LIGHT AND ELECTRON MICROSCOPIC FEATURES OF

REPRODUCTIVE TISSUES OF THE MARE

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PREFACE

This study was designed to investigate the light and electron microscopic features of tissues of the reproductive tract of the mare. Tissues examined with the light microscope included vagina, uterus, uterine cervix, oviduct, and ovary. The uterus, uterine cervix, and vagina also were examined in electron microscopic preparations.

The findings included previously unreported features as well as confirming some features reported by other investigators. These results may serve as the basis for further studies of normal and abnormal conditions of the female equine reproductive system.

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CHAPTER I

INTRODUCTION

An understanding of the functioning of a body system is predicated upon the knowledge of the normal anatomy, physiology, and cytology/histology of the organ(s) of which the system is composed. This forms the basis for recognition of pathologic phenomena. These phenomena may be susceptible to modification by therapeutic methods that attempt to return the system to a state as near that of "normal" as we can perceive.

Current research on the reproductive system of the mare, the subject of this study, is highly sophisticated, including hormonal and biochemical assays. Many areas of microscopic morphology have been examined by only a few investigators and/or on a limited basis. Problems of morphologic, correlative studies of the reproductive system of the mare include a poor reproducibility due to lack of uniformity of sample sites. Inaccessibility of many parts of the reproductive system limits sampling in the living animal. Serial surgeries or slaughter, expensive procedures, may be necessary to follow single animals sequentially under a variety of experimental or natural conditions.

In a review entitled "Perspectives," Reynolds credits Heape with the creation of the science of reproduction as a field of study and mentions the outstanding work of investigators such as F. H. A. Marshall, whose work Marshall's Physiology of Reproduction is known to

many reproductive investigators as a classic text (1, 2, 3). Reynolds cites the work of Stockard and Papanicolaou with guinea pigs and Long and Evans with rats in assessment of the stage of the estrous cycle by study of cells in the vagina smear, considered by many as the impetus for the rapid advancement of experimental studies of reproduction within the last approximately 60 years (1, 4, 5). These studies have resulted in a preliminary understanding of the embryologic development, pre-puberal conditions, mature functioning state, and senile changes of the reproductive systems of some species. Many of these aspects await investigation in the mare.

The reproductive system of the mammalian female, which includes the mare, is composed of the paired ovaries, the uterine tubes or oviducts, the uterus (and uterine cervix), the vagina, the vaginal vestibule, the vulva, and the clitoris (6, 7).

The reproductive tract of the mare has traditionally been carefully examined grossly and by palpation through the rectum. The appearance of the external genitalia may vary with different stages or phases of reproductive activity or impending parturition (8, 9, 10). The vagina and vaginal portion of the uterine cervix and their secretions are accessible for gross examination by use of specula. The uterus and ovaries are palpable through the rectum; oviducts, unless pathologically dilated, are seldom discernible by this means (9). Although some early investigators used biopsy specimens for evaluation of the equine uterus and vagina (11, 12), the current, widespread use of the uterine biopsy resulted from the work of Kenney and Bergman (13, 14, 15, 16). These investigators described the technique, interpretation, and representa-tiveness of the equine endometrial biopsy as a prognostic indicator of

fertility in the mare.

The use of uterine cytology also has increased in recent years in assessment of conditions that may interfere with conception (17, 18, 19, 20, 21, 22, 23, 24, 25). The correlation of the results of equine endometrial biopsy, culture, and aspirate cytology has been the emphasis of the author's research conducted as part of a team effort to explore the clinical, cytologic, histologic, and electron microscopic features of normal and abnormal reproductive conditions in the mare (17, 18, 19, 20, 21, 26).

Ovarian function in the mare has been assessed primarily by rectal palpation or through assays for products which influence or are produced by the ovaries and their effects on observable facets of reproductive activity or function (8, 9, 10, 27, 28, 29, 30). The oviducts have been studied primarily by specimens collected at necropsy (31, 32, 33).

Ultrasonic examination has been used recently for examination and detection of many phenomena in the uterus, ovaries, cervix, and activity of the conceptus that were previously difficult or not possible to evaluate (9). Correlative studies of ultrasonic and histologic/cytologic appearances of reproductive phenomena observed by ultrasound have not been reported.

This study included the light microscopic appearances of ovaries, oviducts, uteri, uterine cervices, and vaginas collected from six mares at necropsy. This provided tissues from many areas not readily accessible for biopsy in the living animal and the opportunity to examine the relationships of features found in each site. The uteri, uterine cervices, and vaginas were examined by electron microscopy to determine the ultrastructural bases of, or correlations with, features observed

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with the light microscope.

As put by Curran and Codling in the introduction to their book The
Pathologic Bases of Medicine:

> Just as knowledge of pathologic processes forms the basis on which
> a sound understanding of disease can be built, so in turn pathology
> itself must be based on a fundamental understanding of the form and
> function of the normal cell, and the abnormalities of these which
> may cause disease, or arise as a consequence of disease. It is now
> widely accepted that to gain this understanding observations with a
> variety of light-microscopic techniques must be combined with
> studies using the electron microscope, with chemical analysis of
> cells (intact and fractionated) and body fluids, and with other
> techniques such as tissue culture (34).

Results of this study may provide the basis for further studies on
infertility in the mare.

CHAPTER II

MATERIALS AND METHODS

Tissues Collected at Necropsy of Six Mares

Tissues were available during necropsy on six mares during July
(three mares) and August (three mares) of 1985. The animals were
Quarterhorses or mixed breed, identified, and aged by examination of the
teeth as follows:

1. #1, 3 years.

2. #2, 3 years.

3. #3, 22 years.

4. #4, greater than 25 years.

5. #5, 9 years.

6. #6, 8 years.

Sections of ovary (including any prominent ovarian structures
recognized grossly), oviduct, uterus, uterine cervix, and vagina taken
for light microscopic examination were fixed in buffered 10% formalin,
embedded in paraffin, sectioned at approximately 5-7 um, and stained
with hematoxylin and eosin. Sections of oviduct were taken from the
oviductal isthmus in four mares, ampulla in five mares, and preampulla
or infundibulum in one mare. Sections of uterus were taken from its
ventral surface in the area of the junction of the body and either horn.
A portion of the ventral aspect of the cervix was taken. The vagina was

represented by a section from the lateral wall several centimeters

caudal to the ectocervix. Additional oviductal, uterine, uterine cervi-

cal, and vaginal tissues were finely minced and fixed in chilled 1.6%

gluaraldehyde in a 0.12-M cacodylate buffer (pH 7.2-7.4) for electron

microscopic preparations. They were rinsed three times in a 0.2-M

cacodylate buffer and post-fixed in 1% osmium in a 0.1-M cacodylate

buffer, rinsed again, dehydrated in a series of ethanol washes, rinsed

with propylene oxide, and embedded in epoxy plastic blocks. The blocks

were sectioned at approximately 0.5 um and stained with azure II/methy-

lene blue (Mallory's stain) for light microscopic examination. Thin

"silver" sections (60-90 nm) were made from selected blocks, and grids

were stained with uranyl acetate and lead citrate and examined by trans-

mission electron microscopy using a JEOL 100 CX II electron microscope.

Problems Encountered in Collection, Processing,

or Interpretation of Specimens

The interpretation of electron microscopic specimens was compli-

cated by problems with orientation of tissues within the blocks so that

sections contained both surface epithelium and underlying structures.

Ten to 12 blocks were made from each tissue specimen. A minimum of

three and maximum of six blocks of each tissue were selected for thick

sections, from which one was chosen for thin sectioning for electron

microscopic examination.

No electron microscopic examination of the oviducts was possible.

Only one section was oriented so that epithelium was present, and that

was lost in preparation of thin sections. Therefore, collections of

oviduct for electron microscopic examination were unsuccessful. Future

attempts may require careful cross sectioning of these tubular struc-
tures or "rolling" of flattened tissues prior to fixation to obtain
epithelial surfaces that are identifiable after processing and that may
be more accurately oriented in the blocks.

Many of the sections of uterus also did not contain large areas of
surface epithelium.

CHAPTER III

RESULTS

Ovary

Anatomy

The ovary of the mare is suspended from the sublumbar region by the mesovarium containing vessels and nerves and attached to its convex dorsal border (6). The ventral, free border contains a prominent depression known as the ovulation fossa, the site of ovulation in the mare (6, 8, 37, 38). The ovulation fossa is lined at its cranial pole by columnar epithelium originating from the fimbriae of the oviduct and along the rest of its surface by cuboidal cells of germinal epithelium (8, 37). The equine ovary is unique among mammals due to the invagination of the cortex into the medulla, resulting in distribution of the reproductive cells within the center of the ovary. The surface of the ovary is covered by thick bands of connective tissue. Fan-like rays of connective tissue occur within the stroma and may help contain developing follicular structures and direct them toward the ovulation fossa (8, 37).

Ovulation in the mare usually occurs on the last two days of estrus or following the end of estrus (8). Ovulation is hypothesized to involve the interaction of LH, cyclic AMP, prostaglandins, steroids, and proteolytic enzymes (39, 40). In the mare, several follicles develop

and enlarge during the cycle but, usually, only one eventually ovulates, the remainder undergoing atresia. Multiple ovulations may occur and are reported to occur frequently in some individuals (8). The mechanisms for selection of the ovulatory follicle(s) and initiation of atresia are not known (8, 27).

The microscopic anatomy of the ovary is extremely complex due to the dynamic changes constantly occurring within this organ (9).

The light microscopic features of the ovary during different stages of development and in the physiologic roles of follicular and luteal production have been described in some detail (8, 27, 29, 41). Ginther states, in reference to the few morphologic studies of the equine corpus luteum (CL), that:

> This is deplorable, especially since the CL is the structure most involved in the rhythmicity and control of the estrous cycle. In contrast, the CL in many other species is being intensively investigated from many angles. More is known about the microscopic anatomy of the CL in red squirrels, for example, than in the mare (8).

Work of Other Investigators

The gross morphology of the structures of the ovary and correlation with rectal palpation has been explored in detail (8, 9). The histologic and histochemical characteristics have not been studied as extensively with the light or electron microscope. Van Niekerk described the light microscopic appearance of developing ovulatory follicles and subsequent luteal structures (41), while the morphologic features of both viable and atretic follicles have been outlined by Kenney, Condon, Ganjam, et al (17). These investigators described viable follicles as containing a bilayered granulosa cell lining that

lost the bilaminar arrangement except for a palisaded basal layer as the follicles enlarged. Mitotic figures were present in granulosa and luteal cells. Thecal cells became luteinized, with a large amount of cytoplasm and vesicular nuclei, just before ovulation. Atretic folli- cles were classified according to a three-stage system. In stage 1, focal karyorrhexis and pyknosis of granulosa cell nuclei occurred. Pyknosis and mitosis were observed to occur simultaneously. They referred to this stage as "incipient atresia." Alternatively, they observed these changes in the granulosa cells with luteinization of thecal cells, a situation they called "incipient atresia with partial luteinization." In stage 2, the granulosa cells underwent massive pyknosis and karyorrhexis, followed by gradual loss by lysis. Only a mono- or bilayer of small, inactive-appearing granulosa cells remained with concurrent thickening and hyalinization of the basement membrane, which occasionally even encircled adjacent veins. Thecal cells gradu- ally disappeared. In stage 3, the separate identity of granulosa and thecal cells was lost. A few connective tissue cells and the thickened basement membrane were the only recognizable remains (27).

Van Niekerk et al (41) described thecal cells as being best developed a few days before ovulation, to regress quickly, with advanced degeneration present by 24 hours following ovulation; these degenerated cells were replaced by fibroblasts. Hypertrophy and luteinization of granulosa cells began by 10 hours after ovulation, with luteal enlarge- ment primarily due to lutein cell hypertrophy. In their study, the granulosa cells were observed to cease dividing three days before ovula- tion. The membrana granulosa changed from a compact to an open, lacey layer of stellate or spindle-shaped cells that secreted a mucoid

substance that lined antrum and ovum at ovulation. The granulosa cells rapidly enlarged following ovulation and appeared luteinized by day 3 postovulation. Maximum hypertrophy was attained by approximately day 9 and maintained until day 12. Then, there was a progressive decrease in size during the next four days, with condensation or fragmentation of the cytoplasm and shrinkage, pyknosis, or karyolysis of the nuclei.

Two types of lutein cells were observed within the CL: large, light cells and small, dark cells. The small, dark cells were observed to increase throughout the development of the CL, accounting for nearly all the cells by day 20. The small, dark cells were hypothesized to be in a resting stage that ceased to be converted to the large, light cells when the latter began to degenerate at approximately day 12 postovulation (41).

The progression of changes involving the central cavity of the follicle, subsequent CL, and surrounding connective tissue layers also were described by Van Niekerk et al (41). After collapse of the follicle at ovulation, numerous macro- and microscopic folds containing a central core of stromal tissue with large vessels projected into the central cavity. Bleeding into the cavity, which did not occur in some cases, began at approximately 14 hours postovulation. By 30 hours postovulation, the structure was distended with blood to approximately two-thirds the size of the original follicle. By 14 hours post-ovulation, trabeculae of proliferating capillaries and hypertrophic fibroblasts began to grow into the granulosa tissue. As the luteinized cells degenerated, intercellular spaces became filled with stromal cells which grew in from the trabeculae. By day 20, a large number of stromal cells were present in the luteal tissue, and the blood vessels

accompanying them were observed to undergo sclerosis and become obliterated (41).

Histologic Features of Ovaries from Six Mares

The histologic assessment of the ovaries from each mare is presented in Table I. The structures present in histologic sections were categorized according to the criteria described by Van Niekerk et al (41) and Kenney et al (27) as to follicular or luteal nature and probable stage of development. With the exception of two sections, all ovaries contained a few to moderate numbers of granulated eosinophils. These were located in the stroma or were adjacent to or within the follicular or luteal structures. One mare (#4) had ovaries without recognizable follicular or luteal structures (grossly or histologically). Sections from one ovary from mares #2 and #6 contained developing follicular structures (Figure 1). Mare #6 had a large, degenerating corpus luteum present on the opposite ovary. Mare #2 had an atretic follicle on the opposite ovary, while ovaries from mares #1 and #5 had only atretic follicular structures (Figure 2). Mare #3 had a recently ovulated follicle on one ovary (Figure 3) and a regressing corpus luteum on the other (Figure 4). A small nodule of adrenocortical tissue was present in the connective tissue surrounding one ovary of mare #1.

