LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE EQUINE UTERUS RELATED TO TREATMENT

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Thesis Adviser

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Roger, and daughter, Ruth, have been outstandingly flexible, supportive, and encouraging throughout the courses, projects, and study required for this degree.

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CHAPTER I

INTRODUCTION

Equine practitioners traditionally have used intrauterine instillation of various agents to treat equine infertility (9, 12, 20, 35, 51, 54). The favorable effect of these treatments was correlated with negative uterine bacterial cultures, decreased inflammatory cells in uterine biopsy and cytology specimens and, ultimately, by the ability of treated mares to conceive and carry a foal to term (9, 54).

Heretofore, the choice of therapeutic agent has been based on clinicians' preferences and sometimes supported by bacterial culture and sensitivity (9, 12, 20, 35, 51, 54). Intrauterine medication can be instilled by repeated daily catheterization or by using indwelling uterine catheters (27, 45, 51).

Recently, investigators have further assessed the effects of uterine and cervical manipulation on the length of the reproductive cycle (23), the distribution of intrauterine medication (31), and the appearance of the luminal surface of the endometrium following intrauterine instillation of various agents (31).

In earlier studies of cytology and biopsy specimens, it was noted that epithelial changes were associated with intrauterine therapy (46, 47). A special equine uterine indwelling catheter, known as the Slusher Equine Uterine Indwelling Catheter, designed by a member of the equine reproductive research team at this college, was used in some of the

fetus is duplicated by the maternal placenta to provide the large surface area necessary to support fetal growth and development (17). Further knowledge of the properties of the uterus, its epithelium, and the roles they play in normal and abnormal processes may help in our understanding of equine infertility.

The current study was designed to investigate the relationship between light and electron microscopic evaluation of cytology and histology specimens from the nongravid equine uterus and its response to various therapeutic agents administered with an indwelling uterine catheter.

(37). Additional smears from each case were stained with Alcian Blue-Periodic Acid-Schiff-Hematoxylin (pH 2.5) (AlcB-PAS-He).

The chilled portion of the endometrial wash for electron microscopic examination was centrifuged for 10 minutes at 150 X G. The supernatant was decanted and the sediment transferred to 75-mm capillary tubes, plugged with clay, and centrifuged in a microhematocrit centrifuge for three minutes. The capillary tubes were scored with a file and broken slightly above the junction of the packed sediment. The plug of clay and adjacent packed sediment were pushed from the capillary tube with a straight wire and deposited into a clean petri dish containing several milliliters of 2% glutaraldehyde in a 0.25-M cacodylate buffer. After fixing for several minutes, the clay and cell sediment plugs were separated using fine forceps under a dissecting microscope. The cell plugs were processed according to previously described procedures (26).

Two endometrial biopsies were taken at each sampling to detect potential differences in distribution of lesions. The method for endometrial biopsy was similar to that described previously (24). One biopsy taken from the right horn of the uterus was divided in half using a dissecting microscope. One-half was fixed in buffered 10% formalin, embedded in hard paraffin, and sectioned at approximately 2 um. The remaining half was finely minced, fixed in glutaraldehyde, and prepared for electron microscopic examination. A second uterine biopsy taken from the body of the uterus was similarly divided and each half fixed in 10% buffered formalin. One-half was embedded in hard paraffin and sectioned at approximately 2 um. The other half was embedded in glycomethacrylate and sectioned at approximately 1.5 um. In some cases, sufficient tissue was available to make more than one glycomethacrylate

the luminal tip of the cytoplasm and conspicuously devoid of chromatin stippling. Nuclei with inapparent cytoplasm (stripped nuclei) were numerous. A fine background of fragments of granular cytoplasm, sometimes surrounding stripped nuclei, was usually present. A few neutrophils and histiocytes were present in smears from mares without clinical evidence or histories of reproductive disease. In some cases, the inactive pattern was recognized even in the presence of moderate to severe inflammation but was not always apparent when other factors such as previous treatment or urine pooling may have influenced epithelial morphology. Some of the features typical of an inactive pattern are shown in Figures 1 and 2.

