, ovarian weights and vascular thickness in 44 dogs ranging in age from one year to 13.1 years of age.

Body weight; data for linear regression, where $Y =$ body weight in kg and $X =$ age in years.

\[
\begin{align*}
N &= 44 \\
Y &= 4.32 + 1.68 \times X; \quad s_Y = 13.47 \text{ kg} \\
b &= 1.68 \pm 0.52 \text{ kg/year}, \quad P < 0.01 \\
\bar{Y} &= 15.25 \pm 2.03 \text{ kg}, \quad P < 0.01 \\
r &= 0.45, \quad P < 0.01
\end{align*}
\]

Ovarian weights; for linear regression, where $Y =$ ovarian weight in gm and $X =$ age in years.

\[
\begin{align*}
N &= 44 \\
Y &= 0.82 + 0.18 \times X; \quad s_Y = 1.60 \text{ gm} \\
b &= 0.18 \pm 0.06 \text{ gm/year}, \quad P < 0.01 \\
\bar{Y} &= 0.40 \pm 0.24 \text{ gm}, \quad P = \text{ NS} \\
r &= 0.41, \quad P < 0.01
\end{align*}
\]

Vascular layer thickness; for curvilinear regression, where $Y =$ vascular layer thickness in microns and $X =$ age in years.

\[
\begin{align*}
N &= 44 \\
Y &= 241.24 + 71.27 X_1 - 5.10 X_2; \quad s_Y = 144.76 \text{ microns} \\
b_{y1.2} &= 71.27 \pm 21.51 \text{ microns/year}, \quad P < 0.05 \\
b_{y2.1} &= -5.10 \pm 1.95 \text{ microns/year}, \quad P < 0.05 \\
\bar{Y} &= 413.0 \pm 21.82 \text{ microns}, \quad P < 0.01 \\
r &= 0.38, \quad P < 0.01
\end{align*}
\]
Graph 3. Epithelial heights and lamina propria thicknesses in 30 dogs ranging in age from 0.02 months to 11 months.

Epithelial height; data for curvilinear regression, where $Y =$ epithelial height in microns and $X =$ age in months.

\[
N = 30 \\
Y = 15.34 - 1.59 X_1 + 0.13 X_2 ; \quad s_y = 3.52 \text{ microns} \\
b_{Y1.2} = -1.59 \pm 0.64 \text{ microns/month}, \quad P < 0.01 \\
b_{Y2.1} = 0.13 \pm 0.05 \text{ microns/month}, \quad P < 0.01 \\
\bar{Y} = 12.00 \pm 0.64 \text{ microns} , \quad P < 0.01 \\
r = 0.43 \quad , \quad P < 0.01
\]

Lamina propria thickness; data for linear regression, where $Y =$ lamina propria thickness in microns and $X =$ age in months.

\[
N = 30 \\
Y = 186.46 + 51.36 X ; \quad s_y = 238.23 \text{ microns} \\
b = 51.36 \pm 13.05 \text{ microns/month}, \quad P < 0.01 \\
\bar{Y} = 479.00 \pm 43.50 \text{ microns} , \quad P < 0.01 \\
r = 0.60 \quad , \quad P < 0.01
\]
Graph 4. Uterine muscle, total thickness and uterine wall total thickness in 30 dogs ranging in age from 0.02 months to 11 months.

Uterine muscle, total thickness; data for linear regression where $Y = \text{total muscle thickness in microns}$ and $X = \text{age in months}$.

- $N = 30$
- $Y = 83.43 X + 96.61; \sigma_Y = 378.94 \text{ microns}$
- $b = \frac{83.43}{20.76} = 4.05 \text{ microns/month}, P < 0.01$
- $Y = 465.00 - 69.18 \text{ microns}, P < 0.01$
- $r = 0.60, P < 0.01$

Uterine wall, total thickness; data for linear regression where $Y = \text{total wall thickness in microns}$ and $X = \text{age in months}$.

- $N = 30$
- $Y = 189.62 + 134.66 X; \sigma_Y = 472.74 \text{ microns}$
- $b = \frac{134.66}{25.90} = 5.20 \text{ microns/month}, P < 0.01$
- $Y = 955.00 - 86.31 \text{ microns}, P < 0.01$
- $r = 0.70, P < 0.01$
Graph 5. Lamina propria thickness and uterine wall thickness in 30 dogs ranging in weight from 0.4 to 11.1 kg.

Lamina propria thickness; data for curvilinear regression where Y = lamina propria thickness in microns and X = body weight in kg.

\[
\begin{align*}
N &= 30 \\
Y &= 167.03 + 126.82 X_1 - 9.46 X_2 \pm s_y = 268.45 \text{ microns} \\
by1.2 &= 126.82 \pm 51.17 \text{ microns/kg}, \ P < 0.05 \\
by2.1 &= -9.46 \pm 4.65 \text{ microns/kg}, \ P = \text{NS} \\
Y &= 497.00 \pm 49.01 \text{ microns}, \ P < 0.01 \\
r &= 0.46, \ P < 0.01
\end{align*}
\]

Uterine wall, total thickness; data for curvilinear regression where Y = uterine wall thickness in microns and X = body weight in kg.

\[
\begin{align*}
N &= 30 \\
Y &= 228.02 + 292.66 X_1 - 21.59 X_2 \pm s_y = 591.86 \text{ microns} \\
by1.2 &= 292.66 \pm 112.81 \text{ microns/kg}, \ P < 0.05 \\
by2.1 &= -21.59 \pm 10.26 \text{ microns/kg}, \ P < 0.05 \\
Y &= 955.00 \pm 108.06 \text{ microns}, \ P < 0.01 \\
r &= 0.48, \ P < 0.01
\end{align*}
\]
Ovarian weights, vascular layer thickness, external longitudinal muscle thickness and total muscle thickness in 74 dogs ranging in weight from 0.4 kg to 88.2 kg.

Ovarian weight; data for linear regression where Y = ovarian weight in gm and X = body weight in kg.

\[ Y = 0.81 + 0.06 X ; \quad s_Y = 1.37 \text{ gm} \]
\[ b = 0.06 \pm 0.01 \text{ gm/kg} \quad \text{, } \quad P < 0.01 \]
\[ Y = 1.50 \pm 0.16 \text{ gm} \quad \text{, } \quad P < 0.01 \]
\[ r = 0.48 \quad \text{, } \quad P < 0.01 \]

Vascular layer thickness; data for linear regression where Y = vascular layer thickness in microns and X = body weight in kg.

\[ Y = 246.56 + 4.69 X ; \quad s_Y = 180.16 \text{ microns} \]
\[ b = 4.69 \pm 1.65 \text{ microns/kg} \quad \text{, } \quad P < 0.01 \]
\[ Y = 302.00 \pm 21.04 \text{ microns} \quad \text{, } \quad P < 0.01 \]
\[ r = 0.32 \quad \text{, } \quad P < 0.01 \]

External longitudinal muscle thickness; data for linear regression where Y = external longitudinal thickness in microns and X = body weight in kg.