Discussion

The lack of recognizable gross or microscopic follicular or luteal structures on the ovaries of mare #4 was consistent with a state of senile atrophy believed to have been present in this aged (greater than

TABLE I

HISTOLOGIC EVALUATION OF OVARIES FROM SIX MARES

| Mare | Ovary #1 | | | Ovary #2 | | |
	Corpus Luteum	Follicle(s)	Other	Corpus Luteum	Follicle(s)	Other
#1	None	Artetic follicle (stage II)	Small adrenocortical nodule, few eosinophils	Developing corpus luteum	None	Few eosinophils
#2	None	Developing follicle	None	None	Atretic follicle (stage III)	Few eosinophils
#3	Regressing corpus luteum	None	Moderate numbers eosinophils	Corpus hemorrhagicum	Two atretic follicles (stages I and III)	None
#4	None	None	Undulating capsular surface, few eosinophils	None	None	Few eosinophils, focal vascular mineralization
#5	None	Two atretic follicles (stages I and II)	Few eosinophils	Regressing corpus luteum	None	Few eosinophils
#6	None	Developing follicle	Few eosinophils	Regressing corpus luteum	None	Few eosinophils
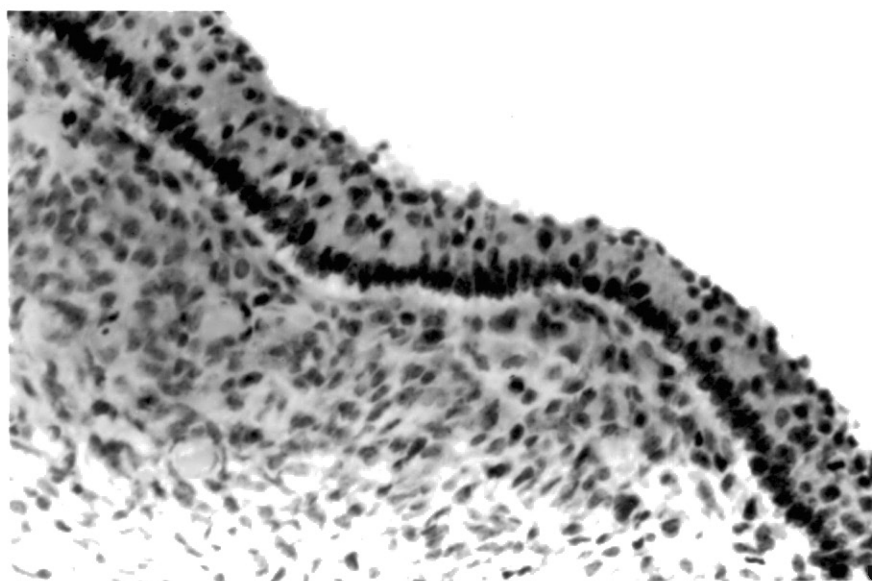
Figure 1. Ovarian stroma and wall of developing ovarian
           follicle from mare #2. Note palisaded basal
           granulosa cells with subnuclear clear spaces
           and adjacent condensed theca cells. Hematoxy-
           lin and Eosin X120.
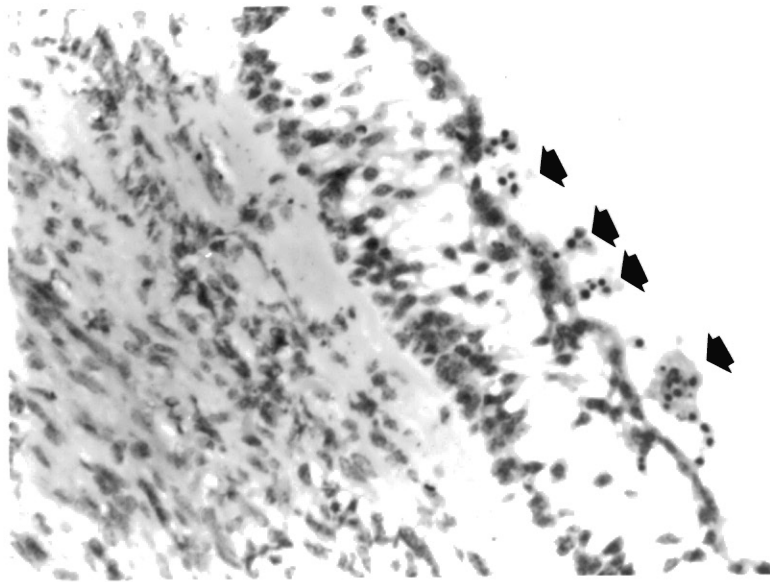
Figure 2. Atretic follicle from mare #3. Massive pyknosis
and lysis (apoptosis) (arrows) of granulosa
cells with beginning thickening and hyaliniza-
tion of basement membrane (B). Hematoxylin and
Eosin X120.
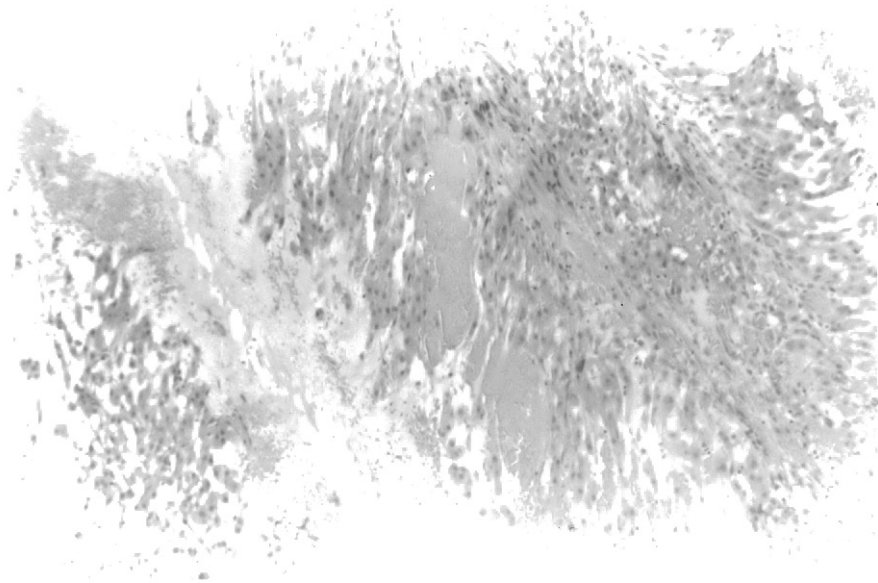
Figure 3.   Stellate and spindloid granulosa cells projecting
            into central cavity filled with blood and
            proteinaceous material in a recently ovulated
            follicle (early corpus luteum or corpus hemor-
            rhagicum) from mare #3.   Hematoxylin and Eosin
            X25.
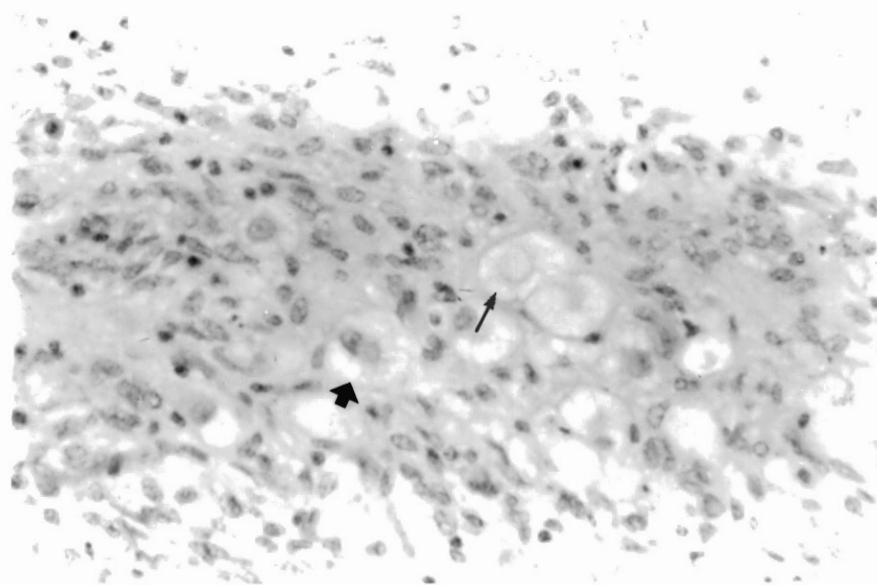
Figure 4. Large vacuolated luteal cells with focally con-
densed cytoplasm (large arrow) or karyolysis
(small arrow) among intervening fibroblasts.
Regressing corpus luteum on ovary of mare #3.
Hematoxylin and Eosin X120.

25 years old) animal. The age of senile ovarian atrophy is extremely variable in the mare. In some mares, ovarian activity and reproductive function may continue at advanced ages (8, 10).

The presence of developing follicles without evidence of atresia in mares #2 and #6 suggests that these mares were actively cycling at the time of collection. Incipient or early atresia cannot be ruled out, since the changes typical of this stage may be focal and occur in the presence of mitotic activity (27). The entire wall of the follicles from these mares was not available for study, so such focal changes may have been missed. The atretic follicle on the opposite ovary of mare #2 and the corpus luteum on the opposite ovary of mare #6 would support the hypothesis of current cyclic activity.

The cellular changes associated with equine ovarian follicular atresia have been described by investigators but not apparently recognized as being a specific type of cellular death known as apoptosis (42, 43, 44). Apoptosis is a morphologic classification of cell death characterized by condensation of the cell, maintenance of organelle integrity, formation of surface protuberances, and absence of inflammation. It characteristically affects single rather than contiguous groups of cells, and the mechanism for its occurrence appears to involve an active process of self-destruction requiring macromolecular synthesis (42, 43, 44). This phenomenon has been described by other investigators as the mechanism of cell death and deletion in mammalian ovarian follicular atresia (42, 43) and has been recognized in equine endometrial specimens by the author (36). The concept of apoptosis is classically associated with hormone-responsive tissues. However, the mechanism by which follicles are designated for subsequent atresia or continued

development and ovulation is not known (8, 27).

The recently ovulated follicle on one ovary and regressing corpus luteum on the other ovary in mare #3 support the presence of current cyclic activity. The presence of only atretic follicular structures in the sections of ovaries from mare #5 suggests that this mare may have been undergoing seasonal transition to winter anestrus. However, since the entire ovary was not examined and reproductive history was not available, other ovarian structures suggestive of current activity may have been missed. The correlation of the ovarian features with those of other tissues will be presented in a later section. It may be possible to gain more confidence in interpretation of state of activity if several tissues reflect changes consistent with the stage hypothesized to exist.

The finding of a small, paraovarian adrenal nodule is not unusual in the mare (13). These are not usually associated with abnormal hormone production and may be an incidental finding at necropsy. Abnormal hormonal activity associated with paraovarian adrenal tissue has been reported to occur rarely (13).

Inflammatory cells are not described in normal ovaries. The presence of eosinophils in sections of ovaries from all mares in this study may reflect immunologic or biochemical mediation by these cells. Such activity may be the result of local factors or other processes occurring in the peritoneal cavity. The parasitologic status and deworming history of these mares was not known, and peritoneal involvement due to parasitic migration cannot be ruled out as a cause of the ovarian eosinophils.

Oviduct

Anatomy

The oviduct of the mare is located in the mesosalpinx, a fold of peritoneum derived from the lateral layer of the broad ligament, and extends from the extremities of the uterine horns to the ovaries (6). The oviduct is divided anatomically into four segments as it proceeds from the uterus to the ovary:

1. Uterotubal junction--the area of the transition between the uterus and the oviduct.

2. Isthmus--the segment located between the uterotubal junction and ampulla that comprises approximately one-third of the length of the oviduct.

3. Ampulla--the cranial two-thirds of the oviduct, which sometimes may be recognized by an increased diameter at the isthmus-ampullary junction, and is the site of fertilization.

4. Infundibulum--the dilated part of the ampulla that contains the opening of the oviduct and whose margin contains finger-like projections known as fimbriae (6, 32, 45, 56).

The classification of the parts of the oviduct based on their microscopic appearance does not always agree with that of the gross anatomy but is thought, by its proponents, to correlate better with the morphology, biochemistry, physiology, and pharmacology of the oviduct (47). Under this classification, based on specimens from women, the oviduct is divided into the following parts:

1. Preampulla--includes the fimbriae and infundibulum; usually contains primarily ciliated cells, has a thin wall, and is concerned

with egg transport.

2. Ampulla--slightly thicker wall, few ciliated cells, provides nutrients, and is the site of fertilization.

3. Isthmus--pronounced muscular coat, more nonciliated than ciliated cells; affects nutrition and transport of sperm and eggs.

4. Junctura--includes some of the extramural as well as the intramural part of the oviduct (not the same anatomical segment as the uterotubal junction); well-developed muscular layers, varying proportion of ciliated and nonciliated epithelial cells, and the narrowest lumen of any segment of the oviduct. A similar microscopic classification has not been reported for the oviduct of the mare.

Some of the fimbriae of the infundibulum of the mare are attached at the ovulation fossa of the ovary, and the remainder are normally closely applied to the ovary to trap ova at the time of ovulation (6, 32, 46).

The oviduct, uterine tube, or Fallopian tube varies in length and tortuosity with species. The mare is considered to have a very tortuous oviduct (46) that is usually 20 to 30 cm long with a diameter that ranges from 2 or 3 to 8 mm (6). Histologically, the oviduct is surrounded by serosal and muscular layers (6, 32). Sisson describes the muscular layers to consist mainly of circular fibers with longitudinal fibers derived from the broad ligament (6). Beck, however, points out that through any given cross-sectional plane of the tube individual smooth muscle fibers or fiber bundles are present in a variety of planes of orientation (32). This may be due to orientation of fibers in a spiral fashion about the circumference of the tube with individual fibers traversing the muscularis from the outside inward. The tightness

of the spiral arrangement may very along the length of the tube and result in variation of the pattern of the smooth muscle layers (32). The mucosa of the oviduct is made of folds which project into the lumen; the number, height, and complexity of these folds varies among species and among segments of the tube (32). Both ciliated and nonciliated cells have been identified in mammalian oviducts by light and electron microscopy (32, 45, 47, 48, 52). Of the nonciliated cells, secretory, intercalary or "peg" cells, and indifferent or basal cells have been recognized with the light and electron microscopes (32, 46, 47, 48, 49). A nonciliated, villous cell, identified by electron microscopy, also has been described by Oberti et al (49).

There is considerable disagreement regarding the functions of these various cells and possible changes in light or ultrastructural morphology relative to the reproductive cycle or cessation of reproductive activity. Some investigators feel that the cilia, along with muscular contractions, are important for movement of ova into the oviduct following ovulation (32, 46, 47). Others question that importance due to the finding of normal fertility in women affected with the immotile cilia syndrome, involving abnormal ciliary structure and function (45). Pauerstein et al state that tubal epithelium probably does not undergo cyclic ciliation and deciliation in response to hormonal stimulation (45), while other investigators have reported extensive activity of this sort at this site in rabbits, pigs, monkeys, and women (48). Different findings have been reported with regard to atrophy of both ciliated and nonciliated cells in postmenopausal women as well (45). Some reports noted atrophy affecting both the ciliated and nonciliated cells, while others noted little or no atrophy until after 60 years of age in women

(45).

Work of Other Investigators

The oviduct is of extreme importance as the site of fertilization, in the capacitation of sperm, ovum cleavage, and nurturing of the zygote (50, 51, 52, 53, 54). Exposure to the oviductal environment may be important for later survival of the embryo, since embryos transferred prematurely from oviduct to uterus fail to develop properly and implant (50).