The transitional pattern was seen in mares during the seasonal transition between periods of relative reproductive activity and inactivity. These occurred during the spring and fall months and, in some cases, continued into the winter months normally associated with anestrus. During fall transition, slender, elongated orange-stained cells and red ciliated cytoplasmic tufts became apparent in smears which had other evidence of normal cyclic activity. A few to moderate number of epithelial cells with hypochromatic nuclei and homogenous cytoplasm usually were present. Early in the fall transitional period, slender, elongated orangophilic cells and red ciliated cytoplasmic tufts were numerous. As the transitional period approached anestrus, the proportion of these cells decreased, and the number of cells with hypochromatic nuclei, stripped nuclei, and granular cytoplasmic fragments increased. By the time of midwinter anestrus, virtually no slender, elongated orange-stained cells nor ciliated cytoplasmic tufts were seen. Features of cytology smears with a transitional pattern associated with

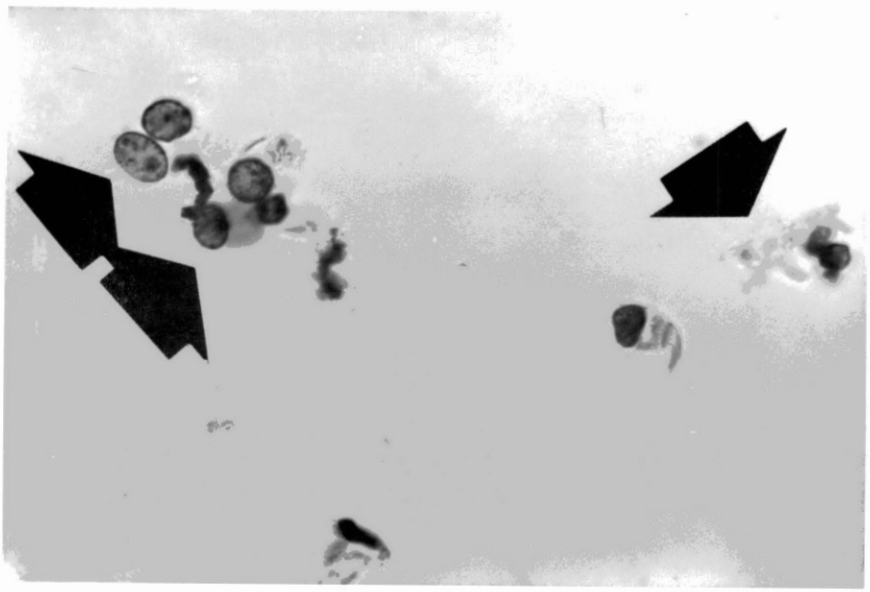


Figure 1. Small cuboidal ciliated epithelial cell, nuclei with inapparent cytoplasm (double arrow), and granular cytoplasmic fragments (single arrow) characteristic of inactive patterns in cytologic smears. Sano Trichrome Stain X320.

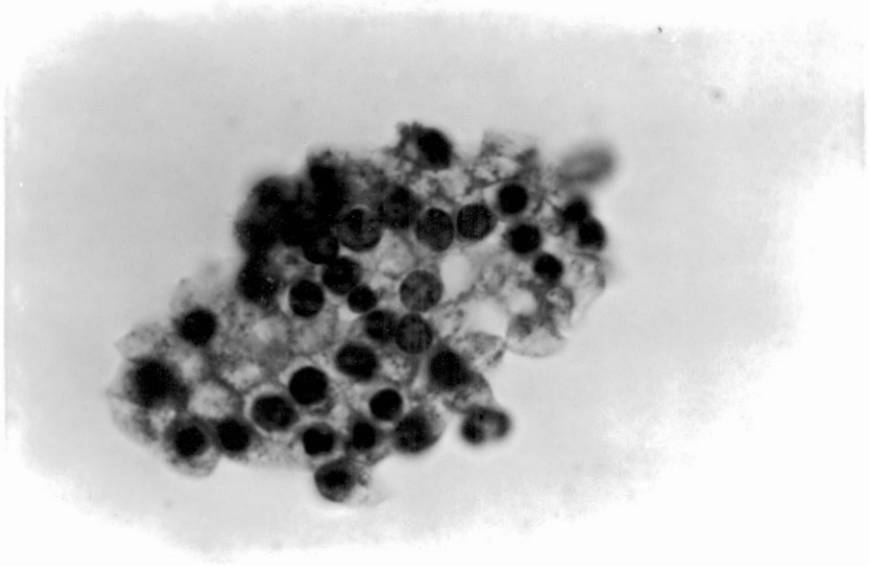


Figure 2. "Honeycomb" of cohesive epithelial cells in cytologic smear from a mare during winter anestrus. Sano Trichrome Stain X320.

fall seasonal transition are shown in Figure 3.