\[ Y = 236.10 + 4.04 X ; \quad s_Y = 202.39 \text{ microns} \]
\[ b = 4.04 \pm 1.85 \text{ microns/kg} \quad \text{, } \quad P < 0.05 \]
\[ Y = 274.00 \pm 23.53 \text{ microns} \quad \text{, } \quad P < 0.01 \]
\[ r = 0.25 \quad \text{, } \quad P < 0.05 \]

Muscle, total thickness; data for linear regression where Y = total uterine muscle thickness in microns and X = body weight in kg.

\[ Y = 770.72 + 11.54 X ; \quad s_Y = 570.84 \text{ microns} \]
\[ b = 11.54 \pm 5.28 \text{ microns/kg} \quad \text{, } \quad P < 0.05 \]
\[ Y = 906.00 \pm 67.29 \text{ microns} \quad \text{, } \quad P < 0.01 \]
\[ r = 0.25 \quad \text{, } \quad P < 0.05 \]
ABSOLUTE OVARIAN WEIGHT

VASCULAR LAYER

EXTERNAL LONGITUDINAL MUSCLE

TOTAL MUSCLE THICKNESS

BODY WEIGHT IN KILOGRAMS

UTERINE TOTAL MUSCLE THICKNESS IN HUNDREDS OF MICRONS

VASCULAR LAYER, EXTERNAL LONGITUDINAL MUSCLE IN HUNDREDS OF MICRONS

ABSOLUTE OVARIAN WEIGHT IN GRAMS

10-10
Graph 7. Gland epithelial height, lamina propria and vascular layer thickness in 30 dogs with combined ovarian weight ranging from 0.13 gm to 2.22 gm.

Gland epithelial height; data for linear regression, where \( Y = \) gland epithelial height in microns and \( X = \) combined ovarian weight in gm.

\[
\begin{align*}
N & = 30 \\
Y & = 11.55 + 4.87 X ; \ sy = 5.55 \text{ microns} \\
b & = 4.87 \pm 2.29 \text{ microns/gm}, \ P < 0.05 \\
\bar{Y} & = 1.45 \pm 1.01 \text{ microns}, \ P = \text{NS} \\
r & = 0.37 \quad P < 0.05
\end{align*}
\]

Lamina propria thickness; data for linear regression where \( Y = \) lamina propria thickness in microns and \( X = \) combined ovarian weight in gm.

\[
\begin{align*}
N & = 30 \\
Y & = 219.96 + 426.92 X ; \ sy = 223.26 \text{ microns} \\
b & = 426.92 \pm 92.06 \text{ microns/gm}, \ P < 0.01 \\
\bar{Y} & = 479.00 \pm 40.76 \text{ microns}, \ P < 0.01 \\
r & = 0.66 \quad P < 0.01
\end{align*}
\]

Vascular layer thickness; data for linear regression where \( Y = \) vascular layer thickness in microns and \( X = \) combined ovarian weight in gm.

\[
\begin{align*}
N & = 30 \\
Y & = 46.35 + 152.36 X ; \ sy = 70.19 \text{ microns} \\
b & = 152.36 \pm 28.94 \text{ microns/gm}, \ P < 0.01 \\
\bar{Y} & = 139.00 \pm 12.81 \text{ microns}, \ P < 0.01 \\
r & = 0.71 \quad P < 0.01
\end{align*}
\]
Graph 8. Internal circular muscle thickness and external longitudinal muscle thickness in 30 dogs with combined ovarian weights ranging from 0.13 to 2.22 gm.

Internal circular muscle thickness; data for linear regression where \( Y = \) internal circular muscle thickness in microns and \( X = \) combined ovarian weight in gm.

\[
\begin{align*}
N &= 30 \\
Y &= 6.01 + 301.80 X ; s_Y = 165.62 \text{ microns} \\
b &= 301.80 \pm 68.29 \text{ microns/gm}, \quad P < 0.01 \\
\bar{Y} &= 189.00 \pm 30.24 \text{ microns}, \quad P < 0.01 \\
r &= 0.64, \quad P < 0.01
\end{align*}
\]

External longitudinal muscle thickness; data for linear regression where \( Y = \) external longitudinal muscle thickness in microns and \( X = \) combined ovarian weight in gm.

\[
\begin{align*}
N &= 30 \\
Y &= -46.47 + 303.09 X ; s_Y = 102.13 \text{ microns} \\
b &= 303.09 \pm 42.11 \text{ microns/gm}, \quad P < 0.01 \\
\bar{Y} &= 137.00 \pm 18.65 \text{ microns}, \quad P < 0.01 \\
r &= 0.81, \quad P < 0.01
\end{align*}
\]
OVARIAN WEIGHT IN GRAMS

UTERINE MUSCLE LAYERS IN HUNDREADS OF MICRONS

- INTERNAL CIRCULAR MUSCLE (•)
- EXTERNAL LONGITUDINAL MUSCLE (○)
Graph 9. Gland epithelial heights, internal circular muscle, vascular layer and external longitudinal muscle layer thicknesses in 74 dogs with combined ovarian weights ranging from 0.13 gm to 9.42 gm.

Gland epithelial height; data for linear regression where $Y =$ gland epithelial height in microns and $X =$ combined ovarian weight in gm.

$N = 74$

$Y = 13.75 + 0.85 X ; s_y = 5.42$ microns

$b = 0.85 \pm 0.41$ microns/gm, $P < 0.05$

$\bar{Y} = 14.60 \pm 0.63$ microns, $P < 0.01$

$r = 0.24$, $P < 0.05$

Internal circular muscle thickness; data for linear regression where $Y =$ internal circular muscle thickness in microns and $X =$ combined ovarian weight in gm.

$N = 74$

$Y = 252.82 + 48.62 X ; s_y = 235.50$ microns

$b = 48.62 \pm 17.76$ microns/gm, $P < 0.01$

$\bar{Y} = 326.00 \pm 27.38$ microns, $P < 0.01$

$r = 0.31$, $P < 0.01$

Vascular layer thickness; data for linear regression where $Y =$ vascular layer thickness in microns and $X =$ combined ovarian weight in gm.

$N = 74$

$Y = 223.20 + 52.39 X ; s_y = 172.39$ microns

$b = 52.39 \pm 13.00$ microns/gm, $P < 0.01$

$\bar{Y} = 302.00 \pm 20.04$ microns, $P < 0.01$

$r = 0.43$, $P < 0.01$

External longitudinal muscle thickness; data for linear regression where $Y =$ external longitudinal muscle thickness in microns and $X =$ combined ovarian weight in gm.

$N = 74$

$Y = 187.21 + 57.64 X ; s_y = 188.61$ microns

$b = 57.64 \pm 14.23$ microns/gm, $P < 0.01$

$\bar{Y} = 274.00 \pm 21.93$ microns, $P < 0.01$

$r = 0.43$, $P < 0.01$
UTERINE GLAND EPITHELIAL HEIGHT
INNER CIRCULAR MUSCLE
VASCULAR LAYER
EXTERNAL LONGITUDINAL MUSCLE

Uterine gland epithelial height in microns

Ovarian weight in grams

Uterine muscle layers in hundreds of microns
Graph 10. Muscle total thickness and uterine wall, total thickness in 30 dogs with combined ovarian weights ranging from 0.13 to 2.22 gm.