The oviduct of the mare has an unusual role in selective retention of unfertilized ova while allowing unimpeded passage of the fertilized ova to the uterus (55, 56). Globular masses, either of desquamated tubal mucosa or from material released from follicles, may contribute to the retention of these eggs (55).

The mare is represented in some of the comparative studies of the oviduct but is not as extensively described or represented as women, food animals, or laboratory animals (32, 48, 57). Veterinary reports have tended to concentrate on pathologic findings in oviductal specimens from mares or enumeration of retained tubal eggs (31, 33, 55, 56). Macroscopic and microscopic lesions have been reported to occur fre-quently and include adhesions, paraovarian cysts, thick fibrous bands, microscopic intraepithelial cysts, focal lymphocytic infiltration, and presence of proteinoid material in the oviductal lumen (globular masses) (31, 33). Complete occlusion of the oviduct is rare (31). The signifi-cance of many of these findings is not known. The adhesions and fibrous bands involved oviducts of pregnant mares as well as nonpregnant mares, suggesting that these lesions may not be detrimental to fertility (31).

The presence of lymphocytic infiltrates in the infundibular and ampullary regions and microscopic intraepithelial cysts may interfere with fertility of the mare since they were found to be more common in non-pregnant mares (31).

Cyclic morphologic changes in the oviducts are reported to be slight. During estrus, subepithelial capillaries and epithelial cells may be enlarged, and there may be leukocytes present in the stroma (8).

Histologic Features of Oviducts from Six Mares

The morphologic appearances of the oviducts from the six mares are summarized in Table II. Oviducts were identified as to probable segment of origin on the basis of luminal diameter, degree of mucosal folding, and thickness and muscularity of the wall. The sections of oviduct all showed pseudostratified to simple columnar and/or cuboidal epithelium. Ciliated and nonciliated cells were identified in all mares.

In sections believed to be isthmus, "clear cells" with unstained cytoplasm and round, nonbasal nuclei were seen (Figure 5). Such cells were seen in sections of isthmus from mares #1, #3, #5, and #6. A large number of these "clear cells" were present in mares #1 and #6, while sections from mares #3 and #5 contained fewer of these cells. Slender cells with elongated nuclei ("peg" cells) were numerous in sections of the isthmus from mare #1 (Figure 5). They were few in the ampullae from mares #1, #4, and #5. A moderate number of "peg" cells were present in the preampulla, ampulla, and isthmus of mare #3. Many of the nuclei of "peg" cells in mares #2 and #3 appeared to be in the process of being extruded into the lumen (Figure 6). The epithelium of the oviductal ampulla of mare #4 contained pseudostratified low columnar to simple

TABLE II

HISTOLOGIC FEATURES OF OVIDUCTS FROM SIX MARES

Mare	Probable Segment(s) of Oviduct Examined	Type of Epithelium	Ciliated Cells	"Peg" Cells	Other
#1	Ampulla	Primarily pseudostratified columnar	Many	Moderate	None
	Isthmus	Simple cuboidal or columnar, pseudostratified columnar	Many	Moderate	Many columnar cells with clear cytoplasm and round, nonbasal nuclei
#2	Ampulla	Pseudostratified to simple columnar	Many	Moderate with nuclei close to luminal surface	None
#3	Preampulla	Simple to pseudostratified columnar	Many	Moderate with nuclei close to luminal surface	None
	Ampulla	Simple to pseudostratified columnar	Many	Moderate with nuclei close to luminal surface	None
	Isthmus	Simple cuboidal or columnar, pseudostratified columnar	Many	Moderate	Few columnar cells with clear cytoplasm and round, nonbasal nuclei

TABLE II (CONTINUED)

Mare	Probable Segment(s) of Oviduct Examined	Type of Epithelium	Ciliated Cells	"Peg" Cells	Other
#4	Ampulla	Pseudostratified low columnar to simple cuboidal, focal areas of flattened or multilayered (squamous) epithelium	Moderate	Very few	Several globular masses of protein
#5	Ampulla	Primarily pseudostratified columnar	Many	Moderate	None
	Isthmus	Simple cuboidal or columnar, pseudostratified columnar	Many	Moderate	Few columnar cells with clear cytoplasm
#6	Isthmus	Simple cuboidal or columnar, pseudostratified columnar	Many	Moderate	Many columnar cells with clear cytoplasm and round, nonbasal nuclei
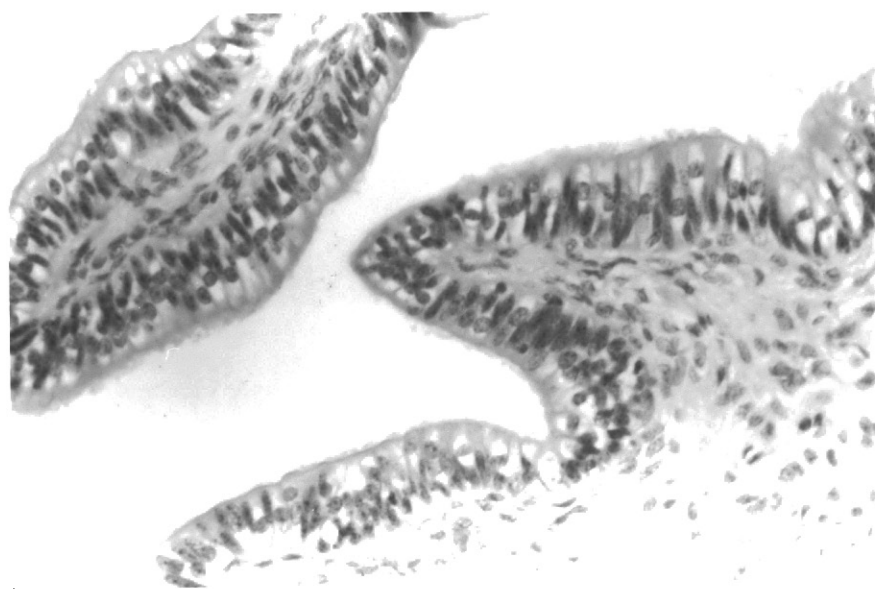
Figure 5. Many "clear cells" lining papillary frond in
           isthmus of oviduct of mare #6. Note clear
           cytoplasm with round, nonbasal nuclei inter-
           spersed with slender cells with elongated
           nuclei ("peg" cells). Hematoxylin and Eosin
           X120.
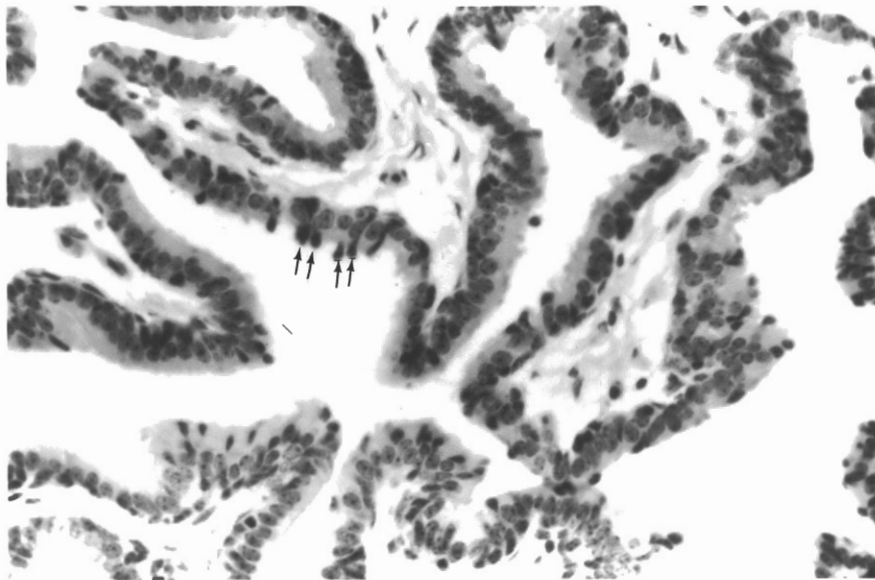
Figure 6.   Oviductal ampulla, mare #3.   Epithelium overlying
            loose stroma and a moderate number of "peg"
            cells with elongated nuclei projecting into the
            lumen (arrows).   Hematoxylin and Eosin X60.

cuboidal epithelium with focal areas of flattened cells. A few foci of multilayered epithelium compatible with incomplete squamous metaplasia were present (Figure 7). Several multilayered, fibrillary proteinaceous masses were present (Figure 8). Focal mineralization of the walls of arteries was recognized in sections of oviduct from mare #4 but not in sections from any other mare.

There was often an appearance of gland-like cross sections of tissue in the connective tissue of the oviduct that may have been an artifact of sectioning or the result of extensions of the epithelium that sometimes become occluded to form a blind pouch similar to the process described in sections of the human uterine cervix (7). The basement membrane was often not distinct, and cells thought to represent basal cells were infrequently identified.

Discussion

The isthmus was similar in four mares from which sections were identified as probably from this segment. There was a difference in the number of "clear cells" seen between mares that may represent a gradation along the oviduct or the influence of ovarian hormones that may have existed at the time of collection. The nature of the process that causes these cells to appear "clear" is not certain. Distinct vacuole formation was not apparent, and the nuclei of the cells did not show margination of chromatin or pyknosis suggestive of degeneration or death.

The basement membrane area was often indistinct, and basal cells, which supposedly rest along it, were not identified with certainty in any light microscopic specimens.
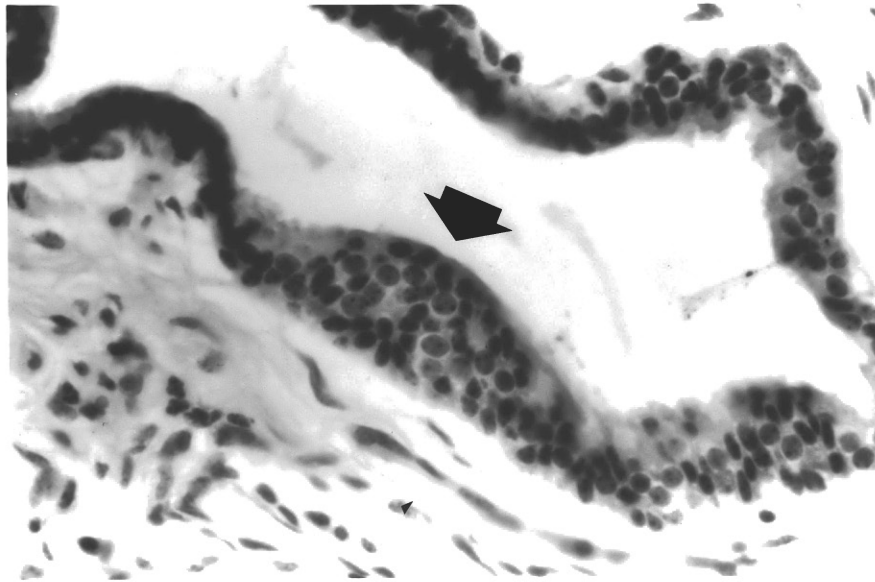
Figure 7. Focus of multilayered epithelium (arrow) com-
           patible with incomplete squamous metaplasia.
           Oviduct of mare #4. Hematoxylin and Eosin
           X160.

Figure 8.   Multilayered fibrillar luminal mass of proteina-
            ceous material (globular mass) in oviductal
            ampulla of mare #4 (same mare as in previous
            figure).   Hematoxylin and Eosin X25.

The ampulla was similar in four of five specimens examined. The slender cells with elongated nuclei correspond to those cells described by other investigators as "peg" cells (32, 47, 48). The function of these cells is not known, but they have been hypothesized to represent exhausted secretory cells (32, 47, 48). Differences in numbers of these cells between mares and among sections from the same mare may represent differences in recent secretory activity. The position of some of the elongated nuclei toward the luminal surface in two mares (#2 and #3) may represent extrusion of these into the lumen following the secretory period similar to the phenomenon reported to occur in the sheep, cow, and pig (32). Secretory cells such as those seen in the uterine, intestinal, or respiratory epithelium were not identified. It may be that electron microscopy or special stains for intracellular products may be necessary to recognize these cells. Alternatively, the "clear cells" seen in some sections and described above may represent secretory cells.

The presumed ampullary tissue from mare #4 was unusual when compared to the other three sections from mares #1, #3, and #5. This mare's sections contained several so-called globular masses of finely fibrillar, proteinaceous material that sometimes contained cells consistent with origin from the lining epithelium. Focal areas of thickened multilayered epithelial cells were present that may represent atypical squamous metaplasia. Squamous metaplasia has not been reported to occur in the oviduct of the mare; the significance of its presence in this case is not known. The epithelial features in this mare's oviduct, including the low cuboidal to flattened cells, as well as possible squamous metaplasia may represent atrophic changes associated with senile ovarian atrophy believed to be occurring in this animal.

The focal mineralization of vascular walls seen in sections of oviduct from mare #4 was similar to that observed by the author in some arteries in uterine biopsies from mares with clinical infertility. The significance of such a change in the oviduct is not known but may be related to the very old age of this mare.

Uterus

Anatomy

The uterus of the mare is attached to the sublumbar region and lateral walls of the pelvic cavity by the broad ligament. It is continuous with the uterine tubes or oviducts cranially and the vagina caudally. It has two horns (bicornuate), a body, and a cervix, a portion of which projects into the vagina (portio vaginalis) (6, 8, 9). The cavity of the nonpregnant uterus is a potential space, largely obliterated by folds of the mucosal lining, which are the source of endometrial biopsies frequently used to evaluate the histologic features of the endometrium (15). The uterus is surrounded by a serous coat, or perimetrium, that is continuous with the broad ligament and a muscular coat consisting of an external longitudinal and thick inner circular layer, which thickens and extends in the area of the cervix, where it forms a sphincter. A vascular and connective tissue layer exists between the two main muscular layers and contains some circular and oblique muscular fibers (6). The mucous membrane of the uterus, the endometrium, is not separated from the underlying layers by a muscularis mucosa; the connective tissue of the lamina propria is adjacent to the inner circular muscle layer. The lamina propria is in two layers, described by Kenney as the stratum compactum and the stratum spongiosum

(15). The stratum compactum lies just beneath the luminal epithelium, has a relatively high density of stromal cells, and has a prominent capillary network beneath the epithelial basement membrane. The stromal cells in this area do not normally produce extracellular collagen that is detectable by light microscopy. The stratum spongiosum is of lower cellular density, with many connecting fibers that create a spongy appearance. Capillaries, venules, small muscular arteries, and lymphatic vessels may be seen in this layer, in addition to numerous endometrial glands which are connected to the luminal surface by ducts. The epithelium of the endometrium lines the luminal surface of the longitudinal mucosal folds and is continuous with the epithelium of the ducts and glands (15).

Work of Other Investigators

The histology of the equine uterus has been described related to pathologic inflammation and fibrosis, and their distribution and correlation with breeding results (8, 13, 14, 15, 16, 58, 59). Changes associated with various stages of reproductive activity and the phases of the estrous cycle have been documented (15). Reports of features of uterine cytologic specimens have dealt primarily with the presence of inflammatory cells and microbial agents (22, 23, 24, 25). The research team with which the author is associated has reported epithelial features of importance in determining the stage of reproductive activity and patterns associated with fungal infection, uterine urine pooling, and response to treatment with an indwelling catheter (17, 18, 19, 20, 26, 36). To date, endometrial cellular features that enable reliable cytologic detection of specific phases of the estrous cycle have not

been detected by the methods used by this research team or by another investigator (60).

