AN ELECTRON MICROSCOPE STUDY OF THE VAGINAL EPITHELIUM OF THE DOG

by

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B. S., Kansas State University, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

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INTRODUCTION

The gross changes of vaginal epithelium of animals possessing classical estrous cycles have been known for many years, but the detailed changes observable with the electron microscope have not been closely followed. A single report, published six years ago when electron microscopy was a new research method to most people, has been concerned with the changes of the vaginal epithelium of a cycling animal, the guinea pig.

Although species differences of vaginal epithelium between this animal and the dog are slight, the longer cycle of the dog and the well worked out determination of stages by vaginal smear allow a more exact determination of cellular conditions at different times. The use of the dog as a laboratory animal by physiologists, endocrinologists, and experimental surgeons heightens the need for increased knowledge of its tissue ultrastructure.

The active portion of the estrous cycle occurs after the low lying non-cornified epithelium of the resting stage is stimulated to increase in thickness to a high squamous condition. The inactive anestrous stage persists for nearly five months out of the six month cycle. During the remaining time the epithelium proliferates to the high squamous condition, the cornified portions of the epithelium sloughs, and the epithelium returns to the low level form of anestrus. Breeding occurs during the time of maximum cornification. In this study tissue was examined from dogs undergoing anestrus; proestrus, the period the epithelium is thickening; and estrus, the time cornification persists. The degenerating epithelium of metestrus was very difficult to handle and

satisfactory results are yet to be obtained with it.

LITERATURE REVIEW

Anatomy and Vaginal Smear

The vagina of the dog is the portion of the female genital tube connecting the vestibule and cervix. Miller (1962) suggested that it is about 10 cm long but that this varies greatly. A mucous membrane, consisting of an epithelium supported by the lamina propria, lines the inner surface of the vagina (Bloom and Fawcett, 1962). A longitudinal fold, usually about 5 mm in width and of variable length, extending caudad from the cervix on the dorsal wall of the vagina, is termed the "dorsal ridge" by Miller (1962).

Vaginal changes in the dog were first reported by Retterer (1892). From the study of a few cases, he judged that significant changes occurred during the latter half of gestation. During this period and the four days following parturition he observed a mucous transformation of the vaginal mucosa cells.

Correlation of the histological condition with the contents of the vaginal fluids was first attempted by Stockard and Papanicolaou (1917a, b) working with the guinea pig. Their pioneer studies presented a method whereby the stage of estrus of an animal could be predicted by examining the stained contents of a fluid sample from the vagina.

This method was subsequently applied to the dog by Evans and Cole (1931) to determine coincident changes in the genital organs during the estrous cycle. They demonstrated that characteristic changes occurred in types and numbers

of cells found free in the vaginal lumen corresponding with the estrous stage of the dog. The histological examination of vaginal tissue revealed a proliferation of epithelial cells prior to estrus with the mucosa achieving maximum height at the beginning of heat. Following the onset of this stage desquamation of the vaginal epithelium occurred returning it to the original condition. They described the vaginal epithelium in the resting condition (anestrus) as low columnar or cuboidal. The stage of formation of the high stratified squamous epithelium was referred to as proestrus. Desquamation during the time of estrus resulted in a low columnar condition in metestrus. This cycle occurs on the average of twice yearly according to Mulligan (1952).

Making a further study on this subject, Gier (1960) used heat records from over 200 dogs to ascertain an exact correlation of the vaginal fluid content with the estrous cycle. No consistent difference in the vaginal fluids of the various breeds of dogs was found. Gier divided the estrous cycle into five stages as determined by the condition of the genital tract. Anestrus was defined as the inactive stage persisting from shortly after parturition until the onset of proestrus characterized by enlargement of portions of the genital tract, including the vagina. During estrus, the period the bitch was attractive to and would accept the stud, the vaginal epithelium reached maximum thickness and began desquamation. Metestrus I was considered to be the stage following the termination of the heat period in which regression of symptoms of estrus occurred. Metestrus II was described as the persisting condition following successful breeding until parturition.

Ultrastructure of Vaginal Epithelium

Electron microscope studies of vaginal epithelium have appeared only twice in the literature. One of these investigations involved a species possessing a classic estrous cycle, the guinea pig, and the other concerned man.

Bahr and Mohberger (1956) were the first to report on this topic, with an investigation of human vaginal epithelium. After examining the upper cell layers they described the epithelial cells as a syncytium connected by cytoplasmic bridges. The cytoplasmic bridges were seen to contain poorly defined structures which they suggested were the nodes of Bizozzero of light microscopists. Intercellular spaces were observed between the cytoplasmic bridges. Tonofibrils were present in the cytoplasm in considerable quantity and thicker tonofibrils communicated from cell to cell across the cytoplasmic bridges. Keratinization was not observed in this tissue.

In a later paper, Burgos and Wislocki (1958) reported on the cyclical changes of the vagina of the guinea pig. During estrus the basal cells (stratum germinativum) seemingly were attached to the basement membrane by half-desmosomes. Adjacent cells of both the basal and prickle cell (stratum spinosum) layer appeared to be joined together by periodic thickenings of their membrane, the desmosomes. Even at these points the cells were separated by a small space. The intercellular space between desmosomes was distended and microvilli extended from the cells. Basal and prickle cells appeared similar internally, nuclei were large and lobulated, and

nucleoli were well defined. The endoplasmic reticulum was poorly defined and dense mitochondria were moderately plentiful. The cytoplasm appeared granular and clusters of cytoplasmic fibrils were seen in the peripheral cytoplasm. In the transition cell layer (stratum granulosum) desmosomes were not apparent, microvilli infrequent, the cells flattened, and the cytoplasm filled with dense filaments. The nucleus was irregular or reduced to a dark strand and mitochondria swollen or disintegrating. The outermost cornified cells (stratum corneum) were flattened and contained little other than dense bundles of filaments.

During metestrus Burgos and Wislocki saw the same layers but a decrease in microvilli in lower layers and an increase of bundles of filaments had occurred. By late diestrus (anestrus) the vaginal epithelium was reduced to three layers of cells: a basal layer, an intermediate layer,and an outer mucous layer. These cells were smaller and more flattened than previously and the intercellular spaces were greatly reduced. Bundles of filaments and free ribonucleoprotein were present in lesser amounts but endoplasmic reticulum was now observable. The outermost mucous cells appeared to be filled with droplets developed within the endoplasmic reticulum.

Ultrastructure of Stratified Epithelia

Basic similarities between vaginal epithelium and other stratified squamous epithelia allow an examination of literature concerning other tissues. Although the existence of the following ultrastructures is agreed upon, the terminology, morphology, and make-up are not well settled.

The basement membrane associated with epithelial surfaces has been a source of disagreement among investigators, even before the advent of electron microscopy. The term "basement membrane" was employed by Porter (1954) to designate the dense submicroscopic membrane. Weiss and Ferris (1954) identified the structure only as a "continuous sharp interface" above the "basement lamellae" of collagen. Yamada (1955) applied the term "limiting membrane" for the comparable dense homogeneous membrane between the lamina propria and the epithelium of the gall bladder. Selby (1955) named the membrane associated with human and rodent skin the "dermal membrane" to distinguish it from the broader basement membrane. Singer and Andrew (1959) suggested the use of a new term, the "adepidermal membrane" or the synonym "adepithelial membrane." Zelickson and Hartmann (1962) proposed yet another name, the "subepithelial membrane." Sognnaes and Albright (1956), Odland (1958), Green (1959), Ashworth, Luibel, and Saunders (1960), Bartoszevicz and Dux (1961), Albright and Listgarten (1962), Gibbins (1962), and Listgarten (1964) referred to the structure as the "basement membrane."

Odland (1958) described the basement membrane as a poorly defined membrane from 150 to 400 angstroms in width separated from the dermal surface of the basal cell membrane by a uniform band of less density 300 angstroms thick. Ashworth, Luibel, and Saunders (1960) related that it is clearly visable and non-laminar. Descriptions of thickness vary from 350 angstroms (Selby, 1955) to 300 to 600 angstroms (Gibbins, 1962). A 450 angstrom space between the membrane and basal cells was seen by

Listgarten (1964). No fibers have been seen to cross it.

Porter (1954) observed that the basement membrane consisted of a layer of granules subadjacent to the plasma membrane of the basal cells, a layer of unorganized material below this, and a lower fabric of fine fibrils. Selby (1956) related histochemical studies to her observations in stating that mucopolysaccharides were present in this area. Salpeter and Singer (1959) and most authors since, have described the basement membrane as an amorphous, moderately dense layer separated from the basal cells by a clear space which Ashworth, Luibel, and Saunders (1960) suggest may be a cement substance.

Odland (1958) reported irregular finger-like processes of the basal epidermal cells of skin projecting toward the dermis. Ashworth, Luibel, and Saunders (1960) noted similar structures associated with the cervix uteri. An irregular cell border adjacent to the basement membrane of human gingival epithelium was described by Listgarten (1964), who also saw an occasional small invagination of the inferior cell border reminiscent of pinocytotic vacuoles.