Muscle total thickness: data for linear regression where $Y =$ uterine muscle total thickness in microns and $X =$ combined weights in gm.

$$
N = 30 \\
Y = 5.89 + 757.75 X ; \; s_y = 325.23 \text{ microns} \\
b = 757.75 \pm 134.10 \text{ microns/gm}, \; P < 0.01 \\
\bar{Y} = 465.00 \pm 59.38 \text{ microns} ; \; P < 0.01 \\
r = 0.73 , \; P < 0.01
$$

Wall total thickness: data for linear regression where $Y =$ uterine wall total thickness in microns and $X =$ combined ovarian weights in gm.

$$
N = 30 \\
Y = 236.80 + 1186.40 X ; \; s_y = 378.80 \text{ microns} \\
b = 1186.40 \pm 156.19 \text{ microns/gm}, \; P < 0.01 \\
\bar{Y} = 955.00 \pm 69.16 \text{ microns} ; \; P < 0.01 \\
r = 0.82 , \; P < 0.01
$$
Uterine wall layers in hundreds of microns

Ovarian weight in grams

Muscle Total

Wall Total

0.5 1.0 1.5
Graph 11. Muscle total thickness and uterine wall total thickness in 74 dogs with combined ovarian weights ranging from 0.13 to 9.42 gm.

Muscle total thickness; data for linear regression where $Y =$ uterine muscle total thickness in microns and $X =$ combined ovarian weights in gm.

\[
N = 74
\]
\[
Y = 671.50 + 156.74 X ; \quad s_Y = 545.26 \text{ microns}
\]
\[
b = 156.74 \pm 41.12 \text{ microns/gm}, \quad P < 0.01
\]
\[
\bar{Y} = 906.00 \pm 63.39 \text{ microns}, \quad P < 0.01
\]
\[
r = 0.41, \quad P < 0.01
\]

Wall total thickness; data for linear regression where $Y =$ uterine wall total thickness in microns and $X =$ combined ovarian weights in gm.

\[
N = 74
\]
\[
Y = 1192.93 + 186.31 X ; \quad s_Y = 711.51 \text{ microns}
\]
\[
b = 186.31 \pm 53.66 \text{ microns/gm}, \quad P < 0.01
\]
\[
\bar{Y} = 1472.00 \pm 82.71 \text{ microns}, \quad P < 0.01
\]
\[
r = 0.38, \quad P < 0.01
\]
UTERINE MUSCLE THICKNESS (○)

UTERINE WALL THICKNESS (●)

OVARIAN WEIGHT IN GRAMS
Graph 12. Internal circular, vascular layer and external longitudinal muscle thickness in 44 dogs with uterine epithelial height ranging from 7 microns to 40 microns.

Internal circular muscle thickness; data for linear regression where $Y = \text{uterine internal circular muscle thickness in microns}$ and $X = \text{uterine epithelial height in microns}$.

$$
N = 44 \\
Y = 221.07 + 10.80 \times X; \\ s_Y = 199.56 \text{ microns} \\
b = 10.80 \pm 3.09 \text{ microns/micron}; \\ P < 0.01 \\
\bar{Y} = 419.00 \pm 30.08 \text{ microns}; \\ P < 0.01 \\
r = 0.48; \\ P < 0.01
$$

Vascular layer thickness; data for linear regression where $Y = \text{uterine vascular layer thickness in microns}$ and $X = \text{uterine epithelial height in microns}$.

$$
N = 44 \\
Y = 260.40 + 8.31 \times X; \\ s_Y = 151.12 \text{ microns} \\
b = 8.31 \pm 2.03 \text{ microns/micron}; \\ P < 0.01 \\
\bar{Y} = 413.00 \pm 19.77 \text{ microns}; \\ P < 0.01 \\
r = 0.53; \\ P < 0.01
$$

External longitudinal muscle thickness; data for linear regression where $Y = \text{uterine external longitudinal muscle thickness in microns}$ and $X = \text{uterine epithelial height in microns}$.

$$
N = 44 \\
Y = 201.76 + 9.00 \times X; \\ s_Y = 157.00 \text{ microns} \\
b = 9.00 \pm 2.43 \text{ microns/micron}; \\ P < 0.01 \\
\bar{Y} = 367.00 \pm 23.67 \text{ microns}; \\ P < 0.01 \\
r = 0.50; \\ P < 0.01
$$
INTERNAL CIRCULAR MUSCLE (○)

VASCULAR LAYER (○)

EXTERNAL LONGITUDINAL MUSCLE (△)

UTERINE EPITHELIAL HEIGHT IN MICRONS

MUSCLE IN HUNDREDS OF MICRONS

10

9

8

7

6

5

4

3

2

1

0

10 15 20 25 30 35 40
Graph 13. External longitudinal muscle, uterine muscle total and uterine wall total thicknesses in 30 dogs with vascular thicknesses ranging from 28 microns to 525 microns.

External longitudinal muscle thickness; data for linear regression where Y = uterine external longitudinal muscle thickness in microns and X = uterine vascular thickness in microns.

\[ Y = -79.79 + 1.56X; \quad s_y = 75.91 \text{ microns} \]
\[ b = 1.56 \pm 0.14 \text{ microns/micron}, \quad P < 0.01 \]
\[ Y = 137.00 \pm 13.86 \text{ microns}; \quad P < 0.01 \]
\[ r = 0.90; \quad P < 0.01 \]

Muscle total thickness; data for linear regression where Y = uterine muscle total thickness in microns and X = uterine vascular layer thickness in microns.

\[ Y = -174.16 + 4.60X; \quad s_y = 135.49 \text{ microns} \]
\[ b = 4.60 \pm 0.26 \text{ microns/micron}, \quad P < 0.01 \]
\[ Y = 465.00 \pm 24.74 \text{ microns}; \quad P < 0.01 \]
\[ r = 0.96; \quad P < 0.01 \]

Wall total thickness; data for linear regression where Y = uterine wall total thickness in microns and X = uterine vascular layer thickness in microns.

\[ Y = 122.97 + 5.99X; \quad s_y = 293.51 \text{ microns} \]
\[ b = 5.99 \pm 0.56 \text{ microns/micron}, \quad P < 0.01 \]
\[ Y = 955.00 \pm 53.59 \text{ microns}; \quad P < 0.01 \]
\[ r = 0.90; \quad P < 0.01 \]
EXTERNAL LONGITUDINAL MUSCLE (○)
UTERINE MUSCLE TOTAL THICKNESS (○)
UTERINE WALL TOTAL THICKNESS (△)
Graph 14. External longitudinal muscle, uterine muscle total and uterine wall total thickness in 44 dogs with vascular layer thicknesses ranging from 163 microns to 788 microns.