Features of Uteri from Six Mares

Light Microscopic Evaluation. The light microscopic evaluation of the uterine tissue included probable stage of reproductive activity (cyclic, anestrus, transitional, or senile atrophy); epithelial, glandular, and stromal features; degree, location, and type of inflammation; presence of nests of glands surrounded by fibrous tissue; and evaluation of vessels and muscle that were present due to collection of a full-thickness section at necropsy. The results of the evaluation are presented in Table III. Of the six mares, four were considered to be actively cycling on the basis of the histologic morphology (#1, #2, #3, and #6). The uterine sections from one mare (#4) were considered to represent senile atrophy (Figure 9), while those from mare #5 were consistent with seasonal transition.

The vessels and muscle of all mares except #4 were without significant abnormalities. A few vessels in the lower stratum spongiosum of mare #4 had diffuse sclerosis of the medial layers. A mild lymphoplasmacytic infiltrate was present in the sections from mare #4, while diffuse lymphocytes were seen in the uteri from mares #1, #2, and #5 and focal accumulations of lymphocytes in mare #3. Eosinophils were present in mare #5 along with many large hemosiderophages containing relatively light, reticular hemosiderin. Eosinophils also were present in uterine sections from mares #1 and #2. The uterine specimens from mares #1, #2, #5, and #6 were free of pathological fibrosis and fibrosed glandular nests. Sections from mare #3 contained a few foci of fibrosed nests,

TABLE III

HISTOLOGIC EVALUATION OF UTERI FROM SIX MARES

Mare	Stage of Reproductive Activity	Luminal Epithelium	Glands	Stroma	Vessels and Muscle	Inflammation	Fibrosis
#1	Cyclic	Columnar	Numerous with open lumens	Moderate edema of SC and SS	NSA	Moderate, diffuse, superficial lymphocytes, few superficial eosinophils	None
#2	Cyclic	Low columnar	Numerous lumens not widely open	Slight condensation of SC, mild edema of SS	NSA	Few superficial lymphocytes and eosinophils	None
#3	Cyclic	Columnar to low columnar foci of abnormally vacuolated columnar cells associated with inflammatory foci	Numerous lumens not widely open, some with inspissated material	Slight condensation of SC, mild edema of SS	NSA	Moderate focal lymphocytes	Few fibrosed nests of dilated glands

TABLE III (CONTINUED)

Mare	Stage of Reproductive Activity	Luminal Epithelium	Glands	Stroma	Vessels and Muscle	Inflammation	Fibrosis
#4	Senile atrophy	Low cuboidal to flattened	Few in a thin layer, many dilated and containing inspissated material	Very condensed SC and SS	Few vessels with sclerotic media, atrophic musculature	Mild, diffuse lymphocytes and plasma cells, moderate foci of neutrophils	Moderate number of fibrotic nests of glands
#5	Transition	Low columnar	Moderate number, straight, lumens not widely open	Slight condensation of SC, mild edema of SS	NSA	Moderate, diffuse, superficial lymphocytes, few superficial and deep eosinophils, many large hemosiderophages	None
#6	Cyclic	Columnar	Numerous lumens open	Moderate edema of SC and SS	NSA	None	None

SC = stratum compactum. SS = stratum spongiosum. NSA = no significant abnormalities.
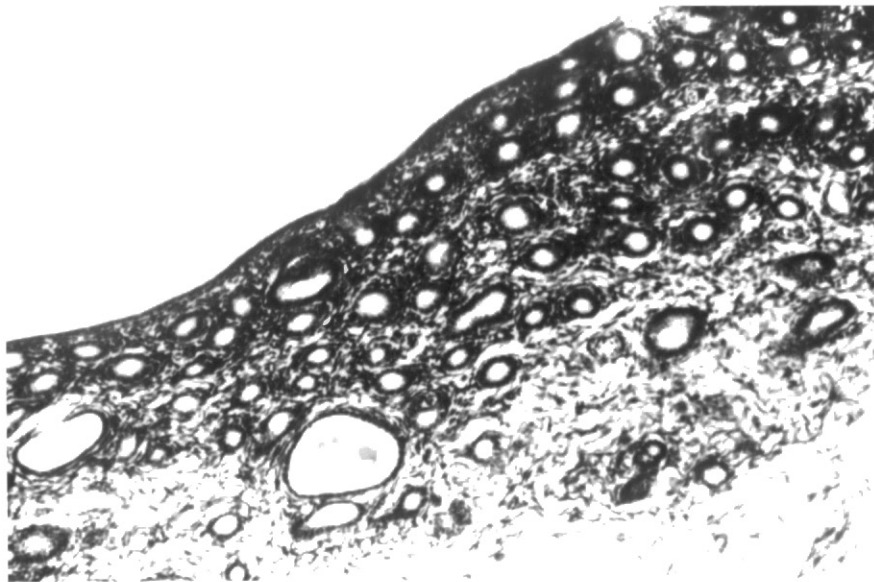
Figure 9. Very condensed stroma, low cuboidal epithelium, and few irregularly dilated glands, indicative of senile uterine atrophy, mare #4. Hematoxylin and Eosin X40.

while sections from mare #6 contained a moderate number of fibrotic

glandular nests. The muscular layers of the uterus of mare #4 showed

signs of atrophy. Cross sections of muscle fascicles were small, very

cellular, and separated by large amounts of connective tissue. The

muscular layers from the other mares were much thicker, and muscle

fascicles were large, less cellular, and less separated by connective

tissue.

Electron Microscopic Evaluation. Luminal epithelium was present

and similar in sections from two mares (#1 and #5). The epithelial

cells were columnar and contained both ciliated and nonciliated cells.

The nonciliated cells had small, sparse microvilli and apical cytoplas-

mic vesicles containing fuzzy, light to moderately dense material

(Figure 10). The luminal surface was arranged in irregular projections

that were more prominent in mare #1 than mare #5. Glands were present

in sections from all mares except #6. Necks or ducts of glands were

lined by ciliated and microvillous cells with granular cytoplasm with

few cytoplasmic vesicles or other recognizable organelles. Deeper

glands contained very few ciliated cells and cytoplasmic vesicles con-

taining globular material similar to that seen in previous studies by

the author (36). These vesicles were more numerous in glands in sec-

tions from mare #2 than in mares #1 or #5. The glands in the sections

from mare #6 had distinct granular cytoplasm with very few vesicles and

some organelles that may be lysosomes or mitochondria.

The glands present in sections from mare #3 were in an area of a

fibrosed glandular nest. The affected glands were dilated and lined by

multiple layers or a single layer of epithelial cells with nuclei that

were sometimes horizontally rather than vertically oriented (Figure 11).
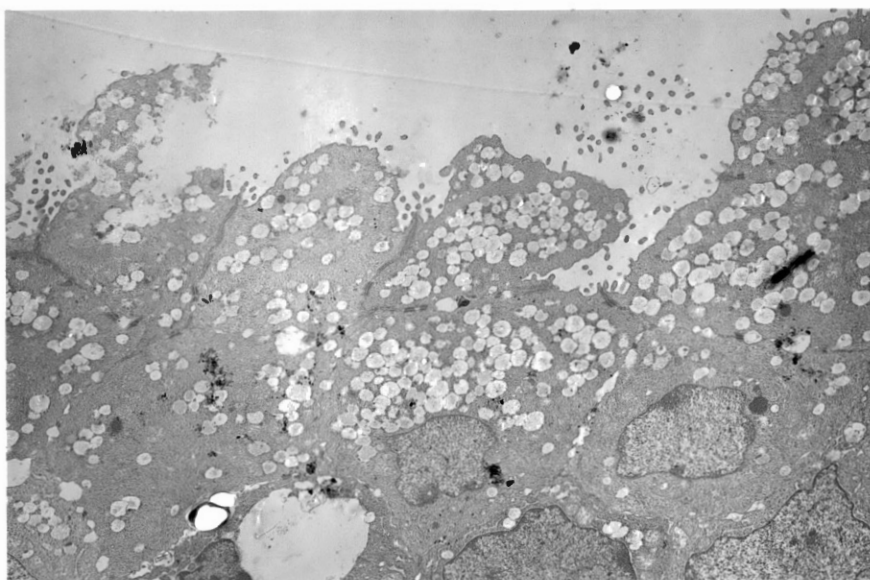
Figure 10. Uterine luminal epithelium in electron microscopic preparation from mare #1. Note irregular luminal surface and numerous secretory cells with sparse, short microvilli and vesicles containing fuzzy, light to moderately dense material. Uranyl Acetate-Lead Citrate X3,600.
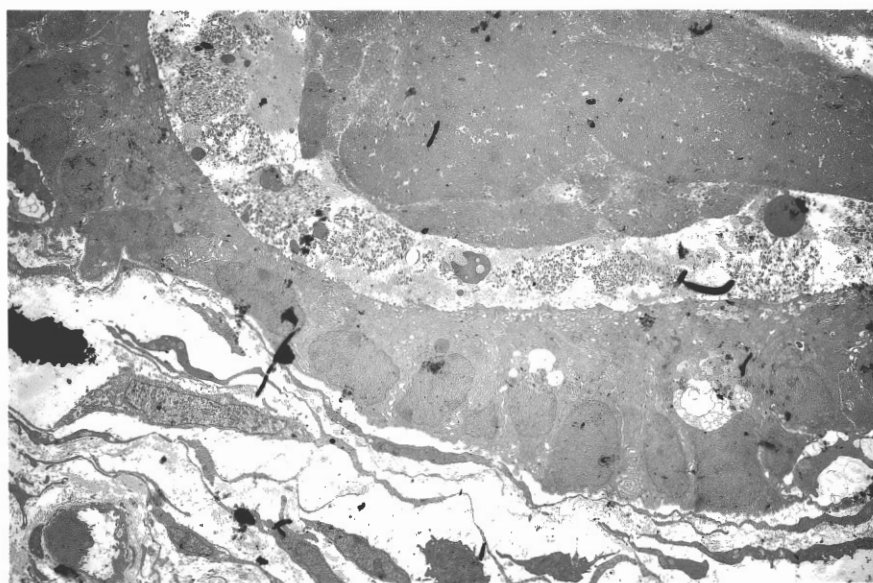
Figure 11. Electron microscopic preparation of abnormal
uterine gland surrounded by fibrosis, mare #3.
Note flattened glandular epithelial cells and
inspissated luminal material. Uranyl Acetate-
Lead Citrate X1,400.

Homogeneous material was present in the center of the lumen of some glands and was surrounded by a peripheral layer of irregular fragments that resembled cellular cytoplasm. The lining cells often contained secretory vesicles containing fuzzy material similar to that seen in luminal epithelial cells but without globular material similar to that seen in glands in other mares. Intercellular junctions between the abnormal glandular cells appeared slightly widened when compared with normal glands in other mares, and distinct, long, interdigitating processes could be seen between the separated cells at their basal aspects (Figure 12).

Only muscle was present in sections from mare #4. It contained distinct vacuolated or granular muscle cells that differed from those seen in other sections of uterine musculature from other mares.

Discussion

Mare #4 was considered to have senile atrophy of the uterus based on the observation by the author of necropsy specimens from one other mare, also of advanced age (estimated by examination of the teeth to be greater than 25 years old). Similar senile changes have been described by Kenney (15). In these mares, the degree of contraction of the tissue, thinness of the glandular layer, and variable distension of glands with retention of inspissated material is sufficiently different from that normally encountered as a result of immaturity or physiologic winter anestrus to be recognizable as a separate entity.

The presence of eosinophils in the equine uterus may be the result of a variety of conditions, including acute inflammation, fungal infections, urine pooling, or pneumouterus (13, 15, 17, 18, 19, 20, 21, 36).
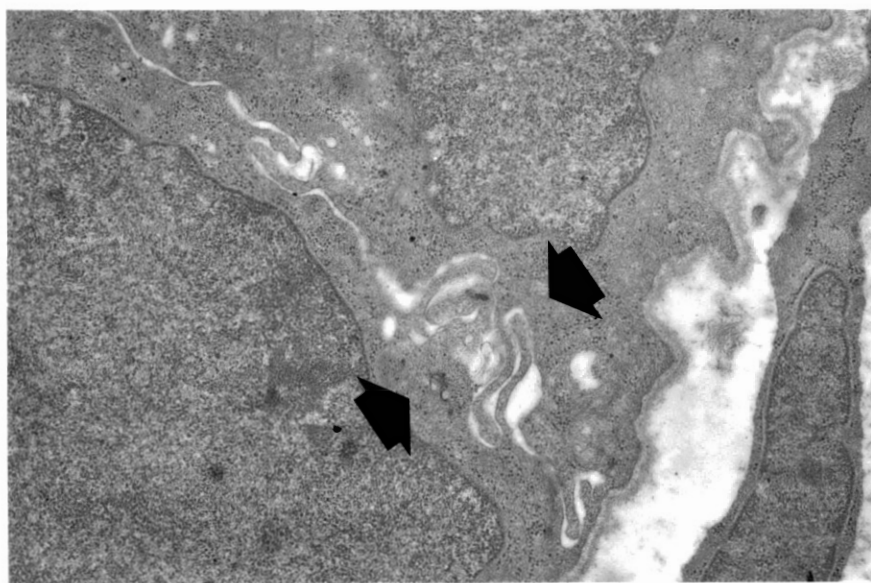
Figure 12. Widened intercellular junction with interdigi-
tating processes (arrows) in abnormal uterine
gland surrounded by fibrosis shown in Figure
11.   Electron microscopic preparation of
uterus from mare #3.   Uranyl Acetate-Lead
Citrate X3,600.

In mare #5, the presence of eosinophils and large, reticulated hemo-siderophages was suggestive of recent foaling, which may have allowed exposure of the endometrium to air and subsequent eosinophilic infiltration. The duration of eosinophil persistence following such hypothesized exposure is unknown. The presence of eosinophils along with moderate, diffuse, superficial lymphocytes in the uterus of mare #1 and with a few superficial lymphocytes in the uterus of mare #2 may be normal or indicative of an inflammatory process. The presence of eosinophils, even with relatively mild inflammation (mares #1 and #2), in the uterine biopsy of a mare with a history of infertility would be considered abnormal.

The diffuse sclerotic changes in the medial layers of some vessels in the uterus of mare #4 differ from focal sclerotic changes seen by the author in five biopsy specimens from mares with histories of infertility and in the vessels of the ovary from this mare. Kenney reports that pathologic vascular changes are uncommon in the endometrium of the mare, and the author's experience confirms this observation (15). Kenney reports the most common vascular changes to be lymphocytic cuffs sur-rounding arterioles and venules. The changes he considers sclerotic, however, are described as hyaline or basophilic degeneration of the tunica media or intima and may differ from those seen by the author in endometrial biopsy specimens. The changes seen in the uterus of mare #4 in this study more closely resemble those described by Kenney (15). It is possible that they may represent a senile change related to the atrophic state of the endometrium or a degenerative change of inflamma-tory or other origin. The focal mineralization observed in the vessels of the ovary of this mare and the uteri of infertile mares may represent

a variant of the same process. The effect of such changes on fertility and the role it plays in mares with clinical infertility is not currently known.