Weiss and Ferris (1954) first described dense thickenings in the inferior basal cell membrane of epidermis, which they called "bobbins." Working with larval amphibian, they described the bobbins as having a heavy electron dense base and top separated by a dense connecting piece. Selby (1955) observed similar dense rodlets associated with human epidermis. Odland (1958) described these as 0.1 to 0.5 microns wide and 0.05 to 0.2 microns apart in human epidermis. Singer and Andrew (1956) and Salpeter and Singer (1959) reported nodular swellings in this area in the newt, Triturus. Thickened areas along the inferior basal cell membrane were seen to give

rise to tonofibrils extending toward the center of the cell by Albright and Listgarten (1962) working with the hamster cheek pouch. In human gingival epithelium, Listgarten (1964) saw thickened areas occurring at intervals of 500 to 2000 angstroms.

A similarity between bobbins and desmosomes has been commented on by several investigators with some referring to the former as half-desmosomes. Setälä, Merenmies, Stjernvall, and Nyholm (1960) described a "desmosome-like" attachment between basal cells and the basement membrane of the interfollicular epidermis of the mouse.

The structure referred to as intercellular bridge or desmosome is usually reported in cell layers above the basal cells and serves to attach cells to each other. Porter (1954) first reported the fine structure of desmosomes, noting dense thickening of opposing areas in epidermal cells of Amblystoma larvae. Selby (1955) demonstrated a similar structure between contiguous cells of human epidermis. In the same tissue Odland (1958) described an attachment plaque on each cell membrane with an intercellular contact layer between. The oval plaques were 100 angstroms thick, about 3000 to 7000 angstroms in diameter and about 350 angstroms apart. Hibbs and Clark (1959) had similar results, noting the appearance of alternate dark and light lamina between the plaques. Karrer (1960) described a desmosome in human cervix with three dense layers between the plaques. Desmosomes have been described by Jakus (1959) in human corneal epithelium, Albright (1960) in human gingiva and cheek mucosa, Ashworth, Luibel, Saunders (1960) in human cervix uteri, and Albright and Listgarten (1962) in hamster cheek pouch epithelium.

The reason for reports of different desmosome structure was indicated to be due to morphologic alteration due to gradual loss of adhesiveness by Listgarten (1964) who examined the different layers of human gingiva. He also stated that present data indicate that the cell membrane is associated with the attachment plaques and the "intercellular contact layer" is indeed extracellular.

A smooth cellular membrane and a small amount of intercellular space was described for human oral mucosa basal cells by Sognnaes and Albright (1956). However, Ashworth, Luibel, and Saunders (1960) reported that intercellular spaces are wider between basal cells than those above in human cervix uteri. Microvilli formed to fill the intercellular spaces of overlying cells of human epidermis according to Odland (1958). Similar alterations occurred in human cervix uteri as stated by Ashworth, Luibel, and Saunders (1960) and in hamster cheek pouch as seen by Albright and Listgarten (1962). Fawcett (1958) and Albright (1960) suggested that these projections may be ridges rather than finger-like. Intercellular space decreased to a narrow cleft in the cornified layer of human epidermis as shown by Brody (1959).

Sognnaes and Albright (1956) reported an amorphous substance between the cells of oral mucosa and presumed it to be of high protein content due to its osmophilia. Similar material is described by Brody (1959), and Ashworth, Luibel, and Saunders (1960), the latter group noting a finely granular composition.

Endoplasmic reticulum is described as being very scarce in human epidermis by Selby (1955). Albright (1960) reported they were not discernable

in human gingiva and cheek mucosa. The general lack of mention of its appearance in epithelial cells by other authors attests to its scarcity.

Mitochondria were abundant in the basal cells but diminished within intermediary and superficial squamous layers of human oral mucosa according to Sognnaes and Albright (1956). The lack of definite internal structure in mitochondria of human gingiva and cheek mucosa was commented on by Albright (1960). They were numerous but not prominent in human cervix uteri studied by Ashworth, Luibel, and Saunders (1960). Transverse type cristae were seen in mitochondria from hamster cheek pouch epithelium studied by Albright and Listgarten (1962).

The Golgi complex of epithelial cells of the epididymis was studied by Dalton and Felix (1954). They noted that in cells showing evidence of secretory or absorptive activity, numerous lamellae were concentrically arranged around the central vacuoles. Elftman (1954) pointed out that the form the Golgi apparatus takes is dependent upon the type of Golgi material present in the cell and the particular chemical environment of the moment. The paucity of Golgi material in epidermis was noted by Selby (1955), the results conflicting with classical silver staining methods which do show a Golgi net, as stated by Montagna (1952). However, recently Zelickson and Hartmann (1962) reported a Golgi apparatus, composed of small vesicles, as well developed and localized at opposite poles of the nucleus in non-keratinizing oral mucosa.

Brody (1960) reported small opaque particles in noncornified epidermial layers of human epidermis which were irregular in shape. Odland (1960)

also mentioned granules in stratum granulosum of human epidermis which disappeared in keratinizing cells and speculated they were degenerating mitochondria. Frei and Sheldon (1961) viewing these small dense bodies in the same tissue suggested they be termed "corpuscula." Albright and Listgarten (1962) reported corpuscula in hamster cheek pouch epithelium and Listgarten (1964) noted the structure in human gingival epithelium.

The chemical components within epithelial cells have received little attention. The presence of lipid droplets within the epithelial cells of epididymis and duodenum is mentioned by Dalton and Felix (1954). Ashworth, Luibel, and Saunders (1960) interpreted moderately dense finely granular material in the cytoplasm of basal cells of human cervix uteri as glycogen. These became more abundant and large and produced a halo like zone around the nucleus in overlying precornified cells. Selby (1955) and Albright (1960) spoke of small particulate matter interspersed throughout human epidermis and human gingiva and cheek mucosa cells respectively. Albright and Listgarten (1962) described fine granules in hamster cheek pouch epithelium which they suggested may represent ribonucleoprotein.

Early workers believed tonofibrils existed solely for the maintenance of epithelial integrity and this accounted for their name, according to Green (1959). Although tonofibrils are found throughout the cytoplasm, Odland (1958) noted that some appeared to attach themselves to the desmosome plaques of human epidermis. Albright and Listgarten (1962) reported that tonofibrils arise from the bobbins of hamster cheek pouch epithelium as well. Singer and Andrew (1956) observed fibrillae to arise from the bobbins of regenerating

skin of Trituris.

Tonofibrils were loosely woven in basal cells of human gingiva, according to Listgarten (1964). In the upper stratum spinosum cells of gingiva and oral mucosa tonofibrils became prevalent, Albright (1960) reported, even to the extent that other cytoplasmic organelles seemed to be pushed aside. In the cornified layer of guinea pig skin, the cells contained little other than tonofibrils (Brody, 1959).

The fine filaments were estimated to be 60 angstroms in diameter by Porter (1954), and Menefee (1957) described alternating densities along the tonofilaments suggesting granularity. Selby (1955) identified the submicroscopic constituents of tonofibrils and called them tonofilaments. He believed they represented the keratin-like fibrous protein epidermin, described by Rudall (1952). Selby (1956) stated that the tonofilaments were the site of the alpha type fibrous protein as identified by x-ray diffraction. Rogers and Filshie (1962) interpreted the tonofibrils as consisting of protofibrils, about 20 angstroms in diameter, arranged in a manner similar to the "9+2" filament pattern of cilia.

Keratinization occurs in most types of stratified squamous epithelium and many theories of how it occurs are available. Menefee (1957) felt there was a relationship of the mitochondria to keratinization because of an apparent continuity of tonofibrils with the mitochondria. Corpuscula were seen as normal components of keratinizing epithelia by Frei and Sheldon (1961) and were thought to play some role in the process. Sognnaes and Albright (1956, 1958) expressed belief that the nuclei held an important position in

keratinization due to noted foldings of the nuclear membrane, nuclear fragmentation, and formation of complexes near the nuclei in keratinizing tissue.

The role of tonofibrils in keratinization is especially actively debated. The alpha-keratin x-ray diffraction patterns of the fibrils, described by Rudall (1952) lends support to the theory. Brody (1959) advanced a hypothesis that keratin is formed from tonofilaments and keratohyaline granules, through a gradual incorporation of tonofilaments into keratohyaline granules. Zelickson and Hartmann (1962) observed that in nonkeratinizing human oral mucosa the tonofilaments are shorter, finer, and fewer in number, and the number of desmosomes is smaller than in keratinizing tissue.

MATERIALS AND METHODS

Adult normal cycling female dogs of mixed breed averaging 16 to 24 pounds were used. These dogs were maintained in the laboratory specifically for reproductive studies and their histories were well known. Tissue was taken by vaginal biopsy from seven dogs during all stages of estrus over a 16 month period. The bioptic sample was removed from the median one-third of the dorsal vaginal ridge and was composed of epithelium supported by a portion of the lamina proporia. The size of the sample recovered was influenced by the estrual stage of each dog and the consequent thickness of the mucous layer. Samples averaged 5 mm across and 2 to 3 mm thick. The stage of estrus was determined by vaginal smear (Gier, 1960) and positive indication of heat by acceptance of the stud.