Based on four specimens, there was indication that the inactive winter smear pattern gradually acquired more of the features associated with active patterns following the onset of spring ovarian activity. The epithelial cells became taller with foamy cytoplasm and had finely stippled chromatin. As these changes took place, a few slender, elongated orange-stained cells and red ciliated cytoplasmic tufts were seen. These features were less conspicuous and of a more subtle nature than those associated with fall transition.

The active pattern was identified primarily by epithelial features that did not correspond to inactive or transitional patterns. One type of active pattern, designated active-normal, was associated with normal reproductive activity and was seen in mares during the spring and summer months corresponding to the physiologic breeding season in the Northern hemisphere. The smears contained primarily nonciliated columnar to tall columnar epithelial cells with slightly foamy cytoplasm. The oval nuclei usually contained finely stippled chromatin and a single small nucleolus. These cells occurred in loose groups or singly. In some smears, green ciliated cytoplasmic tufts were present as well as ciliated and nonciliated cytoplasmic fragments differentiated from ciliated cytoplasmic tufts by their jagged distal borders. A small amount of fine mucus, present in strands or as granular background material, was sometimes seen. A few lymphocytes were often present in mares without clinical evidence or histories of reproductive disease. In some cases, a few groups of epithelial cells with enlarged nuclei and prominent nucleoli were seen.

Abnormal active patterns, designated active-abnormal, were seen in

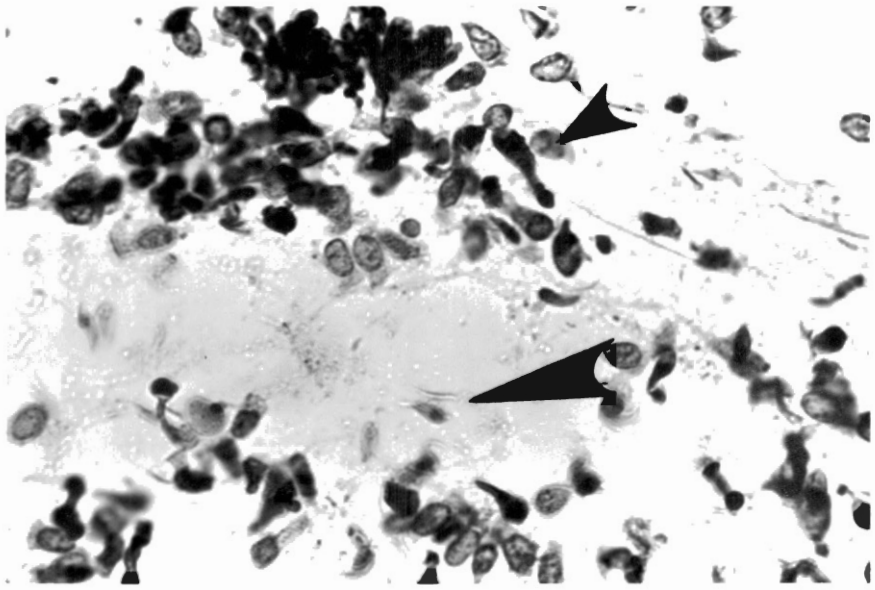


Figure 3. Slender orange-stained cell (small arrow) and red ciliated cytoplasmic tuft (large arrow) amid cuboidal cells with homogeneously hypochromatic nuclei. Cytologic smear pattern typical of seasonal transition. Sano Trichrome Stain X160.

some mares with various degrees of inflammation, pyometra, urine pooling, and following therapy with or without an indwelling uterine catheter. These smears contained varying degrees of epithelial abnormalities and inflammation. In some cases, the epithelial cells were present primarily in large groups in which the majority of the nuclei were enlarged and contained a single large nucleolus. These groups were compatible with cells previously reported to represent regeneration of epithelium in women (4, 10, 30, 53). Single epithelial cells were very tall with foamy cytoplasm and sometimes had nuclear features similar to those of the cells seen in groups. These cells, because of the results of this study, will be referred to hereafter as "reactive epithelial cells." In some cases, the morphology of the epithelial cells was not significantly different from that associated with normal cyclic activity. Interpretation of reproductive pattern was not possible in those cases with significant inflammation and/or moderate to marked epithelial abnormalities. Some features of an active pattern associated with normal cyclic activity are shown in Figure 4.

Interpretation of Specimens

The smears for this report were evaluated for the presence of active-normal, active-abnormal, inactive, or transitional patterns, inflammation, and presence and morphology of cellular or noncellular elements.