The characteristics of the surface epithelium identified with the electron microscope differ from those previously identified and associated with fall seasonal transition by the author (36). The tall columnar cells with luminal vesicles and predominantly microvillous surfaces are consistent with the foamy columnar cells seen in histologic sections and associated with cyclic activity. The glandular epithelial characteristics were similar to those previously seen from mares during fall seasonal transition (36).

The pathologic changes in the dilated glands of the fibrosed nest in sections from mare #3, to the author's knowledge, have not been reported previously. The features suggest that the secretion of affected glands may differ from that of normal glands, since globular material in vesicles, similar to that observed in glands from other mares, was not seen. Instead, flocculent material, similar to that seen in luminal epithelial cells, was present. The intercellular separations suggest that intercellular junctions also may be altered as a result of pathologic processes.

The abnormal, granular, and vacuolated muscle cells seen with the electron microscope in sections of uterus from mare #4 may correspond to those changes in cellularity and size seen in light microscopic specimens and interpreted to represent atrophy.

The features seen in electron microscopic specimens corresponded well with those in histologic specimens. The degree of stromal compaction or edema was not as evident as in histologic sections, and the

small pieces of tissue present in plastic-embedded thick sections were
not considered large enough to form the basis for an interpretation of
phase of reproductive activity.

Uterine Cervix

Anatomy

The uterine cervix is the constricted aspect of the caudal uterus
leading into the vagina. It is thick walled and has a thick layer of
circular muscle rich in elastic fibers. It is relatively easily
dilated, even during periods not corresponding to sexual receptivity
(6). Its internal surface is covered by longitudinal folds of mucosa
which are continuous with those of the uterine body. One of these folds
continues onto the floor of the vagina as a frenulum (6, 8). The portio
vaginalis of the uterine cervix is that part which projects into the
vagina. The reflections of the vaginal mucosa along the front, sides,
and back of the portio vaginalis of the cervix form the vaginal fornices
(6, 7, 8).

Histologically, the cervix is composed of longitudinal folds of
connective tissue covered by mucus-secreting columnar epithelial cells.
Like the large intestine, the subunits of the folds are referred to as
villi and crypts (7). The cervix contains no glands. Cranially, the
epithelium undergoes a gradual transition into that lining the uterine
cavity, but studies delineating the exact features of this transition
area could not be found. Caudally, the columnar cervical epithelium of
the mare meets the stratified squamous epithelium of the vagina, forming
an abrupt, distinct junction (8, 22). The exact location of this
junction relative to the external cervical os is not clear in the

literature.

Work of Other Investigators

Review of the literature indicates the cervix to be the most neglected tissue of the reproductive tract of the mare in relation to light and/or electron microscopic examination. Ginther reports that cyclic histologic changes of the cervix have been little studied but that the epithelial cells may be taller, swollen, and filled with mucus during estrus (8). The gland-like configurations seen in histologic sections from women have been shown to be extensions of crypts which sometimes become occluded or pinched off to form a blind tube or tunnel (7).

Features of Uterine Cervices from Six Mares

Light Microscopic Evaluation. The sections of cervix from each mare were analyzed for tissue structure, cell type, and distribution, as presented in Table IV. No tissue was available from mare #4. The sections had characteristic highly branched, arborous microscopic architecture. The cells of the cervix of mares #3 and #6 ranged from low columnar to columnar, and both ciliated and nonciliated cells were recognized. The majority of cells contained large, plump, vesicular nuclei, located basally. Many were secretory cells with slightly foamy cytoplasm. Some foci contained typical "goblet" cells with distended, clear or finely reticular cytoplasm. A few slender cells with elongated nuclei, resembling "peg" cells of the oviduct, were seen (Figure 13). Sections from mare #3 also contained focally extensive areas of mature, squamous metaplasia and attenuated villi with underlying neutrophils,

TABLE IV

HISTOLOGIC FEATURES OF UTERINE CERVICES FROM SIX MARES

Mare	Type of Epithelium	Ciliated Cells	Nonciliated Cells	"Peg" Cells	Other
#1	Simple low columnar to cuboidal	Many	Few with small, dense basal nuclei	None	None
#2	Simple low columnar to cuboidal	Moderate	Many	Few	Few cells with clear cytoplasm
#3	Simple columnar, focal areas of mature squamous metaplasia with epithelial atypia	Many	Many with large, plump basal nuclei	Few	Many secretory cells, several areas with "goblet" cells, neutrophils in areas of squamous metaplasia
#4	(No tissue)				
#5	Simple cuboidal to low columnar	Few	Many with hypochromic, basal nuclei	Moderate	None
#6	Simple columnar to low columnar	Many	Many with large, plump basal nuclei	Few	Moderate number of secretory cells, several areas with "goblet" cells
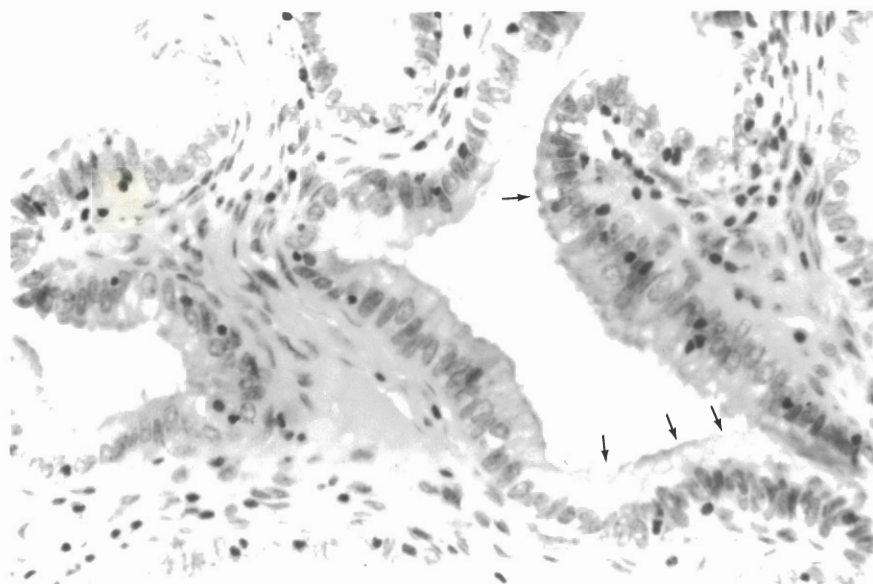
Figure 13. Numerous secretory cells with foamy cytoplasm in
cervix of mare #6. "Goblet" cells are indi-
cated by the arrows. Hematoxylin and Eosin
X120.

lymphocytes, and plasma cells. Many of the superficial squamous cells were vacuolated and separating from the adjacent cells. Intraepithelial neutrophils were present in those areas.

Sections from mares #1 and #2 differed from those of mare #6 in that the cells ranged from low columnar to cuboidal and had smaller, more uniform, and less vesicular nuclei (Figure 14). A greater number of ciliated cells were identified, and the majority of cells had homogenous pink cytoplasm without obvious secretory features.

The cervix from mare #5 contained low columnar to cuboidal cells, few of which appeared ciliated. Many had hypochromatic nuclei similar to those recognized by the author in sections of uterus from mares during fall seasonal transition (36) (Figure 15). Very few cells were obviously secretory, and a moderate number of slender cells with elongated nuclei ("peg" cells) were seen. A few cells contained rounded, clear vacuoles with a round central nucleus. Focal areas were observed in which the usual arborous villous branches of the mucosa were few and short.

Electron Microscopic Evaluation. Satisfactory electron microscopic sections were available from all mares except #4. The sections from mare #3 had an irregular luminal surface lined primarily by secretory cells with a moderate number of microvilli and numerous apical vesicles containing fuzzy material similar to that seen in luminal epithelial cells of the uterus in this study (Figure 16). A few ciliated cells were present. One area contained superficial secretory cells overlying stratified squamous epithelium resembling focal metaplastic epithelium of the cervix of women (7, 61, 62) (Figures 17 and 18). Less secretory activity was apparent in cervical specimens from mares #2 and #6.
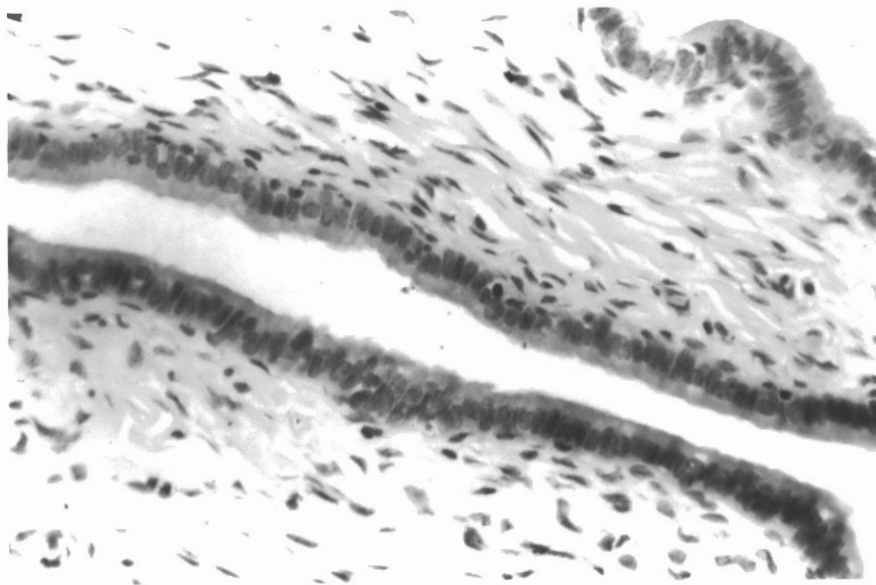
Figure 14. Cervix from mare #1. Nonsecretory cells with
            homogeneous cytoplasm. Compare to secretory
            cells in Figure 13. Hematoxylin and Eosin
            X120.
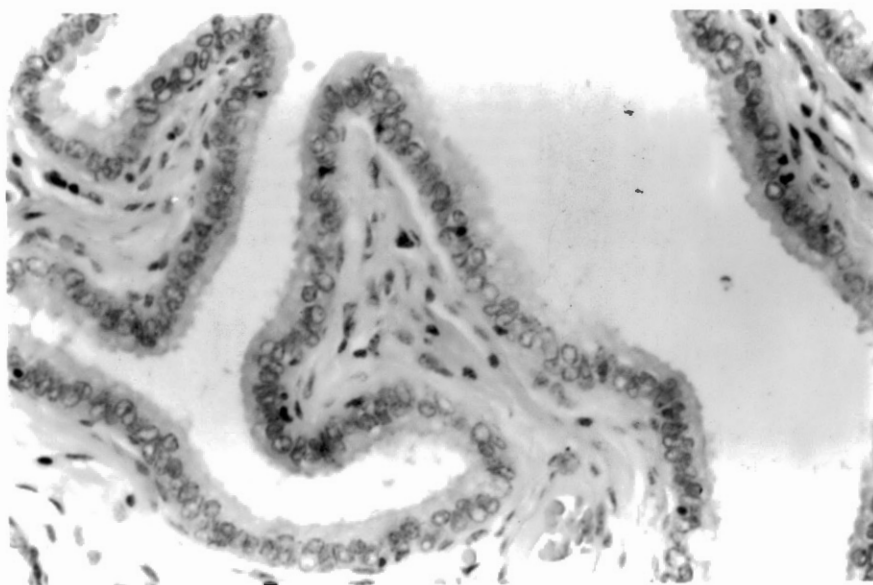
Figure 15. Primarily nonciliated columnar cells with
hypochromatic nuclei. Cervix from mare #5.
Hematoxylin and Eosin X64.
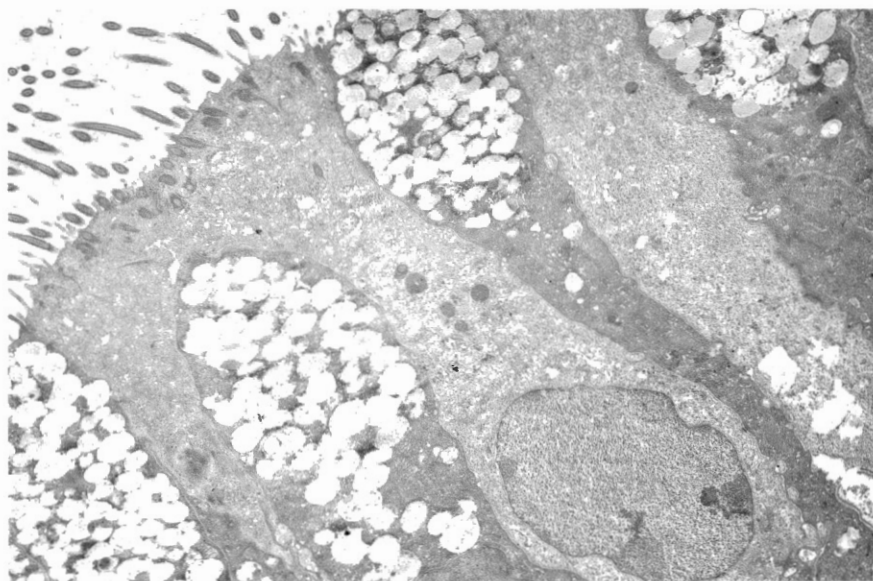
Figure 16. Two ciliated nonsecretory cells surrounding and
adjacent to a fragment of nonciliated secre-
tory cells.   Electron microscopic preparation
of cervix from mare #3.   Hematoxylin and Eosin
X3,600.
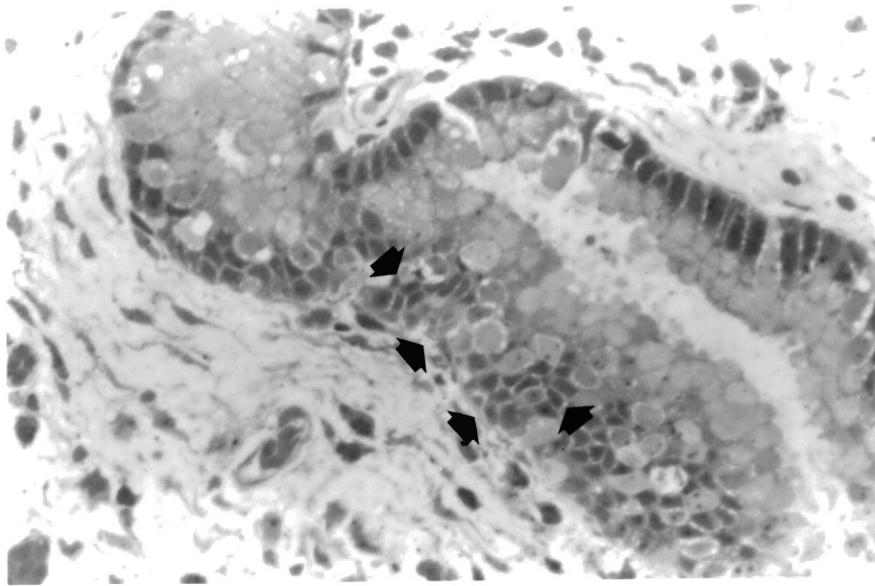
Figure 17. Plastic-embedded, thick section of cervix from
            mare #3. Note focus of incomplete squamous
            metaplasia containing multiple layers of
            intermediate squamous epithelial cells with
            intercellular bridges (arrows) and secretory
            cells resting on them and forming the opposite
            epithelial surface. Mallory's Stain X320.
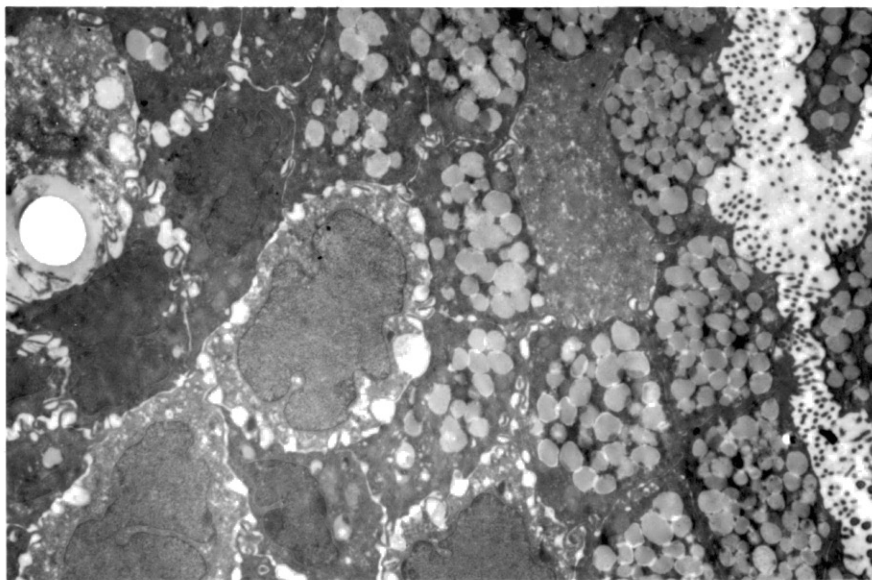
Figure 18. Electron microscopic preparation of cervix
containing superficial secretory cells and
underlying squamous cells with intercellular
bridges. Compare with plastic-embedded, thick
section from same area in Figure 17. Uranyl
Acetate-Lead Citrate X3,600.