External longitudinal muscle thickness; data for linear regression where \( Y = \) uterine external longitudinal muscle thickness in microns and \( X = \) uterine vascular thickness in microns.

\[
N = 44 \\
Y = 115.87 + 0.61 \times X \; ; \; \sigma_Y = 154.36 \text{ microns} \\
b = 0.61 \pm 0.15 \text{ microns/micron}, \; P \leq 0.01 \\
\bar{Y} = 367.00 \pm 23.27 \text{ microns} , \; P \leq 0.01 \\
r = 0.52 , \; P \leq 0.01
\]

Muscle total thickness; data for linear regression where \( Y = \) uterine muscle total thickness in microns and \( X = \) uterine vascular layer thickness in microns.

\[
N = 44 \\
Y = 292.53 + 2.22 \times X \; ; \; \sigma_Y = 330.52 \text{ microns} \\
b = 2.22 \pm 0.33 \text{ microns/micron}, \; P \leq 0.01 \\
\bar{Y} = 1207.00 \pm 49.83 \text{ microns} , \; P \leq 0.01 \\
r = 0.72 , \; P \leq 0.01
\]

Wall total thickness; data for linear regression where \( Y = \) uterine wall total thickness in microns and \( X = \) uterine vascular layer thickness in microns.

\[
N = 44 \\
Y = 819.33 + 2.44 \times X \; ; \; \sigma_Y = 507.00 \text{ microns} \\
b = 2.44 \pm 0.50 \text{ microns/micron}, \; P \leq 0.01 \\
\bar{Y} = 1824.00 \pm 76.43 \text{ microns} , \; P \leq 0.01 \\
r = 0.60 , \; P \leq 0.01
\]
UTERINE EXTERNAL LONGITUDINAL MUSCLE THICKNESS,
UTERINE MUSCLE THICKNESS AND UTERINE WALL
THICKNESS IN HUNDREDS OF MICRONS

- UTERINE VASCULAR LAYER IN HUNDREDS OF MICRONS
- UTERINE MUSCLE THICKNESS (•)
- UTERINE WALL THICKNESS (○)
- EXTERNAL LONGITUDINAL MUSCLE (△)
Graph 15. External longitudinal muscle, uterine muscle total and uterine wall total thickness in 74 dogs with vascular layer thickness ranging from 28 microns to 788 microns.

External longitudinal muscle thickness; data for linear regression where $Y =$ uterine external longitudinal muscle thickness in microns and $X =$ uterine vascular thickness in microns.

$$N = 74$$
$$Y = 24.57 + 0.83X \quad ; \quad s_y = 137.40 \text{ microns}$$
$$b = 0.83 \pm 0.08 \text{ microns/micron}, \quad P < 0.01$$
$$\bar{Y} = 274.00 \pm 15.97 \text{ microns} , \quad P < 0.01$$
$$r = 0.75 \quad , \quad P < 0.01$$

Muscle total thickness; data for linear regression where $Y =$ uterine muscle total thickness in microns and $X =$ uterine vascular layer thickness in microns.

$$N = 74$$
$$Y = 85.85 + 2.72X \quad ; \quad s_y = 296.53 \text{ microns}$$
$$b = 2.72 \pm 0.18 \text{ microns/micron}, \quad P < 0.01$$
$$\bar{Y} = 906.00 \pm 34.47 \text{ microns} , \quad P < 0.01$$
$$r = 0.87 \quad , \quad P < 0.01$$

Wall total thickness; data for linear regression where $Y =$ uterine wall total thickness in microns and $X =$ uterine vascular layer thickness in microns.

$$N = 74$$
$$Y = 511.05 + 3.19X \quad ; \quad s_y = 470.60 \text{ microns}$$
$$b = 3.19 \pm 0.29 \text{ microns/micron}, \quad P < 0.01$$
$$\bar{Y} = 1472.00 \pm 54.70 \text{ microns} , \quad P < 0.01$$
$$r = 0.79 \quad , \quad P < 0.01$$
EXTERNAL LONGITUDINAL MUSCLE THICKNESS (•)
UTERINE MUSCLE THICKNESS (○)
UTERINE WALL THICKNESS (▲)

UTERINE VASCULAR THICKNESS IN HUNDREDS OF MICRONS

UTERINE EXTERNAL LONGITUDINAL MUSCLE MUSCLE
THICKNESS AND WALL THICKNESS IN HUNDREDS OF MICRONS

0.28 1 2 3 4 5 6 7 8
UTERINE VASCULAR THICKNESS IN HUNDREDS OF MICRONS
APPENDIX C

PHOTOMICROGRAPHS
Figure 1. 
Section through cortex and medulla. 
Smooth outer contour. Septate fingers extending through cortex. 
Reticulin stain. 
X 40.

Figure 2. 
Periodic acid Schiff stain. 
X 1000.

Figure 3. 
Dog No. 060. 2 days. Ovary. 
Cortex divided by collagen bands. 
Large medulla. 
Verhoeff's and Van Gieson's stain. 
X 40.

Figure 4. 
Dog No. 060. 2 days. Ovary. 
Cortex with various sizes of oogonia separated by collagen fibers. 
Verhoeff's and Van Gieson's stain. 
X 250.

Figure 5. 
Dog No. 060. 2 days. Ovary. 
Columnar to cuboidal germinal epithelium. Tunica albuginea. 
Various sizes oogonia separated by collagen fibers. 
Verhoeff's and Van Gieson's stain. 
X 1000.

Figure 6. 
Germinal epithelium one to 2 cells in thickness. Basement membrane. Degenerated follicular remnants. 
Periodic acid Schiff stain. 
X 1000.

Figure 7. 
Cortex containing primordial and primary follicles. 
Mallory's triple stain. 
X 400.

Figure 8. 
Dog No. 072. 2.5 months. Ovary. 
Cortex, Invagination of germinal epithelium 3 cells in thickness. Tunica albuginea fibers intermixed with cortical fibers. 
Hematoxylin and eosin stain. 
X 400.
Figure 9.
Dog No. 059. 2.75 months. Ovary.
Tunica albuginea thickened at pole.
Dark stained cells in cortical stroma.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 10.
Dog No. 059. 2.75 months. Ovary.
Germinal epithelium, vertical, low columnar cells. Clumps of cell separated from germinal epithelium.
Hematoxylin and eosin stain.
X 250.

Figure 11.
Dog No. 059. 2.75 months. Ovary.
Young growing follicles, oogonia.
Hematoxylin and eosin stain.
X 250.

Figure 12.
Dog No. 049. 5.5 months. Ovary.
Growing follicle with zona pellucida, granulosa cells and a theca interna.
X 250.

Figure 13.
Dog No. 049. 5.5 months. Ovary.
Growing follicle with antrum, infolded granulosa and a dense theca interna.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 14.
Dog No. 049. 5.5 months. Ovary.
Cortex. Numerous growing follicles.
Hematoxylin and eosin stain.
X 100.

Figure 15.
Dog No. 061. 6 months. Ovary.
Triovular follicle, zona pellucida, theca interna, thecal cone.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 16.
Dog No. 061. 6 months. Ovary.
Cortex. Follicles separated by fibers perpendicular to the ovarian surface. Mild interna thecosis. Definite cortico-medullary junction.
Verhoeff's and Van Gieson's stain.
X 100.
Figure 17.
Dog No. B61. 7 months. Ovary.
Cortex. Atretic follicle with hyaline ring near theca interna.
Growing follicles.
Verhoeff's and Van Gieson's stain. X 100.