Immediately following excision, the sample was placed in cold (4° C) Palade's (1952) fixative (Appendix), sliced into one millimeter cubes, care

being taken to retain the epithelial surface, and transferred to fresh cold fixative where it remained for two hours. After rinsing in chilled Ringer's physiological solution the tissue was dehydrated in an isopropyl alcohol series and embedded in Epon epoxy resin using an adaptation of Luft's (1961) technique (Appendix).

Thin sections, approximately 250 to 400 angstroms in thickness, were cut on a Porter-Blum ultramicrotome. The sections were collected in a trough filled with 20% ethyl alcohol, spread with acetone vapors, and placed on celloidin coated 200 mesh per inch copper screen grids or uncoated electrolytically formed 75/300 mesh per inch copper grids. Viewing was done on an RCA EMU 2C electron microscope.

OBSERVATIONS

Estrus

The following zones could be distinguished in the vaginal epithelium of dog during estrus: stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum.

The Stratum Germinativum. The stratum germinativum or basal cell layer (Fig. 1, 2, 4) was a single tall columnar cell layer at the bottom of dog vaginal epithelium during estrus. Cell height averaged four times the width and nuclei were oriented perpendicular to the basement membrane upon which the basal cells rested. The nucleoplasm was highly granular with granule size varying, an irregular distribution of granules produced areas of uneven

density. Nucleoli differed in shape from round to angular and in number from one to three. They were more dense than the nucleoplasm and contained areas darker and lighter than their general tone. The nuclear membrane was smooth in some regions but heavily corrugated in others. The concentration of granules along the inner surface of the membrane gave the nuclei a dark bordered appearance.

Observation of organelles within the cytoplasm was difficult due to its high density. Lightly staining tonofilaments in a homogeneous matrix comprised a majority of the cytoplasmic content. They ran the long axis of the cell, paralleling the elongated nuclei. A tonofilament-free area lay immediately adjacent to the nuclei and there was no apparent special relationship with the cell membrane.

Dense spherical and oval mitochondria were frequent at either end and along the cell membranes. The matrix stained heavily and only a few transverse cristae were visible. An apparent case of mitochondrial fission was seen (Fig. 1).

A light vacuolar complex appeared in the cytoplasm adjacent to the ends of the nuclei. A membrane limited some portions of the low density region and large spherical bodies could be seen associated with several. The form and location would indicate the complex was the Golgi apparatus. Small amounts of endoplasmic reticulum were visible in regions where tonofibrils did not totally fill the cytoplasm and appeared to be of the smooth membrane type.

The cell membranes of the lower portions of the cells were fairly smooth except for a few short microvillous processes. The upper borders increased in irregularity with microvilli becoming longer and more frequent. A few desmosomes (Fig. 1, 2) appeared along the lower regions of the cells' membranes and increased in number distally. The desomosomes found in the lower one-third were composed of dense attachment zones which were well formed but not as large as those of the higher level. Intercellular space was moderate and of a fairly constant width. It was filled with a homogeneous low density material.

The basement membrane (Fig. 1, 2) followed exactly the inferior undulating contour of the basal cells' membranes and was continuous across the area where two cells came together. It consisted of a light layer approximately three times the thickness of the cell membrane, or about 300 angstroms, adjacent to the cell membrane and a darker layer of comparable width beyond that. Thickenings of the basal cell membrane, known as bobbins (Fig. 1, 2), adjacent to the basement membrane, continued into the cytoplasm as a darkened band with cross striations. This produced an alternate dark and light layering. Direct continuity of tonofibrils with this structure was not observable.

The Stratum Spinosum. The stratum spinosum or prickle cell layer (Fig. 3, 4, 5) began immediately above the basal layer and consisted of a three to four cell thick stratum. The cells were slightly compressed laterally from an irregular rounded shape. Nuclei were similarly affected but retained the same general texture as before. The nucleoli appeared bubbly and the wrinkles in

the nuclear membrane became longer pointed protrusions.

Cytoplasmic inclusions were in general more distinct than in the basal layer. Mitochondria were quite numerous and scattered throughout the cytoplasm. Many were irregularly shaped (Fig. 5, 6) and contained light areas within their matrix. Tonofilaments lacked a regular orientation, running all directions through the cytoplasm. Peripherally they followed the cell membrane closely. They were darker staining than in the lower layer but the matrix surrounding them was less dense. The Golgi complex remained as a vacuolar complex adjacent to the nucleus. Endoplasmic reticulum continued to be quite scarce with only a few scattered tubules and vacuoles visible.

The cell membrane lacked the larger undulations of the lower layer but had many smaller projections into microvilli and desmosomes. Microvilli were bulbous protrusions (Fig. 3, 5, 6) with a homogeneous composition. In some areas they nearly filled the intercellular space and in others, particularly those with many desmosomes, none were seen. An apparent thickening of the cell membrane proved to be the result of a layering of tonofilaments along the inner surface when viewed at higher magnification (Fig. 6).

Desmosomes (Fig. 3, 6) were especially numerous and prominent in the prickle cell layer, being the reason for its name. The projection of each cell membrane was a club shaped protrusion of cytoplasm flattened on one lateral surface. The cell membrane was thickened on the flattened surface forming the attachment plaque, separated by a low density region from the opposing plaque. The intercellular attachment zone between the plaques was approximately perpendicular to the cell surface. No continuity of cytoplasm across

the intercellular attachment zone was observable (Fig. 8). As many as 70 desmosomes could be seen in a single plane of one cell. The intercellular space was about twice the width of that of the basal stratum and was filled with a very light material. Tonofilaments could be seen running into the cellular projections forming the desmosomes.

The Stratum Granulosum. The stratum granulosum (Fig. 7, 10) was the next more superficial layer. In the lowest level little change had occurred other than an orientation of the cells parallel to the surface. Higher level cells had undergone greater squamation and were showing signs of pyknosis.

The nuclei became shrunken and flattened with the cell. Only remnants of the nuclear membrane were seen with the granular nucleoplasm clumped in dark strands. Nucleoli were dark disorganized masses in the lower layer and no longer discernable in the upper layers. The region formerly containing nuclear material appeared structureless and of medium density.

In the top most cells of the stratum the cytoplasm was filled with very densely staining tonofilaments (Fig. 10). The heavily compacted fibrilar cytoplasm was surrounded by a light staining zone adjacent to the cell membrane. The tonofilaments ran roughly parallel to the flattened plane of the cell. There appeared to be two cell types, a dark staining and light staining variety. These were of the same kind, however, the lighter cells being those in which the tonofilaments were sectioned transversely, the darker cells those in which sectioning was longitudinal (Fig. 10). Tonofilaments were occasionally tightly clumped together forming tonofibrils. The supporting matrix surrounding all filaments was the same light homogeneous mass as

before.

Mitochondria were not identifiable but were probably the degenerated source of the scattered dense masses surrounding the nuclear remnants (Fig. 7). Neither Golgi apparatus nor endoplasmic reticulum could be identified in this stratum.

The cell membrane had fewer irregularities than in the previous stage, with only small indentations and projections. Small bulbular invaginations with or without matching projections from the opposing membrane were irregularly grouped along the surface of the cells. Such invaginations were not present in previously described strata. An increase in thickness of the intercellular attachment zone became evident and the processes from the cells supporting the desmosomes were considerably shortened. The attachment plaques had been reoriented until they were approximately parallel to the cell membrane. Desmosomes were smaller than before and did not appear as frequently. The intercellular space was narrowed to about one-sixth as wide as in the previous stratum. The intercellular material stained more darkly than before and had shrunken away from the cell membranes at several locations.

The Stratum Corneum. The stratum corneum or cornified layer (Fig. 9) comprised nearly half of the total depth of the epithelium but was limited in structural content. The cells were flattened parallel to the surface to about eight times as broad as they were thick. The cells were thicker in the region of the nuclear remnants and tapered to a thin edge. The nuclei appeared as washed out areas containing a few dense masses. As in the lower stratum, nuclear membranes were degenerate and the cavity was filled with a medium

density homogeneous material. Nucleoli were not observable.

The cytoplasm was extremely compressed, being further condensed than the stratum below. Mitochondria, endoplasmic reticulum, and the Golgi complex were not identifiable. Tonofilaments and their supporting matrix comprised the majority of the cytoplasm. The filaments were grouped in bundles in some cells and in others they appeared to course independently. Their distribution throughout the cells was regular except for a dense layer along the cell membrane.

The bulbous depressions of the cell membrane of the lower layer were usually replaced by low undulations in the thickened cell membrane (Fig. 9). Desmosomes were still identifiable at the cell contact areas but were closely drawn against the cell. Attachment plaques remained as dense areas of the cell membrane and the intercellular contact zone continued to widen. Frequency was comparable to that of the previous cell layer and size had changed little. The intercellular space continued to decrease and contained mediumly dense material.

Anestrus

The vaginal epithelium in the anestrus condition (Fig. 11) was a two to three cell thick layer of rounded cells with occasional projecting arms. The cells appeared loosely stacked with no exact orientation into layers. The nuclei were generally rounded and large, containing granules of consistent size and density. The granules were distributed in a few large masses, many smaller clumps, and an irregular layer over the nuclear membrane. The

remainder of the nucleus consisted of an opaque supportive substance. Nucleoli were small and not prominent and appeared as granular masses slightly darker than the nuclear granules. The nuclear membrane was occasionally notched and was prominent due to its dark staining and the apposition of nuclear granules.