However, mare #6 had very distinct surface projections with vesicles containing fuzzy material (Figure 19). Other cells were more lightly stained and had homogeneous, granular cytoplasm and cilia.

A moderate number of ciliated cells and few with sparse apical secretory vesicles and microvilli were seen in cervical specimens from mare #2. Focal areas of lighter stained and apparently degenerating cells with perinuclear vacuoles and homogeneous granular cytoplasm were present (Figure 20). Several slender cells with elongated nuclei and luminal microvilli that may correspond to the "peg" cells in light microscopic sections were present.

A moderate number of secretory cells and foci of cells with clear, irregular cytoplasmic vacuoles were seen in specimens from mare #1. Several cells with both secretory vesicles and cilia were present.

The cervix from mare #5 differed from that of the other mares in that distinct surface projections without many secretory vesicles were seen (Figure 20). These resembled projections observed in several uterine cells in a previous study by the author (36). A large number of cells with homogeneous cytoplasm without cilia or microvilli and without cytoplasmic granulation were present.

Discussion

The light microscopic features of the cervical epithelium differed among the mares examined, presumably related to variation in hormonal stimulation and secretion associated with stage of the estrous cycle. The morphology of the epithelial cells with very hypochromatic nuclei in mare #5 was strikingly similar to features seen in the uterus of mares during fall seasonal transition. The focal areas of few, very short
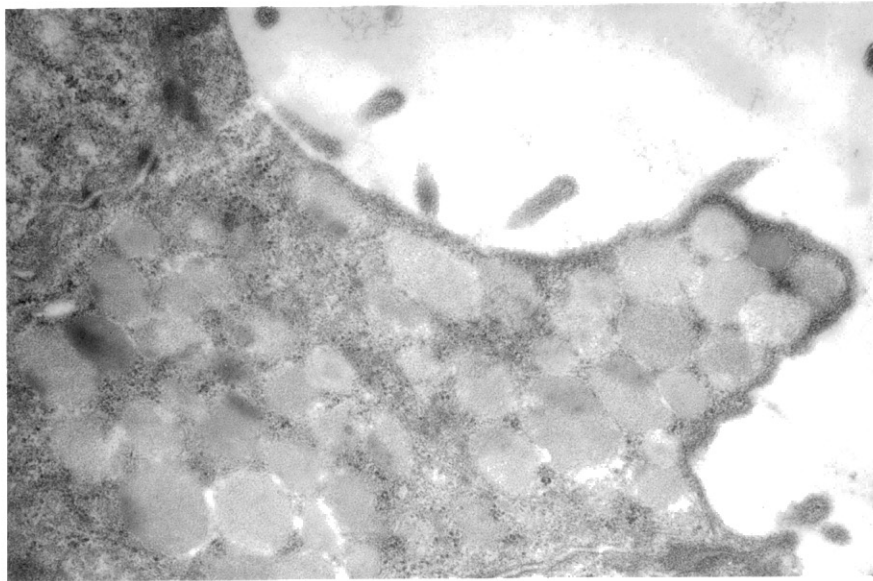
Figure 19. Distinct surface projection containing secretory
vesicles. Electron microscopic preparation of
cervix from mare #6. Uranyl Acetate-Lead
Citrate X19,000.
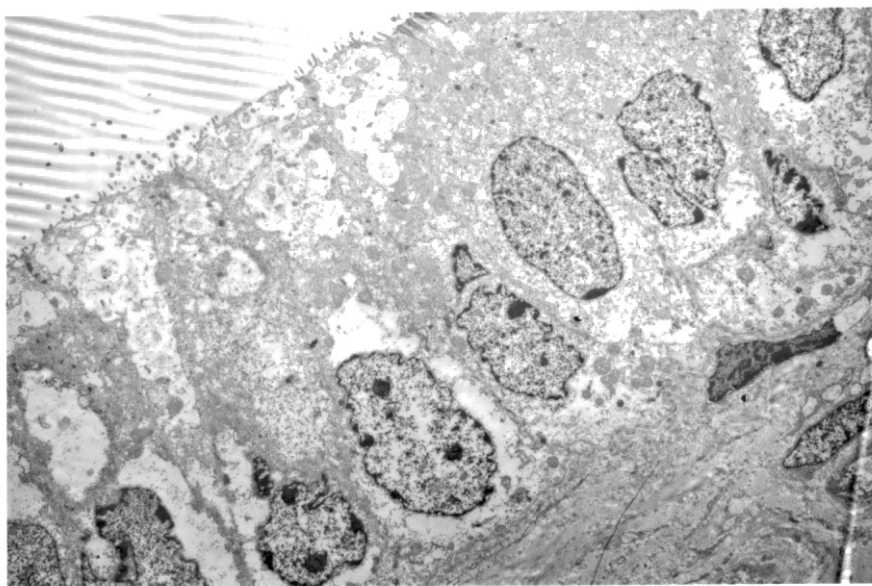
Figure 20.   Focus of lightly stained columnar cells with
             perinuclear vacuoles and granular cytoplasm.
             Electron microscopic preparation of cervix
             from mare #2.   Uranyl Acetate-Lead Citrate
             X1,900.

villi may represent a transitional area between the cervix and uterus or be the result of focal differences in the villous structure of the cervix itself. The large number of "peg" cells seen in this mare was similar to "crowded" cells seen in the uterus of mares during fall seasonal transition (36).

The variation in electron microscopic specimens was obvious. The significance of the nonvesicular cytoplasmic surface projections in mare #5 is not known. These projections were less obvious in corresponding thick sections. Their presence in human uterine cells has been associated with apocrine secretion (1), but mention of them in the human uterine cervix was not found.

The focal changes in small groups of contiguous cells was particularly impressive in thick and thin sections of the cervix. Such a distribution was not appreciated in light microscopic sections. The focal area of squamous metaplasia recognized with the electron microscope in the cervix of mare #3 was not present in paraffin-embedded sections. Similar squamous metaplasia in the cervix of women is seen so frequently that the terms "original squamocolumnar junction" and "metaplastic squamocolumnar junction" are used to differentiate the origin of these changes (7, 61, 62). Various investigators have proposed that ingrowth of squamous epithelium occurs from the margins of the squamocolumnar junction or by differentiation of multipotential reserve cells (7, 62). The metaplastic process, involving conversion of columnar to squamous epithelium, also has been hypothesized to be the result of activity of the subepithelial cells, degeneration of the original basement membrane, and eventual inclusion of modified stromal cells along with the columnar epithelial cells above a newly formed basement

membrane. The remaining columnar cells eventually degenerate, and the activated subepithelial cells transform into the progenitor cells of the squamous epithelium. As this process spreads, adjacent villi may be progressively fused with some retention of variable amounts of columnar epithelium in the depths of crypts and tunnels due to presumed isolation from the irritative phenomena that initiated the squamous metaplasia. The epithelium may progress to a stage of maturity that is histologically undifferentiatable from the original squamous epithelium of the human vagina and ectocervix (7). However, the occurrence of squamous metaplasia is focal, and various degrees of maturity and stages of transformation may exist simultaneously within the same human cervix (7). Squamous metaplasia of the cervix of women is most active during late fetal life, adolescence, and pregnancy, times in which there occurs an eversion of the surface epithelium of the endocervix to an ectocervical position. Exposure to the acidic pH of the vagina is postulated to be the stimulus for this event. In the human, the pH change across the cervicovaginal portio from approximately 8.5 to 4.0 is reported to be one of the most extreme in the body (7).

Occurrence of squamous metaplasia of the uterine cervix of the mare, such as that observed in mare #3, as a physiologic or pathologic process has not, to the author's knowledge, been described previously. Although the vaginal pH of the mare is more alkaline (pH 7.0-8.0) than that reported in women (8), the variation in cervical length and degree of patency during the cycle suggests that some areas of cervical columnar epithelium may potentially be exposed to the vaginal environment and might undergo a metaplasia similar to that described in women. The potential for metaplasia as a result of infectious or inflammatory

processes that may change the cellular environment also exists. The cervix of the mare is subject to laceration at foaling and may be recognized grossly to be inflamed as a result of extension of vaginal or uterine infectious or inflammatory conditions. Squamous metaplasia of the equine cervix has been observed by the author in one other mare and was believed to be the result of a histologically identifiable inflammatory process involving the uterus, cervix, and vagina.

Vagina

Anatomy

The vagina of the mare extends through the pelvic cavity from the uterine cervix to the vulva. A small, anterior part is covered by a reflection of the peritoneum, while the majority is retroperitoneal and surrounded by loose connective tissue containing a venous plexus and fat (6). The lumen of the vagina is obliterated by its walls; a cross section would appear as a transverse slit. The vagina proper continues to the area of the external urethral orifice, where a transverse fold, the hymen, demarcates it from the vaginal vestibule (6, 8). The wall of the vagina contains a thin outer longitudinal muscular layer and a thick inner circular muscular layer. The mucosal surface is covered by stratified squamous epithelium (6, 8). The production of a superficial keratinized layer is reported to be very sparse in the mare (8, 11, 12).

The layers of the squamous epithelium of the vagina have been classified histologically into five zones as follows:

1. Zone 1. Basal layer or stratum cylindricum. This is the zone adjacent to the basement membrane and consists of a single row of small cylindrical cells with relatively large nuclei.

2. Zone 2. Parabasal or prickle cell layer or stratum spinosum profundum. This zone contains several layers of polyhedral cells with fairly large nuclei and distinct intercellular bridges.

3. Zone 3. Intermediate, navicular, or clear layer or stratum spinosum superficiae. Cells of this zone are slightly flattened and have glycogen-rich cytoplasm that may appear vacuolated.

4. Zone 4. Intraepithelial or condensation zone. This zone is of variable thickness and may not be recognized. It contains closely packed polyhedral cells containing keratohyaline granules.

5. Zone 5. Stratum corneum. This zone is the most superficial and contains flattened cells with small pyknotic nuclei and a large amount of cytoplasm (7).

Work of Other Investigators

The cytologic and histologic appearance of the vagina of the mare has not been investigated extensively. Previous studies, conducted primarily prior to 1950, gave contradictory results (11, 12). Changes in the histologic appearance of the equine vagina related to the estrous cycle have been reported (11, 12). In one study, there was an increase in epithelial thickness up to 5 to 10 cell layers deep during estrus. The minimum thickness of the epithelium was reported to occur during the interestrual period and early and late pregnancy. The nonestrual thickness was indicated to range from four to nine cell layers deep, a range which considerably overlaps that reported by the same investigators during estrus (12). These investigators separated the vaginal epithelium into three zones: a basal zone, a middle zone of varying numbers of irregular polyhedral cells, and a superficial layer of flattened,

nucleated squamous cells. The basal zone was reported to become more prominent shortly prior to and during estrus, with increased mitotic figures and more deeply stained, elongated nuclei whose long axis was perpendicular to the basement membrane. The polyhedral cells of the middle zone made up the bulk of the epithelium. Distinct layers of flattened surface cells which formed the third zone were never prominent except during estrus. During the first 40 days of pregnancy, the condition of the vaginal epithelium was similar to estrus (12).

Other investigators emphasized the degree of folding of the vagina, indicating that secondary folds and their thickness increased toward the end of estrus (11). These investigators reported that prior to estrus the epithelium was thin with only one dark-stained and one flattened layer and that the thickness increased with the duration of heat. Intraepithelial leukocytes were most prominent in their study during the last day of estrus (11).

No reports of changes in the vaginal cytologic or histologic appearance due to annual variation in reproductive activity could be found.

Features of Vaginas from Six Mares

Light Microscopic Evaluation. The results of the histologic analyses of vaginal specimens collected as a part of this study are presented in Table V.

The degree of folding of the vaginal mucosa varied among mares, with mares #1 and #5 having many folds, #2, #3, and #6 a moderate number, and #4 a few. The numbers of cell layers varied from 3 to 10, with the greatest number of cell layers observed in mare #6 (Figure 21).