Figure 18.
Dog No. A46. 9 months. Ovary.
Cortex. Prominent tunica albuginea.
Growing follicles. Stromal thecosis.
Verhoeff's and Van Gieson's stain. X 100.

Figure 19.
Dog No. B79. 10 months. Ovary.
Verhoeff's and Van Gieson's stain. X 100.

Figure 20.
Dog No. C8. 11 months. Ovary.
Verhoeff's and Van Gieson's stain. X 100.

Figure 21.
Dog No. C8. 11 months. Ovary.
Verhoeff's and Van Gieson's stain. X 250.

Figure 22.
Dog No. C8. 11 months. Ovary.
Verhoeff's and Van Gieson's stain. X 400.

Figure 23.
Mallory's triple stain. X 100.

Figure 24.
Dog No. 032. 3.1 years. Ovary.
Verhoeff's and Van Gieson's stain. X 100.
Figure 25.
Dog No. 017. 3.3 years. Ovary.
Cortex. Thick tunica albuginea.
Numerous follicular remnant cells.
Cyst. Regressed corpus luteum.
Cyclic sclerosis.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 26.
Dog No. 017. 3.3 years. Ovary.
Follicular remnant cells.
Hematoxylin and eosin stain.
X 100.

Figure 27.
Dog No. D27. 6.1 years. Ovary.
Cortex invaginated. Lobulated ovary. Regressed corpus luteum.
Atretic follicles.
Verhoeff's and Van Gieson's stain.
X 40.

Figure 28.
Dog No. D27. 6.1 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 29.
Dog No. B117. 6.4 years. Ovary.
Reticulin stain.
X 100.

Figure 30.
Dog No. 7BA. 6.4 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 31.
Dog No. B117. 6.4 years. Ovary.
Periodic acid Schiff stain.
X 100.

Figure 32.
Dog No. B117. 6.4 years. Ovary.
Cortex. Germinal epithelium.
Basement membrane.
Periodic acid Schiff stain.
X 1000.
Figure 35.  
Dog No. 7BA, 6,4 years. Ovary.  
Cortex. Granulosa cell islands.  
Zona pellucida.  
Periodic acid Schiff stain.  
X 250.

Figure 34.  
Dog No. 1F. 6,6 years. Ovary.  
Cortex. Follicular remnant cells.  
Verhoeff's and Van Gieson's stain.  
X 100.

Figure 55.  
Dog No. B18, 6,5 years. Ovary.  
Cortex. Atretic follicle with zona pellucida and hyaline membrane.  
Periodic acid Schiff stain.  
X 100.

Figure 36.  
Dog No. M47. 8,3 years. Ovary.  
Cortex lobulated. Combined thecosis.  
Dark stained germinal epithelium.  
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 37.  
Dog No. M47. 8,3 years. Ovary.  
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 38.  
Dog No. M47. 8,3 years. Ovary.  
Verhoeff's and Van Gieson's stain.  
X 250.

Figure 39.  
Dog No. M53. 9 years. Ovary.  
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 40.  
Dog No. M53. 9 years. Ovary.  
Verhoeff's and Van Gieson's stain.  
X 100.
Figure 41.

Figure 42.

Figure 43.

Figure 44.

Figure 45.

Figure 46.

Figure 47.

Figure 48.
Figure 49.
Dog No. B64. 10 years. Ovary.
Cortex. Combined thecosis.
Verhoeff's and Van Gieson's stain. X 100.

Figure 50.
Dog No. B64. 10 years. Ovary.
Growing follicles.
Verhoeff's and Van Gieson's stain. X 100.

Figure 51.
Dog No. M5. 10.2 years. Ovary.
Cysts.
Verhoeff's and Van Gieson's stain. X 40.

Figure 52.
Dog No. M22. 11 years. Ovary.
Cortico-medullary junction. Decreased follicular activity.
Edematous-like area.
Verhoeff's and Van Gieson's stain. X 100.

Figure 53.
Dog No. M22. 11 years. Ovary.
Cortico-medullary junction.
Edematous-like area. Macrophages.
Verhoeff's and Van Gieson's stain. X 400.

Figure 54.
Dog No. M22. 11 years. Ovary.
Cortico-medullary junction. Edematous-like area. Macrophages.
Periodic acid Schiff stain. X 400.

Figure 55.
Dog No. M42. 11.2 years. Ovary.
Verhoeff's and Van Gieson's stain. X 100.

Figure 56.
Dog No. M45. 12 years. Ovary.
Verhoeff's and Van Gieson's stain. X 100.
Figure 57. Dog No. B73. 11.9 years. Ovary. Medulla. Cystic rete ovarii with hyalinized collagen. Verhoeff's and Van Gieson's stain. X 100.


Figure 60. Dog No. M32. 13.1 years. Ovary. Cortex. Mucopolysaccharide secretion in germinal epithelium. Periodic acid Schiff stain. X 100.


Figure 63. Dog No. B105. 12 hours. Ovary. Cortex. Fibers parallel with outer ovary. Reticulin stain. X 100.

Figure 64. Dog No. B60. 2 days. Ovary. Medulla. Collagen fibers among arterioles and fibroblasts. Verhoeff's and Van Gieson's stain. X 400.
Figure 65.

Figure 66.

Figure 67.
Dog No. 059. 2.75 months. Ovary. Medulla. Some vessels engorged others empty. Hematoxylin and eosin stain. X 100.

Figure 68.

Figure 69.

Figure 70.

Figure 71.

Figure 72.
Figure 73.
Dog No. C8. 11 months. Ovary.
Medulla. Cyclic sclerosis.
Vessels near regressed corpus luteum.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 74.
Dog No. C8. 11 months. Ovary.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 75.
Dog No. 640. 1.3 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 76.
Dog No. B96. 1.4 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 77.
Dog No. B24. 2.3 years. Ovary.
Medulla. Artery. Reticulin just beneath internal elastic membrane and in tunica media.
Reticulin stain.
X 400.

Figure 78.
Dog No. B24. 2.3 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 400.

Figure 79.
Dog No. B24. 2.3 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 80.
Dog No. 032. 3.1 years. Ovary.
Verhoeff's and Van Gieson's stain.
X 100.
Figure 81.

Figure 82.

Figure 83.

Figure 84.

Figure 85.

Figure 86.

Figure 87.

Figure 88.
Figure 89. Dog No. B42, 8.6 years. Ovary. Medulla. Dense collagen, elastic fibers and stromal cells. Verhoeff's and Van Gieson's stain. X 250.


Figure 94. Dog No. M29, 9 years. Ovary. Medulla. Rete ovarii with amyloid. Periodic acid Schiff stain. X 400.


Figure 97.

Figure 98.

Figure 99.

Figure 100.

Figure 101.

Figure 102.

Figure 103.
Dog No. M43. 11.4 years. Ovary. Medulla. Same as Figure 102. Periodic acid Schiff stain. X 400.

Figure 104.
Dog No. M43. 11.4 years. Ovary. Medulla. Same as Figure 102. Reticulin stain. X 400.
Figure 105.
Dog No. 060. 2 days. Oviduct.
Wall. Uniform epithelium.
Indistinct boundary between lamina propria and muscularis.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 106.
Dog No. 065. 1 week. Oviduct.
Isthmus. Poor vascularization.
Hematoxylin and eosin stain.
X 100.