An evenly distributed granular cytoplasm contained many substructures. Mitochondria were especially prevalent throughout the cells, most being round but some oval or elongate. Although not prominent in the dark grained cytoplasm, they appeared to contain a dense granular matrix. A considerable variance in size was evident and transverse cristae were faintly visible in several. The epithelial fibrillar elements were present in low amounts. A few short poorly stained tonofilaments were evenly distributed throughout the cytoplasm. They were oriented roughly parallel to the nuclear membrane.

The endoplasmic reticulum was present throughout the cytoplasm (Fig. 12) with vesicles especially prominent in the surface cells. The vesicular droplets appeared to join together near the luminal surface, producing larger light staining masses. The droplets were located near the cell membrane indicating they were probably mucous substance ready to be released onto the serous epithelial surface.

The cell membrane was poorly defined and difficult to follow, especially on the epithelial surface. The arm-like extensions radiating into surrounding intercellular spaces were of the same apparent composition as the remainder of the cytoplasm. The membrane was free of alteration of its smooth surface in most areas; a few microvilli and desmosomes occurred. Small, poorly

developed desmosomes were found occasionally, eight or ten surrounding the non-surface cells. The thickened areas of the cell membrane forming the attachment plaques were not an extension but part of the smooth membrane surface (Fig. 11, 12). The intercellular contact zone was the same approximate width as described in the stratum spinosum of estrus. Tonofibrils could be seen radiating out of the desmosome area.

The cell surfaces presented to the vaginal lumen were coated with debris, the mucous exudate serving to trap loose particles at this margin. Microvilli or cytoplasmic protrusions resulting from release of mucous also entangled luminal detritus.

Intercellular spaces were apparent occasionally where three cells came together; cell surfaces were usually closely apposed. The substance in the intercellular space was a low density material staining slightly darker than the epoxy within the vaginal lumen.

Proestrus

The vaginal epithelium of proestrus was arbitrarily divisable into three regions: the stratum germinativum, the intermediate stratum, and the partially cornified stratum.

The Stratum Germinativum. This basal cell layer (Fig. 14) was composed of very dense distinctly flattened cells. The flattened nucleus was completely filled with dark staining granules and generally lacked an identifiable nucleolus. Areas of varying intensity were present throughout the nucleus and a clumping of granules along the nuclear membrane gave it a darkened edge.

The cytoplasm presented characteristics of both the preceeding and following stages. The dark staining tonofilaments were more distinct than in the germinativum during estrus and were oriented generally in the same plane as the flattened cells (Fig. 14). The tonofilaments were supported by an equally dark staining matrix, making them difficult to view individually. Mitochondria and Golgi complexes were clearly visable in the dense cytoplasm. Mucous droplets distended the endoplasmic reticulum at scattered locations throughout the cell. The membranes surrounding the droplets were wrinkled as if they were shrinking.

Cell membranes lacked extensive projections but had many smaller protrusions (Fig. 14). Irregular microvilli were plentiful over the lateral and superior cell surface. The inferior surface against the basement membrane was thrown into sharply folding protrusions. Only a few small desmosomes were visible closely drawn against the cell body. The lightly staining attachment plaques were not on the long cytoplasmic stalks as in estrus, but were incorporated in small bulges of the cell membrane. The intercellular space was expanded by a very light staining material.

The basement membrane followed the irregular basal cell surface closely and was continuous across intercellular spaces. Bobbins were faintly visible along the basement membrane but appeared to have a similar structure to that described before.

The Intermediate Stratum. The intermediate stratum (Fig. 13, 16) was a layer three to four cells thick with the cells irregular and flattened and cytoplasm dense, much like that of the stratum germinativum. Those on the

upper level (Fig. 16) were considerably thinner and more elongate than those adjacent to the basal layer. Nuclei were better differentiated than in the basal layer with nucleoli visible in some (Fig. 13). The flattened nuclei had occasional bulging protrusions of the nuclear membrane but lacked the corrugated appearance of the other stages.

The cytoplasm was densely filled with tonofilaments while mitochondria and the Golgi complex remained distinguishable (Fig. 16). Flattened mucous droplets persisted with some taking on a frothy appearance. The cell membrane was quite irregular with an increasing number of desmosomes evident. Intercellular space was decreasing but the intercellular matrix was unchanged in staining characteristics.

The Partially Cornified Stratum. The topmost partially cornified stratum (Fig. 15) was sharply defined from the next lower stratum by the sudden appearance of quite flattened smooth bordered cells with little space between them. The horizontally elongate nuclei contained large granuled nucleoplasm and several dense nucleoli. The nuclear membrane was very smooth and rimmed by a layer of nuclear granules.

The cytoplasm was fibrillar with the exception of a few large deposits of mucous droplets and an occasional mitochondria. The tonofilaments ran parallel to the squamate cell surface and were evenly distributed throughout the cytoplasm.

The greatly reduced intercellular space contained a darker staining material than in lower strata. Desmosomes were present in greater frequency but were difficult to discern. Adjacent cell borders were free of projections

except for the areas of desmosomes. A few knobular protrusions of the cell membrane were evident along the luminal surface.

DISCUSSION

The Epithelial Strata

Although the ultrastructure of dog vaginal epithelium was similar to that described in another species with an estrous cycle and to other tissues as well, many points were in conflict with descriptions by previous authors or had been totally overlooked.

In estrus the stratified epithelium consisted of four definite layers. Although not an ideal method of naming the layers, the author chose the classification most recently used in human epidermis (Listgarten, 1964). The terms stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum or their popularized form (Burgos and Wislocki, 1958) have become internationally accepted. All were found descriptive of the actual tissue appearance with the exception of stratum granulosum. In the dog vaginal epithelium this was not a granular layer, but a stratum of partially flattened cells showing signs of pyknosis and filled with fibrils (Fig. 10). Tissues showing similarity to the vaginal epithelium of this stage were epidermis (Selby, 1955), gingiva and cheek mucosa (Albright, 1960), and palate (Gibbins, 1962). Displaying a very close resemblance was the nonkeratinizing mucosa of the inner surface of human lower lip (Zelickson and Hartmann, 1962).

Examination of vaginal fluid during early metestrus revealed a consider-

able amount of debris in addition to desquamated cornified cells. Debris was evident on the surface cells during anestrus (Fig. 11). These findings would tend to disagree with the observations of Sognnaes and Albright (1956) on oral mucosa. They stated that desquamation of this type of epithelium occurred in toto, unlike that of skin.

The low epithelium of anestrus was similar to that described for the guinea pig in late diestrus (Burgos and Wislocki, 1958). However, the author did not find the surface cells to be greatly distended with mucous droplets as they had reported; instead several large flocculant drops were observed in the peripheral cytoplasm of the cells nearest the lumen (Fig. 11). A definite delimitation into three cell layers, as reported by Burgos and Wislocki (1958), was not observed either; instead a very irregular orientation of the rounded cells was seen. The only other tissue reported that was similar to this stage was the interfollicular epidermis of the mouse (Setala, Merenmies, Stjernvall, and Nyholm, 1960) which, however, did show signs of keratinization on the surface of the low stratified epithelium.

There has been no previous description in the literature of electron microscopy of an epithelium like that described in proestrus. The flattened basal cells (Fig. 14) were reminiscent of those seen in the guinea pig vagina during late diestrus (Burgos and Wislocki, 1958) and the intermediate cells (Fig. 13, 16) were similar to those of stratum spinosum of epidermis (Odland, 1958), but the flattened precornified surface cells (Fig. 15) which retained their substructures were different from those of other epithelia.

The Vaginal Ultrastructure

The basement membrane retained a constant structure throughout the estrous cycle (Fig. 1, 2, 14). It consisted of a light band adjacent to the inferior basal cell membrane about 300 angstroms thick and a darker band of the same width beyond that. This agreed with the observations of epidermis (Odland, 1958). It remained constantly against the cell membrane except where an intercellular space occurred. Here the basement membrane continued across to the next cell without following the cells' membranes upward (Fig. 2). The irregular cell border which it was forced to follow was similar to that described in gingival epithelium (Listgarten, 1964).

The author adopted the term "basement membrane" from the myriad names proposed because it was the most widely used. As has been repeatedly pointed out (Selby, 1955; Singer and Andrew, 1959; and Zelickson and Hartmann, 1962) this was a term pirated from light microscopist which was used for a different structure and is not particularly descriptive of the actual item. It has had such wide usage over the last ten years, however, that a name change now would be highly improbable. The application of the term basement membrane to nearly every epithelial surface and organ has planted it firmly in the literature. It would seem more probable a new name should be devised for the upper layer of the lamina propria, formerly known exclusively as the basement membrane.

Another substructure closely associated with the basement membrane was the so-called bobbin (Weiss and Ferris, 1954). Seen clearly in estrus (Fig. 1, 2) and less so in proestrus (Fig. 14), it appeared to consist of a thicken-

ing of the basal cell membrane over an area about 1200 angstroms in diameter. Some appeared to have a structure similar to those described by Weiss and Ferris (1954), a dense top and bottom separated by a less dense intermediate layer. Others appeared to be fashioned of alternate dark and light bands repeatedly stacked one on top of the other (Fig. 2). Tonofibrils were not seen to have an association with this structure, as reported in human epidermis (Selby, 1955) and hamster cheek pouch epithelium (Albright and Listgarten, 1962).