TABLE V

HISTOLOGIC FEATURES OF VAGINAL TISSUE FROM SIX MARES

Mare	Degree of Mucosal Folding	Number of Epithelial Cell Layers/ Thickness	Basal Cells	Middle Cells	Surface Cells	Inflammatory Cells	Other
#1	Very folded	3-6/ moderately thick	Focally prominent, vertically oriented nuclei	Moderately plump	Nucleated, variably flattened cells over raised folds to cuboidal in depths of folds	Few foci, lymphocytes, and plasma cells, few intraepithelial neutrophils	None
#2	Moderately folded	3-5/ moderately thick	Focally prominent, vertically oriented nuclei	Moderately plump	Nucleated, variably flattened cells over raised folds to cuboidal in depths of folds	Few submucosal plasma cells	None

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TABLE V (CONTINUED)

Mare	Degree of Mucosal Folding	Number of Epithelial Cell Layers/ Thickness	Basal Cells	Middle Cells	Surface Cells	Inflammatory Cells	Other
#3	Moderately folded	3-5/ moderately thick	Foci of vertically oriented nuclei, some plump nuclei blending with underlying inflammatory cells	Moderately plump	Focally extensive areas of vacuolated, loosely attached nucleated cells	Mild to severe, focal lympho- cytes and plasma cells, few foci, neutrophils, some intraepithelial	Mild, diffuse submucosal edema
#4	Few folds	3-4/ very thin	Few foci of vertically oriented nuclei, majority small and flat	Small and flat	Predominately flattened nucleated cells	Few submucosal foci of lymphocytes	Few accu- lations of protein material in depths of folds

TABLE V (CONTINUED)

| Mare	Degree of Mucosal Folding	Number of Epithelial Cell Layers/ Thickness	Basal Cells	Middle Cells	Surface Cells	Inflammatory Cells	Other
#5	Very folded	3-5/ moderately thick	Focally prominent, vertically oriented nuclei	Moderately plump	Nucleated, variably flattened cells over raised folds to cuboidal in depths of folds	Moderate, diffuse lympho-cytes and plasma cells, few intra-epithelial neutrophils	None
#6	Moderately folded	5-10/ very thick	Focally prominent, vertically oriented nuclei	Large and plump	Nucleated, variably flattened cells over raised folds to cuboidal in depths of folds	Mild to moderate, diffuse lympho-cytes and plasma cells, many intra-epithelial neutrophils	None
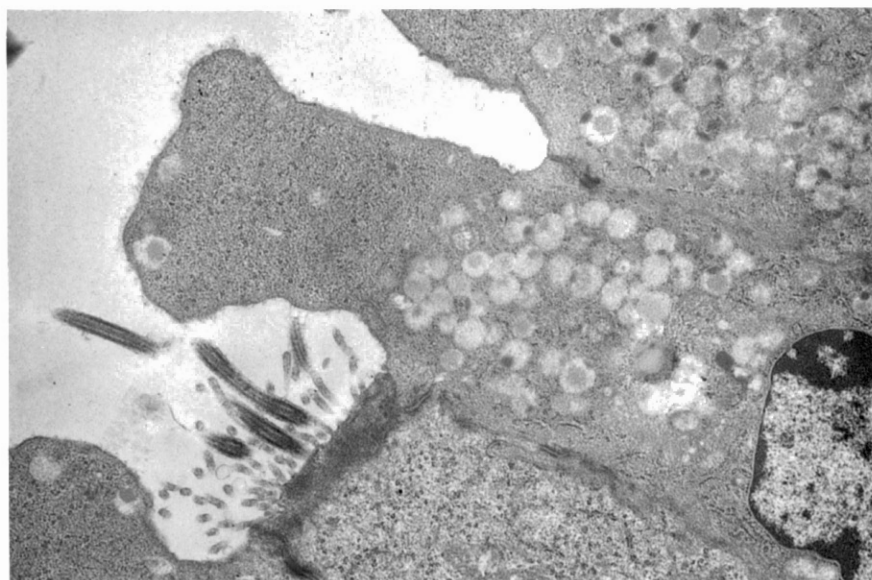
Figure 21. Surface projections without many secretory vesi-
cles. Electron microscopic preparation of
cervix from mare #5. Compare with similar
projection containing many secretory vesicles
in Figure 18. Uranyl Acetate-Lead Citrate
X7,200.

The remainder of the mares had epithelia three to five cell layers thick, but the thickness of the epithelium did not depend just on numbers of cell layers. The size of the cells also contributed to the thickness (Figure 22).

A basal zone with elongated, vertically oriented nuclei was seen in mares #1, #2, and #6. Mare #3, whose vagina contained significant inflammatory and epithelial changes, had foci of vertically oriented basal nuclei; in other areas, the basal cells were plump, without elongated nuclei, and blended with the underlying inflammatory infiltrate. In mare #4, the basal cells with horizontal, flattened nuclei differed only slightly from more superficial cells (Figure 22).

The surface layer of the epithelium varied within sections and between mares. In all mares except #4, nucleated epithelial cells lining the surface generally were more flattened over the raised mucosal folds and less flattened within the depths of the folds (Figure 23). In mare #4, the surface was more uniformly flattened.

A mild to moderate lymphoplasmacytic infiltrate was present in the submucosa of all mares, and a few neutrophils or lymphocytes could be seen migrating through the epithelium. Mare #3, in addition to the lymphoplasmacytic infiltrate, had significantly more pronounced focal infiltrates of plasma cells and neutrophils and vacuolation and separation of surface epithelial cells, consistent with an inflammatory process (Figure 24). A vaginal epithelial zone containing keratohyaline granules (zone 4) similar to that described previously was not seen in any specimens. Anucleated, keratinized surface cells were not seen in any of the sections examined with the light microscope.

Electron Microscopic Evaluation. Tissues from all mares except #4
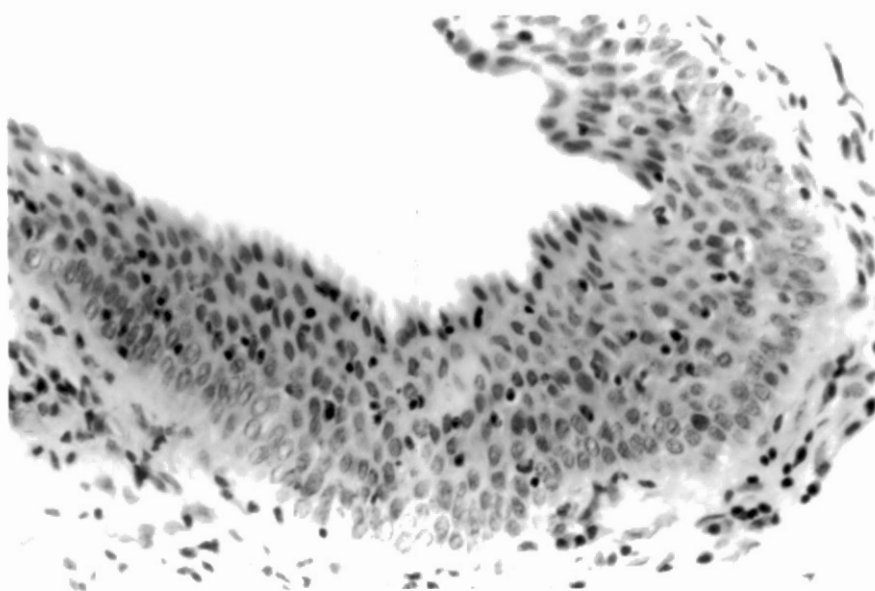
Figure 22.   Multilayered, thick vaginal epithelium with a focus of individual cuboidal to low columnar cells projecting into the lumen.   Vagina from mare #6.   Hematoxylin and Eosin X120.
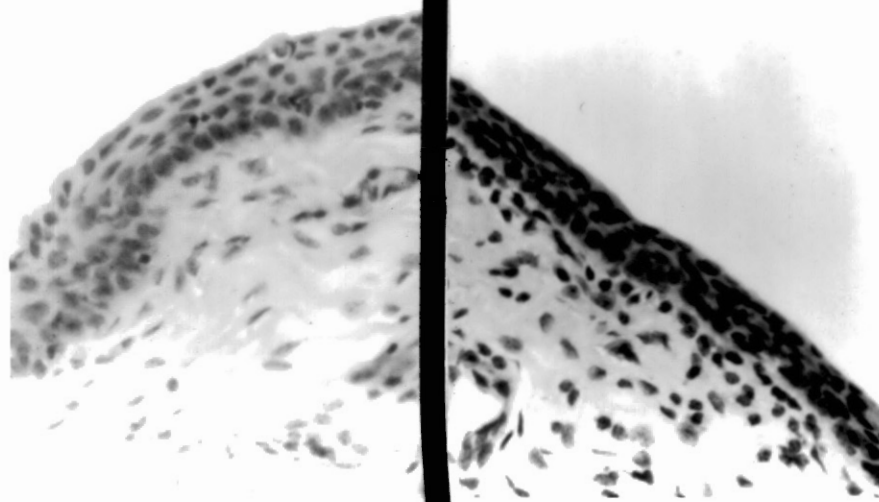
Figure 23. Composite showing vaginal epithelia of three to five cell layers but with differing overall thickness. Vaginas from mares #4 (right) and #1 (left). Hematoxylin and Eosin X64.
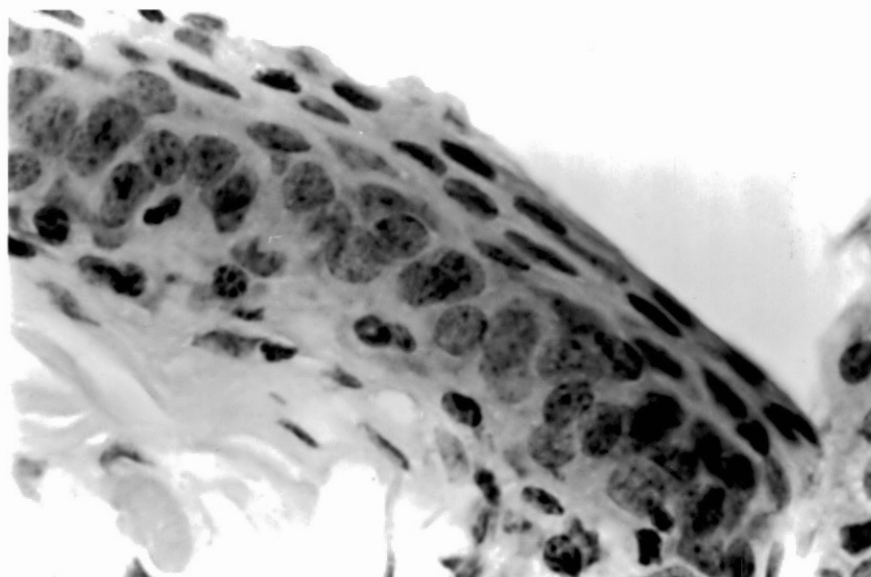
Figure 24. Flattened nucleated superficial squamous epithe-
            lial cells overlying raised vaginal fold.
            Vagina from mare #3. Compare with cuboidal
            projecting epithelial cells in depth of vagi-
            nal fold in Figure 22. Hematoxylin and Eosin
            X320.

were satisfactory for electron microscopy.

The vertical orientation of basal nuclei was not as apparent as in the light microscopic sections; it was sometimes apparent in thick sections. Surface epithelium contained numerous, short microvilli (Figure 25). Intercellular bridges were evident in thick sections as straight projections and with the electron microscope as tortuous villous structures (Figure 26). Granular cytoplasmic particles in middle-layer cells consistent with glycogen were seen. Surface cells in all the mares contained short, sparse microvilli (Figure 27). A few surface cells in mare #6 contained perinuclear clearing and chromatin margination suggestive of cell death (Figure 28). In some areas, presumed to be the depths of a fold, surface cells bulged into the lumen and had slightly longer and more numerous microvilli. Subepithelial plasma cells were recognized in some sections, and a few intraepithelial inflammatory cells were seen. No anucleated surface material was seen in any of the sections. In mare #3, vacuolation and separation of the surface cells were prominent in thick sections as well as thin sections examined with the electron microscope (Figure 29).