Figure 107.
Dog No. 065. 1 week. Oviduct.
Infundibulum. Vascularization more pronounced than in isthmus.
Hematoxylin and eosin stain.
X 100.

Figure 108.
Dog No. 065. 1 week. Oviduct.
Hematoxylin and eosin stain.
X 400.

Figure 109.
Dog No. 072. 2.5 months. Oviduct.
Wall. Increased density of growing lamina propria. Increased collagen content.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 110.
Dog No. 072. 2.5 months. Oviduct.
Epithelium. Three cell types.
Lamina propria with more dense collagen.
Verhoeff's and Van Gieson's stain.

Figure 111.
Dog No. 049. 5.5 months. Oviduct.
Wall. Estrous. Increased thickness of lamina propria.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 112.
Dog No. 049. 5.5 months. Oviduct.
Epithelium. Estrous.
Verhoeff's and Van Gieson's stain.
X 1000.
Figure 113.

Figure 114.

Figure 115.

Figure 116.

Figure 117.

Figure 118.

Figure 119.

Figure 120.
Dog No. 053. 2.7 years. Oviduct. Wall. Increased density of nuclei in muscularis. Verhoeff’s and Van Gieson’s stain. X 250.
Figure 121.  

Figure 122.  

Figure 123.  

Figure 124.  

Figure 125.  

Figure 126.  

Figure 127.  

Figure 128.  
Figure 129.

Figure 130.

Figure 131.

Figure 132.

Figure 133.

Figure 134.

Figure 135.

Figure 136.
Figure 137.
Dog No. 060, 2 days. Uterus.
Epithelium, small indentations.
Indistinct boundary between lamina propria and muscularis.
Hematoxylin and eosin stain.
X 40.

Figure 138.
Dog No. 060, 2 days. Uterus.
Wall. Pseudostratified columnar epithelium. Indistinct boundary between lamina propria and muscularis.
Hematoxylin and eosin stain.
X 250.

Figure 139.
Dog No. 060, 2 days. Uterus.
Wall. Reticulin fibers sparse in lamina propria.
Reticulin stain.
X 250.

Figure 140.
Dog No. 060, 2 days. Uterus.
Pseudostratified columnar epithelium. Definite basement membrane.
Periodic acid Schiff stain.
X 400.

Figure 141.
Early invagination of uterine glands. Early muscle layer differentiation.
Hematoxylin and eosin stain.
X 100.

Figure 142.
Increased reticulin in lamina propria.
Reticulin stain.
X 100.

Figure 143.
Dog No. 072, 2.5 months. Uterus.
Wall. Epithelial glands deeper.
Three layers to muscularis.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 144.
Dog No. 049, 5.5 months. Uterus.
Wall. Glands down to muscularis.
Prominent muscle layers.
Verhoeff's and Van Gieson's stain.
X 40.
Figure 145. Dog No. 049. 5.5 months. Uterus. Dense reticular network in all parts of uterine wall. Especially circular muscle. Reticulin stain. X 40.

Figure 146. Dog No. 049. 5.5 months. Uterus. Wall. Dense mature type tissue. Verhoeff's and Van Gieson's stain. X 100.

Figure 147. Dog No. 049. 5.5 months. Uterus. Epithelium pseudostratified columnar. Secretion in glands and uterine lumen. Incomplete basement membrane. Periodic acid Schiff stain. X 250.

Figure 148. Dog No. B66. 6.5 months. Uterus. Internal circular muscle penetrated by artery from vascular layer. Verhoeff's and Van Gieson's stain. X 250.

Figure 149. Dog No. M56. 7.1 months. Uterus. Vascular layer. Localized intimal change. Verhoeff's and Van Gieson's stain. X 400.

Figure 150. Dog No. A46. 9 months. Uterus. Wall. Longitudinal ridge of tissue opposite mesometrial border. Verhoeff's and Van Gieson's stain.


Figure 153.
Dog No. C17. 11 months. Uterus.
Lamina propria 2 weeks postpartum. Sloughing epithelium.
Involution incomplete.
Periodic acid Schiff stain.
X 100.

Figure 154.
Dog No. C17. 11 months. Uterus.
Vascular layer. Cyclic sclerosis.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 155.
Dog No. C17. 11 months. Uterus.
Epithelium, popcorn. 2 weeks postpartum.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 156.
Wall. 3 weeks postpartum.
Hyalinized mass of collagen in lamina propria.
Verhoeff's and Van Gieson's stain.
X 25.

Figure 157.
Wall. 3 weeks postpartum.
Hyalinized mass of collagen in lamina propria.
Verhoeff's and Van Gieson's stain.
X 40.

Figure 158.
Same as figure 157.
Reticulin stain.
X 40.

Figure 159.
Same as figure 157.
Periodic acid Schiff stain.
X 40.

Figure 160.
Dog No. 2BD. 1.1 year. Uterus.
Wall. 1 month postpartum.
Cystic endometrial hyperplasia.
Verhoeff's and Van Gieson's stain.
X 40.
Figure 161.
Dog No. 2BD. 1.1 year. Uterus.
1 month postpartum.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 162.
Dog No. 499. 1.2 years. Uterus.
Muscularis. Arteries surrounded by collagen. Intimal thickening.
Verhoeff's and Van Gieson's stain.

Figure 163.
Dog No. 053. 2.7 years. Uterus.
Wall. 3 months postpartum.
Hemosiderin. Prominent vascular layer.
Periodic acid Schiff stain.
X 40.

Figure 164.
Dog No. 053. 2.7 years. Uterus.
Epithelium and lamina propria.
As figure 163.
Periodic acid Schiff stain.
X 100.

Figure 165.
Dog No. 053. 2.7 years. Uterus.
Vascular layer. Collagen infiltration of thickened intima.
Elastoid.
Verhoeff's and Van Gieson's stain.

Figure 166.
Dog No. 053. 2.7 years. Uterus.
Vascular layer. As figure 165.
Hematoxylin and eosin stain.
X 100.

Figure 167.
Dog No. B87. 3.1 years. Uterus.
Wall. Folded external longitudinal muscle.
Verhoeff's and Van Gieson's stain.
X 25.

Figure 168.
Dog No. B87. 3.1 years. Uterus.
Epithelium and lamina propria.
Proliferative stage.
Verhoeff's and Van Gieson's stain.
X 100.
Figure 169.

Figure 170.

Figure 171.

Figure 172.

Figure 173.

Figure 174.

Figure 175.

Figure 176.
Figure 177.
Dog No. M29. 9 years. Uterus. 
Lamina propria. Cystic endometrial hyperplasia. 
Verhoeff's and Van Gieson's stain. X 100.

Figure 178.
Dog No. M29. 9 years. Uterus. 
As figure 177. Periodic acid Schiff stain. X 100.

Figure 179.
Dog No. M29. 9 years. Uterus. 
As figure 177. Basement membrane. 
Periodic acid Schiff stain. X 400.