Bobbins were described as "half desmosomes" by Burgos and Wislocki (1958) and would seem to have a similar function. Since it has been pointed out that melanocytes possess bobbins but lack tonofibrils (Odland, 1958), it seems likely that they have an application more valuable than as a membrane attachment site for tonofilaments. The author would agree with Odland (1958) that they most likely serve as a membrane attachment site, binding the basal cells to the basement membrane.

Desmosomes were found in varying quantities associated with cells of every level of every estrous stage. They were generally of low numbers in the basal layer and increasingly plentiful until cells began cornification, except in anestrus when they were few at all levels. Estrus was characterized by a few desmosomes in the basal cell layer (Fig. 1, 4), a greatly increased number in the prickle cell layer (Fig. 3, 5), a leveling off in the granulosum layer (Fig. 7, 10), and an apparent decrease in the cornified layer (Fig. 9). This differs substantially from the observations by Burgos and Wislocki (1958) on the guinea pig vagina during estrus. They described

an approximate equal number of desmosomes in basal and prickle cells, none in the granulosa or cornified layers.

The intercommunication of cytoplasm across the desmosomes, forming a syncytium as reported by Bahr and Mohberger (1956) in human vaginal epithelium, appears quite unlikely from observations of this study (Fig. 8). It was seen that the "attachment plaques" (Odland, 1958) were thickened areas of the opposing cell membranes separated by an intercellular attachment zone. This agreed with many recent findings, including that in human gingiva (Listgarten, 1964). The terms "desmosome" and "intercellular bridge" have been used interchangeably by many authors. With the term intercellular bridge implying a syncytium condition it would seem logical to prefer desmosome as the better usage.

The intercellular spaces were distended in the lower cell levels of estrus and proestrus (Fig. 1, 5, 14) and compressed in the upper cell strata of these stages (Fig. 9, 10, 15). This agreed with the observations during estrus on the guinea pig (Burgos and Wislocki, 1958) and with human cervix uteri (Ashworth, Luibel, and Saunders, 1960). During anestrus the intercellular space in the dog vaginal epithelium was not distended but appeared incidentally at the angle of three cells coming together (Fig. 11). Burgos and Wislocki noted that cells of this stage were closely apposed and extensively interdigitated.

Cell processes were limited on the membrane of basal cells during estrus (Fig. 1) but were numerous in this stratum during proestrus (Fig. 14). Generally, they increased as the intercellular space increased and the cell moved

away from the basement membrane. This was likewise observed in human epithelium (Odland, 1958) and in hamster cheek pouch (Listgarten, 1962). It appeared that as the cell moved farther away from its source of nutrient, diffusion through the basement membrane, the cell surface area was increased in an attempt to enhance absorption. As the cells continued movement away from the basement membrane the intercellular space decreased, microvilli disappeared and the cell died.

A relationship between microvilli and desmosome formation has not been suggested in the literature. It seemed apparent to the author that since they both appear in quantity at the same time, the formation of one is influenced by the other. A desmosome was little other than a thickening of the cell membrane on a bulbous protrusion, as seen during the time of greatest concentration in the stratum spinosum during estrus (Fig. 5, 6). It appeared they originated by two microvilli coming together, a local thickening of membranes occurring, and a bond being formed. The stimulus for desmosome formation then seemed to be the increasing need of the cells for food products.

Little could be determined of the intercellular substance, an amorphous material, lighter in the lower layers (Fig. 1, 5, 14) darker in the upper (Fig. 9, 10, 15). Sognnaes and Albright (1956) suggested a high protein content due to its osmophilia, but the opposite conclusion would be drawn by the author. The light staining characteristics would indicate a lack of complex proteins and lipid material in the substance.

The dense nature of the tonofilament-filled cytoplasm made observation of the endoplasmic reticulum difficult. Endoplasmic reticulum was seen in

small amounts during estrus (Fig. 1) but was more obvious during anestrus (Fig. 11, 12) and proestrus (Fig. 15, 16) when portions were distended by mucous. Ribosomes were not identifiable on the endoplasmic reticulum in any preparations studied. Endoplasmic reticulum has been reported scarce in human epidermis (Selby, 1955) and not discernable in human gingiva and cheek mucosa (Albright, 1960), but was probably masked by tonofilaments in these epithelia.

Mitochondria were plentiful but obscured by the tonofilament-filled cytoplasm. They were reported abundant in the basal cells but diminishing in upper strata of human oral mucosa (Sognnaes and Albright, 1956) and seemed to follow this general pattern in dog vaginal epithelium. The internal structure could not be clearly observed in cells of estrus (Fig. 1, 6) or anestrus (Fig. 11, 12), agreeing with a description of human gingiva and cheek mucosa (Albright, 1960). Cristae were well resolved in the mitochondria of proestrus (Fig. 16), extending approximately four-fifths of the way across the mitochondria.

A structure suggested to be the Golgi complex was described in estrus (Fig. 1, 4, 5) and proestrus (Fig. 14). Its polar location and vesicular nature would agree with the report by Zelickson and Hartmann (1962) of a Golgi apparatus, composed of small vesicles, located at opposite poles of the nucleus in human non-keratinized oral mucosa. Other authors have not reported a Golgi apparatus in epithelial cells but it has been described when the classical silver staining methods were used with light microscopy (Montagna, 1952).

The small dense bodies in non-cornified cells of epidermis, termed corpuscula (Frei and Sheldon, 1961) were not seen in this tissue. Odland (1960) mentioned a similar structure in stratum granulosum of human epidermis and speculated they may be degenerating mitochondria. They may have had such an origin or were associated with keratinization, since they do not appear in non-keratinizing epithelium.

The level of activity of the cell could be determined by its staining nature. After the epithelium reached greatest height in estrus, the basal cells were less dense staining (Fig. 1) than those cells in proestrus when multiplication and growth was at a maximum (Fig. 14). The epithelial cells of proestrus appeared muddy due to the great amount of proteinaceous material in the cytoplasm. Whether this was due to the tonofilaments and their supportive matrix or fine granules of ribonucleoprotein as suggested by Albright and Listgarten (1962) remains to be seen.

The dominant structures of vaginal epithelial cells were tonofibrils. In the thicker epithelia they comprised the majority of the cytoplasm of the basal cells and continued to increase relatively until nothing else remained in the top-most strata. They have been described as loosely woven in basal cells of human gingiva (Listgarten, 1964) and totally dominant in the cornified layers of guinea pig skin (Brody, 1959), agreeing with this observation. Some were seen to originate in the area of desmosome plaques (Fig. 5) agreeing with descriptions of human epidermis (Odland, 1958). Tonofibrils were not seen definitely arising from bobbins, as reported in the hamster cheek pouch epithelium (Albright and Listgarten, 1962).

Tonofibrils were composed of submicroscopic constituents (Fig. 5, 10) known as tonofilaments (Selby, 1955). Although much has been written about their structure and composition, very little definite information could be determined in this investigation. It was seen that they were light staining and indefinite in the basal layers (Fig. 2, 14) but became darker and more regular in the intermediate strata (Fig. 5, 10, 16). The staining characteristic was probably due to a protein, as suggested by Selby (1955).

The terms "keratinization" and "cornification" have been used interchangeably by many authors. From observation of this tissue, guinea pig vagina (Burgos and Wislocki, 1958), and the inner surface of human lower lip (Zelickson and Hartmann, 1962),all of which underwent cornification (i.e., formation of a horny stratified squamous epithelium) without the presence of keratohyaline granules, it appeared that this was a misusage. Keratohyaline granules have been judged as precursors of keratin (Brody, 1959), formed by gradual incorporation of tonofilaments into the granules. The term keratinization should be limited, then to the process involved in transformation of stratified epithelia possessing keratohyaline granules, and cornification to the condensation of those lacking the granules.

SUMMARY

In a study of the ultrastructure of dog vaginal epithelium, tissue taken from seven dogs during the stages of estrus, anestrus, and proestrus was examined. The epithelial strata were photographed on the electron microscope.

The epithelium of estrus consisted of four layers: stratum germinativum,

stratum spinosum, stratum granulosum, and stratum corneum. The stratum germinativum was a tall columnar layer one cell high resting on the basement membrane. The cytoplasm contained frequent dark mitochondria and many tonofilaments in a dense matrix. A vacuolar complex adjacent to the end of the nuclei was identified as the Golgi apparatus. The cell borders had a few microvilli and an occasional desmosome.

The stratum granulosum was the next more superficial layer. The cells were more flattened and the cytoplasm contained little else than tonofibrils. The nuclei showed signs of pyknosis and the intercellular space was reduced. The cell membrane was considerably flattened and desmosomes were shortened. The top-most layer, the stratum corneum, displayed an extreme form of these conditions. Only tonofibrils were seen in the cytoplasm, following the orientation of the squamate cells. Nuclear granules were clumped in dark strands and the former area of the nuclei was a structureless expanse. Cell membranes were regular and closely apposed. Desmosomes were still visible but were closely drawn against the cells.