Discussion

The numbers of cell layers in the vaginal epithelium of mares in this study were consistent with those reported by other investigators (8, 11, 12). The orientation of the basal layer of cells with elongated, vertical nuclei is interesting; in human vaginal specimens, this is apparently a consistent finding, regardless of the stage of the cycle (7, 61). The increased prominence of this layer and increased mitotic activity associated with estrus reported by other investigators (12)
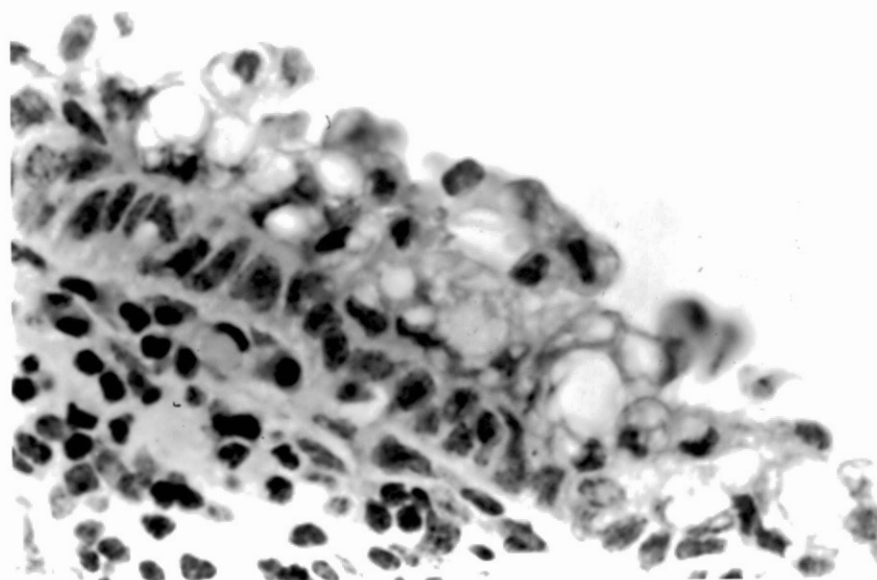
Figure 25. Vacuolation and separation of superficial squamous epithelial cells of the vagina of mare #3 and consistent with vaginitis. Hematoxylin and Eosin X160.
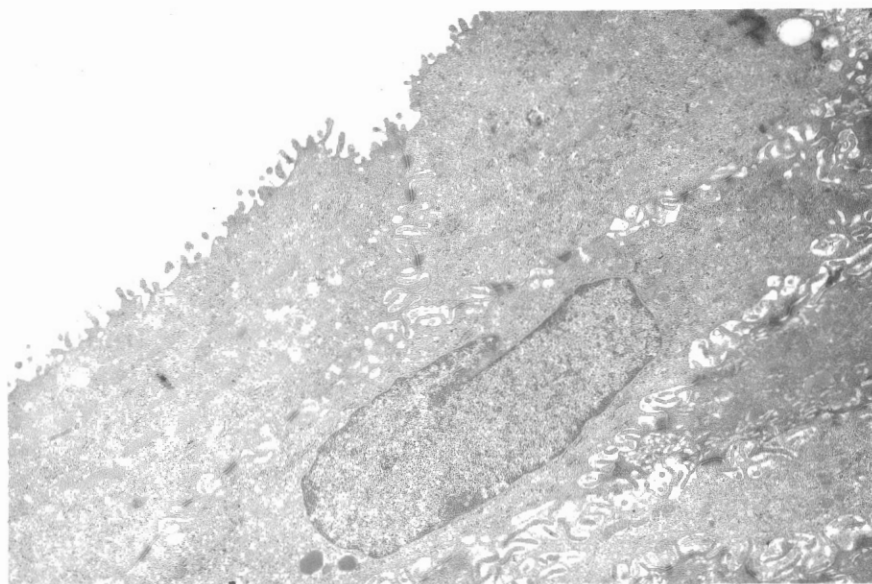
Figure 26. Electron microscopic preparation of vagina from
            mare #2. Note numerous tortuous villous
            intercellular projections and granular cyto-
            plamic particles consistent with glycogen.
            Uranyl Acetate-Lead Citrate X7,200.
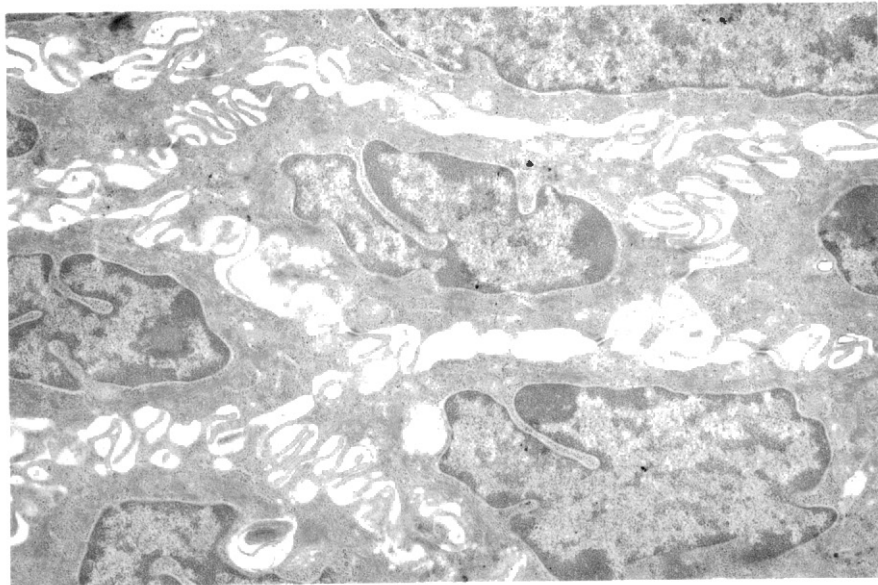
Figure 27. Superficial vaginal epithelial cells containing numerous short microvilli. Electron microscopic preparation of vagina from mare #6. Uranyl Acetate-Lead Citrate X4,800.
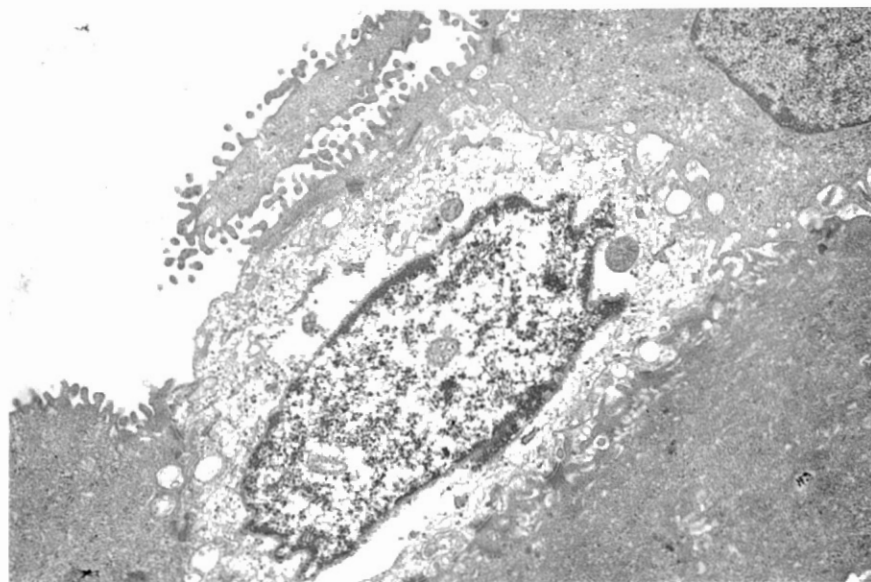
Figure 28. Isolated surface vaginal epithelial cell with
            perinuclear clearing and chromatin margina-
            tion, suggestive of cell death. Compare with
            unaffected surface epithelial cells in Figure
            26 and group of cervical cells in Figure 20.
            Uranyl Acetate-Lead Citrate X5,800.
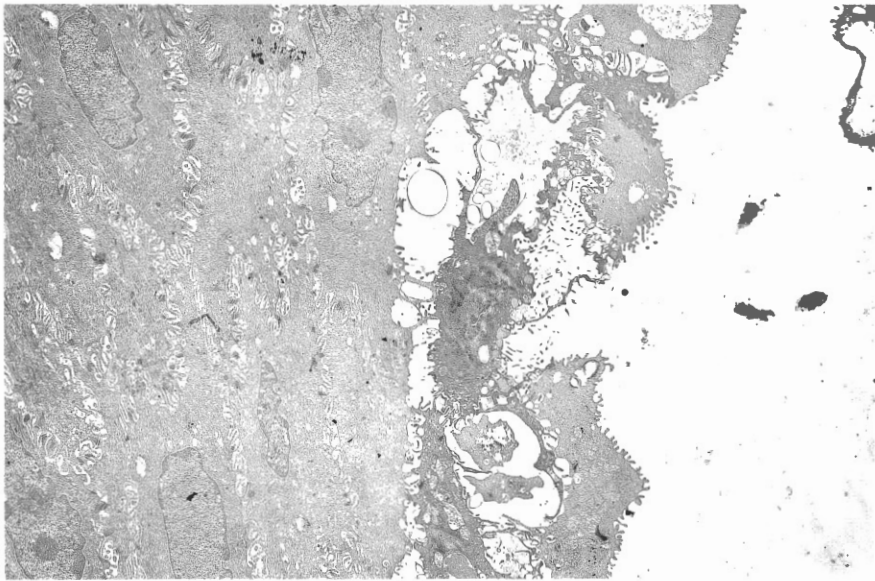
Figure 29.   Vacuolation and separation of vaginal surface
             epithelial cells consistent with vaginitis.
             Compare with similar features in histologic
             section pictured in Figure 25.   Uranyl
             Acetate-Lead Citrate X2,900.

could not be documented by this study, since stage of the cycle was not identified clinically or by other means prior to necropsy.

The variation in flattening of the surface epithelial cells related to position on the vaginal folds seen in this study has not been reported by other investigators. This may be a factor contributing to the lack of uniformity in the cytologic interpretation of equine vaginal smears (8, 11, 12). The possible identification of bulging cells in microscopic sections that might correspond to these cells was fortuitous. These cells contained longer and more numerous microvilli, but the significance of these cells and structures in the vagina is not known.

A lymphoplasmacytic infiltrate was present in the submucosa of all mares' vaginal tissues, suggesting that this was a normal cell population and undoubtedly reflects the antigenic stimulation of this mucosal surface. The degree of inflammation and concurrent epithelial atypia in mare #3 was consistent with a true vaginitis.

The tortuous nature of the villous projections between the vaginal epithelial cells differed from the straight, spiney appearance of "intercellular bridges" of stratified squamous epithelium in the thick sections. The importance of membrane events in the pathogenesis of abnormalities of the stratified squamous epithelium was more easily appreciated in the electron microscopic sections in which the great surface area involved, extensive interdigitations and junctions, and capacity for "cell communication" were more easily recognized.

Previous reports of "cornified" layers of cells in equine vaginal specimens imply that anucleated layer(s) of keratin were present (11, 12). No such layers were seen in specimens in this study, but focal

cornification might easily have been missed. Other sources for such cells in vaginal smears (11, 12) may represent contamination from the vaginal vestibule or skin. Review of the pictures supposedly illustrating limited cornification of vaginal tissue in previous reports was not convincing of the presence of an anucleated surface layer (11, 12).

<div align="center">

Summary of the Features Observed in Tissues of

the Reproductive System of the Mare

</div>

A summary of the histologic evaluations of the ovaries, uteri, uterine cervices, and vaginas of the six mares in this study is presented in Table VI. An interpretation of the state of reproductive activity based on the histologic features is included.

Interpretation of phases in individual tissues corresponded well with other tissues from the same animal. Also, the same phase of the cycle had similar features in tissues from different mares.

Oviducts were deleted due to the lack of published information concerning their structure, the lack of uniformity in specimen collections between mares, and the variation in morphology at different sites within the oviduct.

Active ovarian structures, cyclic interpretation of uterine tissue, and moderately thick vaginal epithelium in mares #1, #2, and #3 are compatible with current cyclic activity. The secretory activity of the cervix in mare #3 may be due to recent estrus (corpus hemorrhagicum present) or irritation associated with observed inflammation. Ovarian follicles, cervical secretory cells, and very thick vaginal epithelium in mare #6 are suggestive of estrus. The cuboidal epithelium, moderate stromal compaction, and mild glandular atrophy in the uterus and only

TABLE VI

SUMMARY OF HISTOLOGIC INTERPRETATION OF OVARIES, UTERI, UTERINE CERVICES, AND VAGINAS OF SIX MARES

Mare	Ovarian Structures	Uterine Reproductive Activity	Uterine Cervical Epithelium	Vaginal Mucosa	Overall Interpretation
#1	Atretic follicles, corpus luteum	Cyclic	Nonsecretory	Very folded 3-6 cell layers, moderately thick	Cycling
#2	Developing follicle, atretic follicle	Cyclic	Nonsecretory	Moderately folded 3-5 cell layers, moderately thick	Cycling
#3	Corpus luteum, atretic follicles, corpus hemorrhagicum	Cyclic	Secretory; foci of mature squamous meta-plasia and epithelial atypia	Moderately folded 3-5 cell layers, moderately thick, marked epithelial atypia with neutrophils	Cycling, recent ovulation, vaginitis and cervicitis
#4	None	None	No tissue	Few folds, 3-4 cell layers, thin	Senile atrophy

TABLE VI (CONTINUED)

Mare	Ovarian Structures	Uterine Reproductive Activity	Uterine Cervical Epithelium	Vaginal Mucosa	Overall Interpretation
#5	Atretic follicles	Transition	Nonsecretory, many cells with hypochromatic nuclei	Many folds, 3-5 cell layers, moderately thick	Seasonal transition
#6	Developing follicle, corpus luteum	Cyclic	Secretory	Moderately folded, 5-10 cell layers, very thick	Currently cycling, secretory activity of cervix, and very thick, multi-layered vaginal epithelium, suggestive of estrus

atretic ovarian follicular structures of mare #5 are consistent with fall seasonal transition. The hypochromatic nuclei of the uterine cervix resembled those in the corresponding uterine specimen from this mare and other mares during fall transition. The similarity between cervical and endometrial epithelial nuclei is likely, since both epithelia share a common embryologic source (7).

Atrophic changes in the uterus (unlike those seen during winter anestrus), nodular inactive ovaries, and very thin vaginal epithelium are compatible with senile atrophy of the reproductive system of mare #4.

All mares had some inflammatory cells within the uterus, but inflammation was found in adjacent segments of the reproductive system of only one mare (#3). This mare had vaginitis, cervicitis with squamous metaplasia, and moderate endometritis. The relationship of uterine, cervical, and vaginal processes may depend on patency of the cervix, the nature of the initiating insult, or inherent properties of local defense mechanisms in individual tissues.

CHAPTER IV

CONCLUSION

The completion of this project represents not only a step toward
the completion of an educational goal (Ph.D.) but also an additional
step in increasing my knowledge of the equine reproductive system, the
relationships of its component tissues, and the features that may be
observed by a variety of methods (paraffin sections and plastic sec-
tions, electron microscopic preparations).

This project was observational rather than experimental and dealt
with specimens from a limited number of animals. As such, the results
did not provide evidence to support or negate a particular hypothesis.
However, it provided the opportunity to examine multiple tissues from
the reproductive tract which fluctuate with hormonal influence. The
literature deals with such changes, concentrating primarily on single
tissues. Even so, the information on equine cervix and oviduct is
sparse. This project correlated the multiple tissues from the same
animal and integrated information regarding the reproductive anatomy
using light and electron microscopy.

It was expected that electron microscopic features would correspond
with those seen with the light microscope, based on studies conducted in
other species (1, 7, 32, 36, 45, 46, 47, 48, 49, 57). The significance
of some of the electron microscopic observations of this study of the
uterus, such as epithelial cytoplasmic projections and altered glands in

areas of fibrosis, is not clear.   Other observations of previously

unreported features in equine reproductive tissues include some changes

associated with senile atrophy, "peg" cells described in the oviducts

but not the cervix, the "clear" cells of the isthmus of the oviduct,

focal squamous metaplasia of the uterine cervix, and some vascular

alterations.

Specimens collected at necropsy emphasize the importance of muscu-

lature and vasculature of the reproductive system, and the uterus in

particular.   These elements may be absent or present only in small

amounts in biopsy specimens.   The importance of the vasculature of the

uterus is expressed in the following excerpt from Wynn's book on the

cellular biology of the human uterus:

> Most of the blood vessels of the body maintain a high degree of
> stability once they are fully formed.   Changes associated with
> advancing years creep on slowly, almost imperceptibly, and only
> dramatic episodes of disease or trauma call forth the vessels'
> inherent capacity for regeneration.   The blood vessels of the
> uterus, however, and particularly those of the endometrium, form an
> exception to this generalization.   In them, stability gives place
> to a high degree of variability.   The variability is of two sorts,
> for the regularly recurring interruptions of vascular pattern asso-
> ciated with the menstrual cycle are themselves interrupted at
> irregular intervals by the vascular upheavals occasioned by preg-
> nancy.   The efficiency with which the vessels adapt to this
> demanding schedule forms the dividing line between normal function
> and pathology.   The factors that produce the vascular variability
> also control the cyclic hanges in the parenchymatous tissues of the
> endometrium, in large part secondarily via the blood vessels.   The
> latter may therefore with justice be regarded as dynamic deter-
> minants of endometrial activity and as such merit a detailed
> consideration of their anatomy and physiology (1).

The dynamic events which occur in the ovary are difficult to

evaluate histologically.   The inclusion of an entire life cycle of a

structure from the single-cell stage, through a variety of differentia-

tions, and final disposition to a form that may be a scar or difficult

to differentiate from normal stroma is dramatic. And, such a proccess occurs with regularity and repeatedly during the cycles throughout the reproductive lifespan of the animal. The mechanisms of ovulation, selection of ovulatory versus atretic follicles, invasion of existing basement membranes by proliferating cells, and conversion of one cell type to another with concurrent changes in types of cell products represent fascinating processes, the bases of many of which are not only unknown but unexplored in the mare.

The appearances and some of the functions of the oviduct and uterine cervix of the mare also remain largely unexplored and represent important components of the reproductive system.

It is hoped that future studies of the equine reproductive system will address some of the questions raised as part of this study. Such studies could be of benefit, not only in approaching the problem of infertility in the mare, but also in investigation of the maintenance or promotion of reproductive health and in understanding the cellular phenomena that may be important in a variety of species or systems.

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