Figure 180.
Dog No. M40. 9.7 years. Uterus. 
Endometrial hyperplasia. Increased collagen and elastic fibers in muscularis. 
Verhoeff's and Van Gieson's stain. X 40.

Figure 181.
Dog No. M50. 10.3 years. Uterus. 
Hematoxylin and eosin stain. X 25.

Figure 182.
Dog No. M5. 10.2 years. Uterus. 
Internal circular muscle. Focal endometrioses (adenomyosis). 
Hematoxylin and eosin stain. X 250.

Figure 183.
Dog No. M46. 10 years. Uterus. 
Endometrial hyperplasia. 
Verhoeff's and Van Gieson's stain. X 100.

Figure 184.
Dog No. B64. 10 years. Uterus. 
Vascular layer. 8 month postpartum. 
Arteries and veins thickened walls. Collagen infiltration and elastoid. 
Intimal thickening. 
Verhoeff's and Van Gieson's stain. X 100.
Figure 185.
Dog No. M30. 11.5 years. Uterus.  
Muscularis. Leiomyoma in external longitudinal muscle.  
Verhoeff's and Van Gieson's stain.  
X 100.

Figure 186.
Dog No. M30. 11.5 years. Uterus.  
As figure 185. Pyknotic nuclei and decreased cytoplasm of longitudinal muscle.  
Verhoeff's and Van Gieson's stain.  
X 400.

Figure 187.
Dog No. B73. 11.9 years. Uterus.  
Internal circular muscle.  
Leiomyoma.  
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 188.
Dog No. B73. 11.9 years. Uterus.  
As figure 187. Whorls evident.  
Verhoeff's and Van Gieson's stain.  
X 100.

Figure 189.
Dog No. B73. 11.9 years. Uterus.  
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 190.
Dog No. M32. 12.8 years. Uterus.  
Epithelium regressed. Glands semi-active.  
Hematoxylin and eosin stain.  
X 250.

Figure 191.
Verhoeff's and Van Gieson's stain.  
X 40.

Figure 192.
Muscularis. Increased density. Arteries surrounded by collagen. Intimal thickening.  
Verhoeff's and Van Gieson's stain.  
X 100.
Figure 193.
Dog No. 060. 2 days. Cervix, Scan. No definite demarcation between lamina propria and muscularis.
Hematoxylin and eosin stain. X 40.

Figure 194.
Dog No. 060. 2 days. Cervix. Wall. Reticulin most prominent in area of future internal circular muscle.
Reticulin stain. X 100.

Figure 195.
Dog No. 060. 2 days. Cervix. Epithelium, pseudostratified. Early indentations.
Hematoxylin and eosin stain. X 250.

Figure 196.
Periodic acid Schiff stain. X 1000.

Figure 197.
Dog No. 065. 1 week. Cervix. Epithelial indentations prominent. Lamina propria more cellular.
Verhoeff's and Van Gieson's stain. X 100.

Figure 198.
Verhoeff's and Van Gieson's stain. X 400.

Figure 199.
Verhoeff's and Van Gieson's stain. X 40.

Figure 200.
Verhoeff's and Van Gieson's stain. X 40.
Figure 201.
Dog No. E05. 3 months. Cervix.
Epithelium. 2 rows of nuclei.
Clear cells. Lamina propria
with mature collagen and
numerous mononuclear cells.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 202.
Dog No. E05. 3 months. Cervix.
Epithelium. Migrating mono-
nuclear cells.
Hematoxylin and eosin stain.
X 1000.

Figure 203.
Dog No. 049. 5.5 months. Cervix.
Cervical pillar with lighter
stained lamina propria.
Edematous.
Verhoeff's and Van Gieson's stain.
X 40.

Figure 204.
Dog No. 049. 5.5 months. Cervix.
Epithelium. Right, cervical
pillar side, dark stained epi-
thermal with edematous lamina
propria. Left, cervical body,
opposite.
Verhoeff's and Van Gieson's stain.
X 400.

Figure 205.
Dog No. B61. 7 months. Cervix.
Epithelium with rete pegs. Dense
lamina propria.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 206.
Dog No. M59. 7.1 months. Cervix.
Epithelium. Cervical pillar side
with proliferations and numerous
clear cells. Cervical pillar
muscle.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 207.
Dog No. 79. 8 months. Cervix.
Epithelium two plus layers of
cells. Estrous.
Hematoxylin and eosin stain.
X 400.

Figure 208.
Dog No. A46. 9 months. Cervix.
Epithelium secretory. Late
estrous or early metestrous.
Verhoeff's and Van Gieson's stain.
X 400.
Figure 209.
Epithelium with proliferation of basal zone. Metestrus.
Verhoeff’s and Van Gieson’s stain.
X 100.

Figure 210.
As figure 209. Mucopolysaccharide secretion. Continuous basement membrane.
Periodic acid Schiff stain.
X 250.

Figure 211.
As figure 209. Numerous migrating leucocytes.
Hematoxylin and eosin stain.
X 400.

Figure 212.
Dog No. B79. 10 months. Cervix.
Epithelium cornified. All zones present. Basement membrane.
Periodic acid Schiff stain.
X 250.

Figure 213.
Dog No. B79. 10 months. Cervix.
Epithelium. Early estrous. No migrating leucocytes in epithelium.
Keratohyaline granules.
Hematoxylin and eosin stain.
X 250.

Figure 214.
Dog No. B79. 10 months. Cervix.
Epithelium. As figure 213. Physiological acumenthesis.
Keratohyaline granules.
Hematoxylin and eosin stain.
X 1000.

Figure 215.
Epithelium infiltrated with leucocytes. Metestrus.
Verhoeff’s and Van Gieson’s stain.
X 250.

Figure 216.
Epithelium degenerated. Late metestrus. Clear cells.
Verhoeff’s and Van Gieson’s stain.
X 250.
Figure 217.

Figure 218.

Figure 219.

Figure 220.

Figure 221.

Figure 222.

Figure 223.

Figure 224.
Figure 225.
Dog No. 499. 1.2 years. Cervix.
Epithelium regenerating. Lamina propria with collagen walled vessels. Late anestrous.
Verhoeff's and Van Gieson's stain. X 250.

Figure 226.
Dog No. 499. 1.2 years. Cervix.
Vascular layer. Late anestrous.
Artery with thick wall. Vein with smooth muscle in wall.
Verhoeff's and Van Gieson's stain. X 100.

Figure 227.
Dog No. B46. 1.4 years. Cervix.
Verhoeff's and Van Gieson's stain. X 100.

Figure 228.
Dog No. 053. 2.7 years. Cervix.
Vascular layer. Arteries and veins with prominent collagen infiltration.
Verhoeff's and Van Gieson's stain. X 100.

Figure 229.
Dog No. 053. 2.7 years. Cervix.
Verhoeff's and Van Gieson's stain. X 400.

Figure 230.
Dog No. 053. 2.7 years. Cervix.
Verhoeff's and Van Gieson's stain. X 1000.

Figure 231.
Dog No. M53. 9 years. Cervix.
Epithelium proliferative. Lamina propria with cystic hyperplasia in cervical pillar.
Verhoeff's and Van Gieson's stain. X 40.