The vaginal epithelium of anestrus was a two to three cell layer of loosely stacked rounded cells. Mitochondria were especially prevalent, most being round but some oval or elongate. Endoplasmic reticulum was visible in the low density cytoplasm and was distended into many small vacuoles by small mucous droplets. Larger droplets were located adjacent to the membranes on the luminal surface indicating they were probably in preparation for release onto the serous surface. Cell membranes were indistinct and covered by debris on the luminal surface.

Proestrus epithelium was divisable into three layers: the stratum germinativum, the intermediate stratum, and the partially cornified stratum. The stratum germinativum was a dense, flattened cell layer adjacent to the basement membrane. Indistinct tonofibrils were embedded in a dark matrix, following the same plane as the cell. Mitochondria and Golgi complexes were clearly visible and mucous droplets distended the endoplasmic reticulum at scattered sites. Cell membranes had many microvilli and the intercellular space was well expanded by a light staining material.

The intermediate stratum possessed many of the characteristics of the previous layer. However, cells were more flattened, desmosomes more plentiful, nucleoli visible, and the intercellular space was decreased. The partially cornified stratum was sharply defined from the lower layer by the sudden appearance of flattened smooth bordered cells with little space between them. Nuclei remained intact to the top layer although tonofibrils filled the cytoplasm. Desmosomes were more plentiful but difficult to discern in the dense intercellular material. A few large deposits of mucous droplets were visible in the cytoplasm.

ACKNOWLEDGEMENTS

Sincere gratitude and acknowledgement is extended to Dr. H. T. Gier, Department of Zoology, for his guidance, advice, and understanding during the course of this investigation; also for permission to acquire tissue from animals maintained for dog reproduction studies in his laboratories at Kansas State University.

The author is indebted to Dr. D. S. Folse, Department of Pathology, for use of equipment and laboratory space essential to the success of this study.

Appreciation is given to Dr. R. D. Dragsdorf and Mr. M. F. Roth, Department of Physics, for the training and maintenance of operational equipment in the Electron Microscope Laboratory.

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APPENDIX

Palade (1952) Fixative, Buffered Osmium Tetroxide

1. Stock buffer solution:

Sodium acetate	9.7 gm
Sodium veronal (barbital)	14.7 gm
Distilled water to make	500 ml

2. Stock osmium tetroxide solution:

Osmium tetroxide	1.0 gm
Distilled water to make	50 ml

3. Fixative:

Buffer	5 ml
0.1 N HOI	5 ml
Distilled water	2.5 ml
2% osmium tetroxide solution	12.5 ml

Correction of the fixative to a pH of 7.4 can be made by adding a few drops of buffer or acid. Both the buffer and the fixative should be kept in the refrigerator.

Luft (1961) Epoxy Resin Embedding Method

1. The tissue blocks are dehydrated in alcohol and then put through two successive changes of propylene oxide for 10 to 15 minutes each.
2. Fresh propylene oxide replaces the previous changes and an equal quantity of the mixed resin is added and mixed by gentle swirling. This is allowed to remain two hours, swirling occasionally.
3. This is replaced by undiluted resin and allowed to stand six to eight hours.
4. One quarter inch of undiluted resin is placed in an aluminum foil boat formed over the end of a rubber stopper. The tissue blocks are touched to a filter paper to remove excess resin from the previous step and placed

in the boat.

5. The resin is polymerized according to the following schedule:

   (a) Incubation six to eight hours at 35° C.
   (b) Incubation ten to eighteen hours at 45° C.
   (c) Incubation twelve to forty-eight hours at 60° C.

6. The sheet of polymerized resin is trimmed into small cubes containing the tissue blocks. The cubes are glued on with household cement to the end of a one-half inch length of 7 mm lucite rod for sectioning.

7. The resin components are as follows:

(a) Epon 812	6.9 ml
(b) Dodecenyl succinic anhydride (DDSA)	4.2 ml
(c) Nadic methyl anhydride (NMA)	3.6 ml
(d) 2,4,6-tri (dimethylaminomethyl) phenol (DMP-30)	0.3 ml

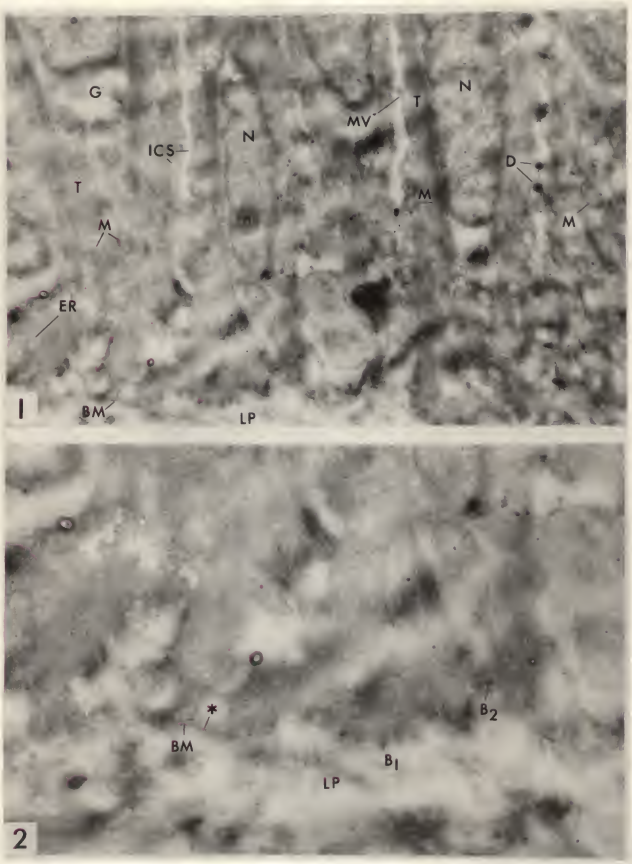
   These should be mixed at low speed for two hours in a glass beaker using a magnetic stirrer. It is suggested a glass covered stirring magnet be used. The same mixture is used for the two steps of infiltration and for embedding. The NMA is increased for greater hardness if this is desired.

EXPLANATION OF PLATE I

Fig. 1. The stratum germinativum of vaginal epithelium during estrus. The basement membrane (BM) separates the lamina propria (LP) from the inferior surface of the epithelium. Elongate nuclei (N) containing nucleoli (n) are seen within the tonofibril (T) filled cells. Mitochondria (M) and a small amount of endoplasmic reticulum (ER) are also seen along with the suggested Golgi apparatus (G) and a dividing mitochondria (M). Microvilli (MV) and desmosomes (D) are occasionally associated with the cell membranes which are separated in most places by the intercellular space (ICS). Magnification 12,650X.

Fig. 2. An enlarged view of the lower left portion in Fig. 1. The basement membrane (BM) separating the lamina propria (LP) from the stratum germinativum is seen. Two types of bobbins are present: bobbins (B_1) with the alternate multiple dark and light layers and bobbins (B_2) with two major dark portions separated by a lighter region. The area in which the basement membrane is continuous across an intercellular space is indicated by *. Magnification 22,770X.

PLATE I

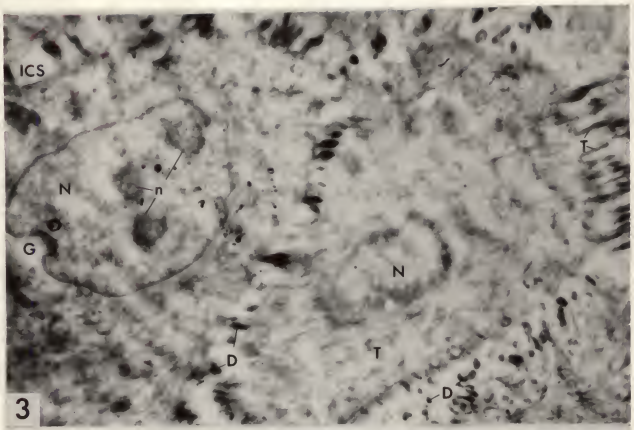


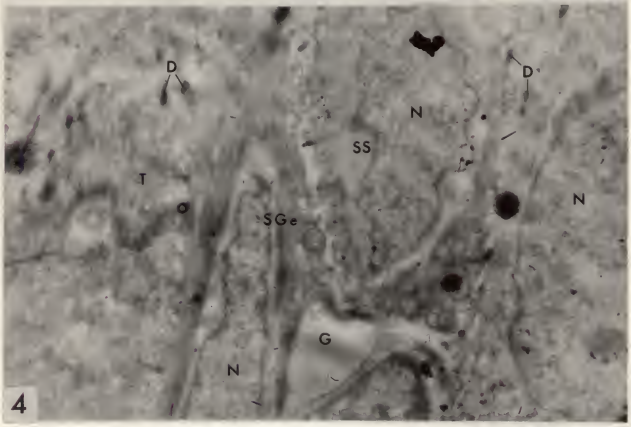
EXPLANATION OF PLATE II

Fig. 3. The stratum spinosum of vaginal epithelium of estrus. Rounded nuclei (N) with bubbly nucleoli (n) are present with the apparent Golgi apparatus (G) immediately adjacent. Desmosomes (D) are quite numerous across the expanded intercellular space (ICS) with tonofibrils (T) seen associated with them and throughout the cytoplasm. Magnification 12,650X.

Fig. 4. Transition from the stratum germinativum (SGe) at bottom to stratum spinosum (SS) at top of vaginal epithelium of estrus. Elongate nuclei (N) give way to rounded nuclei with a similar change in cell shape occurring. A polar Golgi complex (G) is seen along with tonofibrils and an increase in desmosomes (D) in the upper layer. Magnification 12,650X.

PLATE II



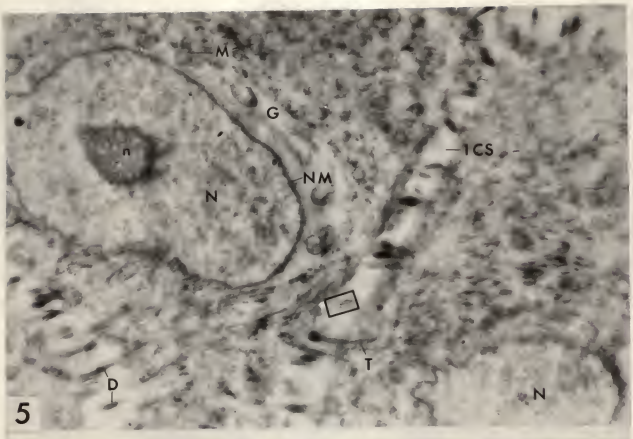


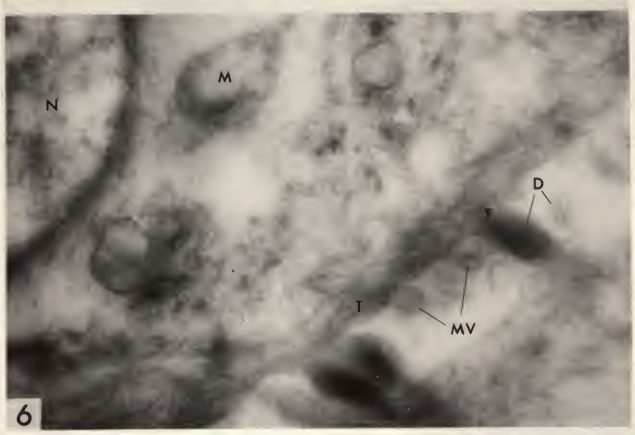
EXPLANATION OF PLATE III

Fig. 5. The stratum spinosum of vaginal epithelium of estrus. Nuclei (N) with a nucleolus (n) show both corrugated and smooth areas of their membrane. Numerous desmosomes (D) are prominent in the intercellular space (ICS) with tonofibrils (T) associated. Mitochondria (M) and the apparent Golgi apparatus (G) can be seen. Magnification 12,650X.

Fig. 6. An enlarged view of region above rectangle in Fig. 5. The nucleus (N) is seen to have an inner layer of granules along the membrane. The cytoplasm contains irregularly shaped mitochondria (M) and many tonofibrils (T) along the cell membrane and associated with the desmosomes (D). Bulbular microvilli (MV) are frequent. Magnification 47,440X.

PLATE III



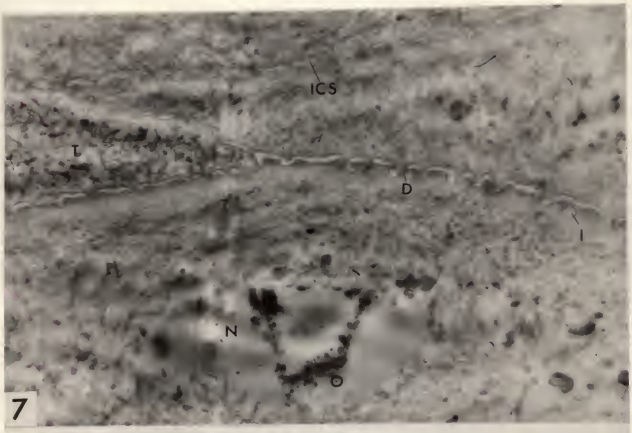


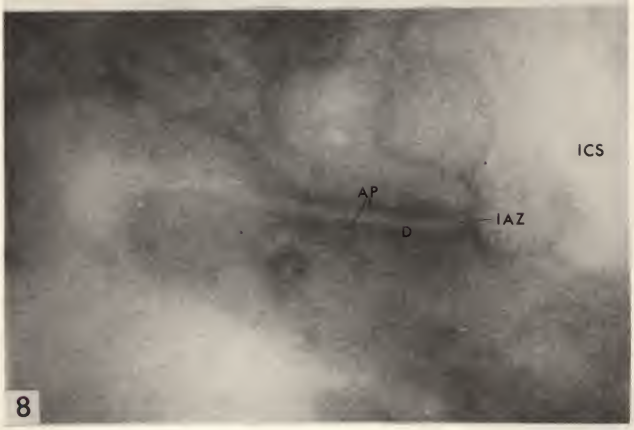
EXPLANATION OF PLATE IV

Fig. 7. The stratum granulosum of vaginal epithelium of estrus. The flattened cells contain a pyknotic nucleus (N) and many tonofibrils (T). The intercellular space (ICS) has been considerably reduced and desmosomes (D) are no longer on stalk-like projections. Bulbous indentation (I) of the cell membrane are seen. Magnification 12,650X.

Fig. 8. A greatly enlarged view of the region of Fig. 5 within the rectangle. The desmosome (D) is seen to be composed of two attachment plaques (AP), one from each contingent cells, separated by an intercellular attachment zone (IAZ). The intercellular space (ICS) is seen to either side of the desmosome. Magnification 194,000X.

PLATE IV



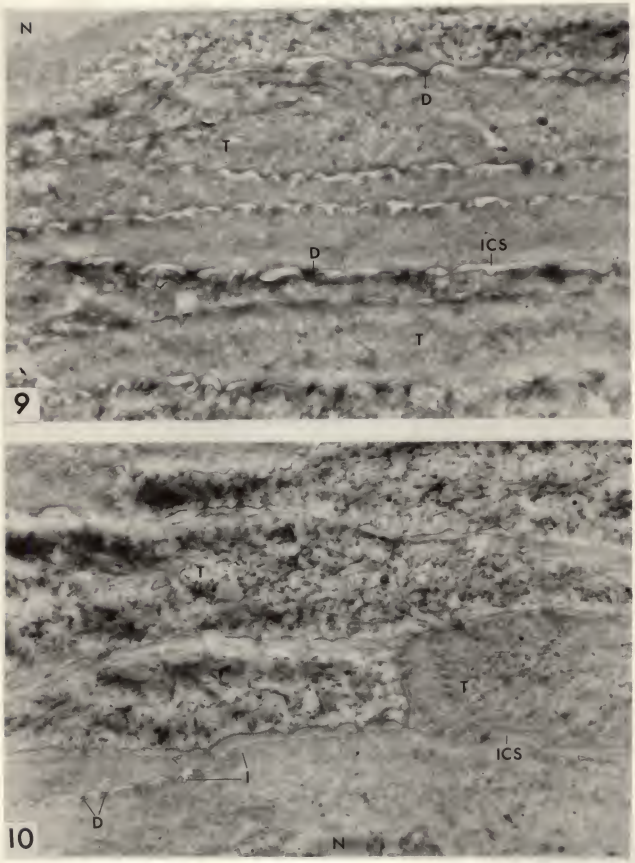


EXPLANATION OF PLATE V

Fig. 9. The stratum corneum of vaginal epithelium of estrus. The greatly flattened cells are filled with tonofibrils (T) and a few degenerate remains of nuclei (N). Desmosomes (D) are drawn against the cellular membrane and the intercellular space (ICS) is quite narrow. Magnification 12,650X.

Fig. 10. The stratum granulosum of vaginal epithelium of estrus. Tonofibrils (T) are seen as cut transversely and longitudinally. Desmosomes (D) span the intercellular space (ICS) and bulbular invaginations (I) of the cell membrane are seen. A nucleus (N) is highly pyknotic. Magnification 12,650X..