Figure 232.
Dog No. M53. 9 years. Cervix.
Epithelium. As figure 231. With numerous leucocytes. Hematoxylin and eosin stain. X 400.
Figure 233.
Dog No. M29. 9 years. Cervix.
Wall. Dense lamina propria.
Muscularis.
Verhoeff's and Van Gieson's stain. X 40.

Figure 234.
Dog No. M29. 9 years. Cervix.
Verhoeff's and Van Gieson's stain. X 250.

Figure 235.
Dog No. M54. 9 years. Cervix.
Verhoeff's and Van Gieson's stain. X 100.

Figure 236.
Dog No. M54. 9 years. Cervix.
Verhoeff's and Van Gieson's stain. X 250.

Figure 237.
Dog No. M40. 9.7 years. Cervix.
Hematoxylin and eosin stain. X 250.

Figure 238.
Dog No. M40. 9.7 years. Cervix.
Verhoeff's and Van Gieson's stain. X 250.

Figure 239.
Dog No. B33. 9.1 years. Cervix.
Muscularis. Increased density of muscles. Edematous vascular layer. Late anestrous.
Verhoeff's and Van Gieson's stain. X 40.

Figure 240.
Dog No. M50. 10.3 years. Cervix.
Cervical pillar. Increased density. Infiltration of muscle with collagen.
Verhoeff's and Van Gieson's stain. X 100.
Figure 241.  
Dog No. M43. 11.4 years. Cervix. 
Muscularis. All layers with increased density of collagen infiltration. 
Verhoeff's and Van Gieson's stain. X 40.

Figure 242.  
Cervical pillar. Collagen infiltration between muscle cells. 
Verhoeff's and Van Gieson's stain. X 100.

Figure 243.  
Dog No. M30. 11.5 years. Cervix.  
Vascular layer. Large vein with prominent collagen infiltration. 
External longitudinal muscle with pyknotic nuclei.  
Verhoeff's and Van Gieson's stain. X 250.

Figure 244.  
Dog No. M30. 11.5 years. Cervix.  
As figure 243.  
Periodic acid Schiff stain. X 250.

Figure 245.  
Periodic acid Schiff stain. X 1000.

Figure 246.  
Verhoeff's and Van Gieson's stain. X 100.

Figure 247.  
As figure 246. Collagen walled vessel. Elastic fibers in lamina propria.  
Verhoeff's and Van Gieson's stain. X 400.

Figure 248.  
Muscularis. Hypertrophied muscle cells. Much elastic tissue in collagen of the vascular layer.  
Verhoeff's and Van Gieson's stain. X 400.
Figure 249.
Dog No. 060, 2 days. Vagina.
Wall. Early indentation of epithelium. Early differentiation of lamina propria from muscularis.
Verhoeff's and Van Gieson's stain. X 100.

Figure 250.
Dog No. 060, 2 days. Vagina.
Epithelium pseudostratified. Hematoxylin and eosin stain. X 400.

Figure 251.
Dog No. 060, 2 days. Vagina.
As figure 250. Continuous basement membrane.
Periodic acid Schiff stain. X 400.

Figure 252.
Dog No. 065, 1 week. Vagina.
Scan. Early differentiation of layers.
Verhoeff's and Van Gieson's stain. X 40.

Figure 253.
Dog No. 065, 1 week. Vagina.
Epithelium no change. Increased cellularity of lamina propria.
Hematoxylin and eosin stain. X 250.

Figure 254.
Wall. Increased density and easy differentiation of all layers.
Verhoeff's and Van Gieson's stain. X 250.

Figure 255.
Wall. Mature type morphology.
Verhoeff's and Van Gieson's stain. X 40.

Figure 256.
Dog No. 049, 5.5 months. Vagina.
Wall. All layers well differentiated. Collagen walled vessels in lamina propria.
Verhoeff's and Van Gieson's stain. X 40.
Figure 257.

Figure 258.

Figure 259.

Figure 260.

Figure 261.

Figure 262.

Figure 263.

Figure 264.
Figure 265.
Dog No. C17. 11 months. Vagina.
Wall. Concentration of reticulin under epithelium. 2 weeks postpartum.
Reticulin stain.
X 40.

Figure 266.
Dog No. C17. 11 months. Vagina.
Outer lamina propria with collagen walled vessels. 2 weeks postpartum.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 267.
Dog No. C17. 11 months. Vagina.
As figure 265.
Periodic acid Schiff stain.
X 40.

Figure 268.
Dog No. C17. 11 months. Vagina.
Epithelium with concentration of mucopolysaccharides. 2 weeks postpartum.
Periodic acid Schiff stain.
X 400.

Figure 269.
Lamina propria densely cellular.
3 weeks postpartum.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 270.
Vascular layer. As figure 269.
Artery with intimal alterations.
Verhoeff's and Van Gieson's stain.
X 250.

Figure 271.
Dog No. 499. 1.2 years. Vagina.
Lamina propria with collagen embedded vessels. Anestrous.
Verhoeff's and Van Gieson's stain.
X 100.

Figure 272.
Dog No. 053. 2.7 years. Vagina.
Lamina propria with collagen embedded vessels. Anestrous.
Verhoeff's and Van Gieson's stain.
X 100.
Figure 273.  

Figure 274.  

Figure 275.  

Figure 276.  
Dog No. 7BA, 6.4 years. Vagina. Vascular layer. Arteries with well stained internal elastic membrane and intimal changes. 3.5 months postpartum. Verhoeff's and Van Gieson's stain. X 250.

Figure 277.  

Figure 278.  

Figure 279.  

Figure 280.  
Figure 281.  
Dog No. M40. 9.7 years. Vagina.  
Wall. Lamina propria dense with reticulin fibers. Estrous.  
Reticulin stain. X 40.

Figure 282.  
Dog No. M40. 9.7 years. Vagina.  
As figure 281. Estrous.  
Verhoeff's and Van Gieson's stain. X 100.

Figure 283.  
Dog No. M40. 9.7 years. Vagina.  
Epithelium and lamina propria.  
As figure 281. Estrous  
Continuous basement membrane.  
Periodic acid Schiff stain. X 100.

Figure 284.  
Dog No. M54. 9 years. Vagina.  
Epithelium sloughing. Lamina propria edematous with foci of mononuclear cells. Late estrous.  
Hematoxylin and eosin stain. X 100.

Figure 285.  
Dog No. M51. 9.3 years. Vagina.  
Epithelium and lamina propria.  
Much elastoid with the collagen.  
Verhoeff's and Van Gieson's stain. X 250.

Figure 286.  
Dog No. M51. 9.3 years. Vagina.  
Vascular layer increased in thickness. Arteries infiltrated with collagen.  
Verhoeff's and Van Gieson's stain. X 100.

Figure 287.  
Dog No. M46. 10 years. Vagina.  
Wall. Increased cellularity and increased collagen, especially around vessels. Anestrous.  
Verhoeff's and Van Gieson's stain. X 100.

Figure 288.  
Dog No. M46. 10 years. Vagina.  
As figure 287. Definite continuous basement membrane.  
Periodic acid Schiff stain. X 400.
Figure 289.

Figure 290.

Figure 291.

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