PLATE V

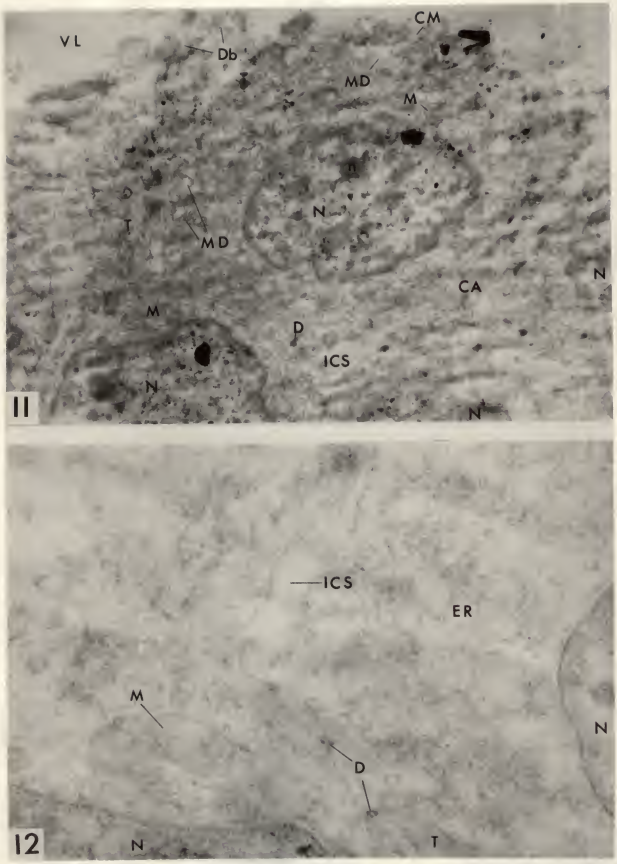


EXPLANATION OF PLATE VI

Fig. 11. The vaginal epithelium of anestrus. The rounded cells contain distinctly patterned nuclei (N) with their nucleoli (n). The cytoplasm is filled with mitochondria (M), tonofibrils (T), endoplasmic reticulum (ER) and mucous droplets (MD). The cellular membrane (CM) is difficult to discern on the debris (Db) covered surface facing the vaginal lumen (VL). The intercellular space (ICS) is limited and cellular arms (CA) project between the cells. Magnification 12,650X.

Fig. 12. Enlargement of lower center region of Fig. 11. Small desmosomes (D) bridge the intercellular space (ICS). The cytoplasm is filled with endoplasmic reticulum (ER), mitochondria (M), and a few tonofibrils (T). Nuclei (N) stain prominently. Magnification 31,600X.

PLATE VI

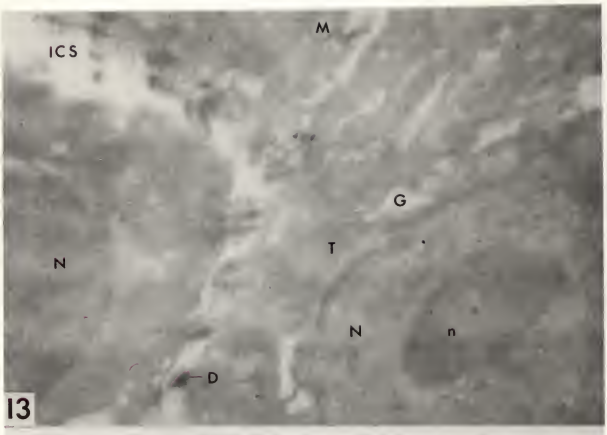


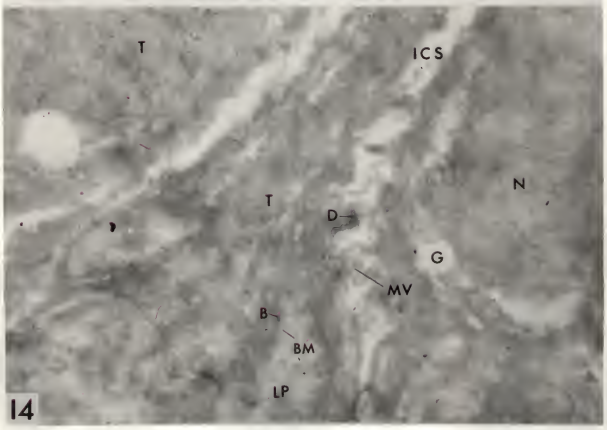
EXPLANATION OF PLATE VII

Fig. 13. The intermediate stratum of vaginal epithelium of proestrus. Dark nuclei (N) with dense nucleoli (n) are present in the slightly flattened cells. The suggested Golgi apparatus (G) lies along the nuclear membrane and mitochondria (M) appear in the dense tonofibril (T) filled cytoplasm. A few desmosomes (D) are seen connecting cells across the expanded intercellular space (ICS). Magnification 12,650X.

Fig. 14. The stratum germinativum of vaginal epithelium of proestrus. The basement membrane (BM) separates the lamina propria (LP) from the irregular projections of the basal cells. Bobbins (B) attach the basal cells to the basement membrane and desmosomes (D) connect contingent cells across the intercellular space (ICS). Nuclei (N) appear homogeneous and the Golgi apparatus (G) is apparent in the tonofibril (T) filled cytoplasm. Many microvilli (MV) project into the intercellular space. Magnification 12,650X.

PLATE VII



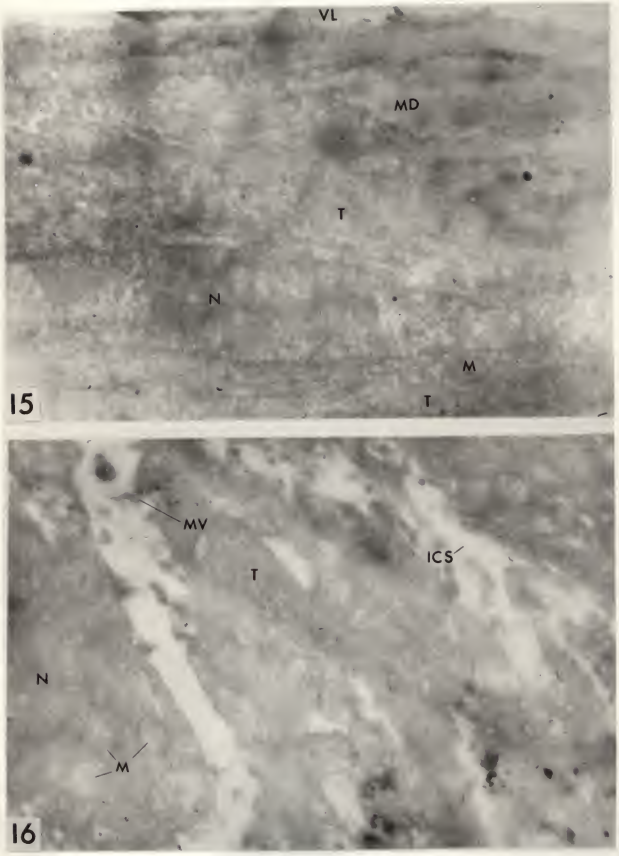


EXPLANATION OF PLATE VIII

Fig. 15. The partially cornified layer of vaginal epithelium of proestrus. Flattened nuclei (N) are seen in the flattened cells. Tonofibrils (T) run parallel to the plane of the cells and mitochondria (M) remain visible. Mucous droplets (MD) are seen near the vaginal lumen (VL). Magnification 12,650X.

Fig. 16. The intermediate stratum of vaginal epithelium of proestrus. The intercellular space (ICS) is expanded and microvilli (MV) project into it. Mitochondria (M) with the double membrane of cristae visible within them share the cytoplasm with many tonofibrils (T). A nucleus (N) appears homogeneous. Magnification 12,650X.

PLATE VIII



AN ELECTRON MICROSCOPE STUDY OF THE VAGINAL EPITHELIUM OF THE DOG

by

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B. S., Kansas State University, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

ABSTRACT

Few studies have been made of the ultrastructure of vaginal epithelium. In a single instance each, the literature contained a description of this tissue of human and guinea pig. The changes occurring throughout the estrous cycle were observable only in the latter species.

Tissues were collected by biopsy from seven dogs during the stages of estrus, anestrus, and proestrus, fixed in osmic acid and embedded in epoxy resin. Ultrathin sections were examined and photographed on an electron microscope.

The epithelium of estrus consisted of stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum. The stratum germinativum was a single layer of tall columnar cells resting on a basement membrane. The cytoplasm contained many tonofilaments in a dense matrix, frequent dark mitochondria, and a polar vacuolar complex identified as the Golgi apparatus. Smooth cell borders possessed a few microvilli and an occasional desmosome. In the more rounded cells of the stratum spinosum, tonofibrils increased in number and density, mitochondria were more irregular, Golgi complex remained, and the endoplasmic reticulum was scarce. An increase in microvilli and desmosomes was accompanied by a widening of the intercellular space. Tonofibrils were associated with the desmosomes. The stratum granulosum consisted of more flattened cells containing mostly tonofibrils. The nuclei were pyknotic, intercellular space reduced, the cell membrane more regular, and desmosomes were shortened. The covering stratum corneum cells visibly

contained only tonofibrils and nuclear remnants. Desmosomes were drawn against the closely apposed cell membranes without evident projection.

Anestrus epithelium was a two to three cell layer of loosely stacked rounded cells. Mitochondria were prevalent and the endoplasmic reticulum was distended throughout the cytoplasm by many small mucous vesicles. Larger droplets were accumulated adjacent to the serous epithelial surface preparatory for release.

The proestrus epithelium was comprised of stratum germinativum, intermediate stratum, and partially cornified stratum. The stratum germinativum was a dense, flattened layer adjacent to the basement membrane. Indistinct tonofibrils in a dense matrix did not obscure mitochondria and Golgi complexes. Mucous droplets were scattered through the endoplasmic reticulum. Many microvilli extended into the well expanded light staining intercellular space. The intermediate stratum was much the same except cells were more flattened, desmosomes more plentiful, nucleoli visible, and the intercellular spaces reduced. The partially cornified layer contained flat cells with smooth borders and little intercellular space. Substructure continued to be visible, desmosomes and tonofibrils were increased, and mucous droplets remained.