The bases for assessing the stage of reproductive activity and stage of the cycle in histologic specimens from the equine uterus have been outlined by other investigators (5, 20, 21, 26). The endometrial biopsies for this report were evaluated for the stage of reproductive

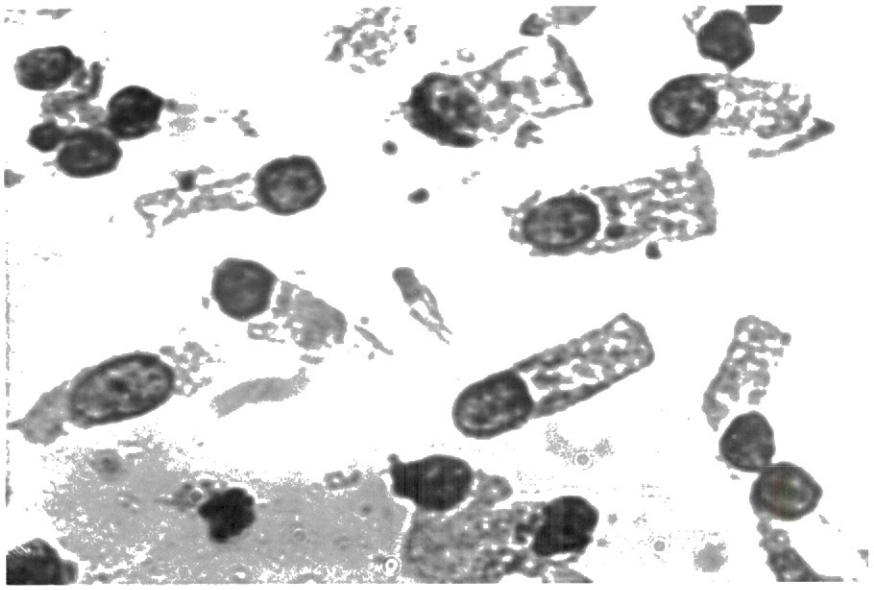


Figure 4. Tall columnar epithelial cells with foamy cytoplasm. Single green ciliated cytoplasmic tufts in center. Cytologic smear with an active-normal pattern consistent with cyclic reproductive activity. Sano Trichrome Stain X400.

activity, presence of inflammation, fibrosis, glandular and luminal epithelial morphology, and presence of other normal or abnormal cellular and noncellular elements. The correlation of these features in the biopsy specimens from different locations was noted.

CHAPTER III

RESULTS

Initial Collection

Cytology Specimens

Cytology Smears

Initial cytology smears from five of the eight mares had transitional patterns. One mare's smears (No. 8--Group D) did not fit into those associated with various stages of reproductive activity. This smear was interpreted as probable early transition. Mare 4 (Group B) and Mare 7 (Group D) had active-normal and active-abnormal patterns, respectively. Groups of reactive epithelial cells were not seen. The following text describes the inflammation and other features present in initial cytology smears for each group. Table I outlines the interpretation of reproductive activity in cytology and biopsy specimens in this study. Table II presents the data concerned with the degree of reactive epithelium seen in cytology smears and paraffin sections in this study. Table III details inflammation and epithelial abnormalities seen in cytology smears at each collection. Inflammation in cytologic and histologic specimens at each collection is presented in Table IV.

Group A (Controls--Mares 1 and 2). The smears from Mare 1 had a moderate number of neutrophils and very few eosinophils. A few small-

TABLE I

INTERPRETATION OF REPRODUCTIVE ACTIVITY IN CYTOLOGIC AND HISTOLOGIC SPECIMENS

	Groups							
	A			B		C		D
	Mare No.							
	1	2	3	4	5	6	7	8
Initial Collection								
Cytology Smears	T	T	T	A-N	T	T	A-A	PT
Biopsy Specimens (Paraffin Sections of Body, Right Horn, and Glycomethacrylate Section)	T	T	T	T*	T	T	T	T
Posttreatment (Five-Day) Collection								
Cytology Smears	A-A	A-A	A-A	A-A	A-A	A-A	A-A	A-A
Biopsy Specimens (Paraffin Sections of Body, Right Horn, and Glycomethacrylate Section)	T**	T	T	T	T	T	T	T
40-Day Collection								
Cytology Smears	A-A	T	T	T	T	T	T	T
Biopsy Specimens (Paraffin Sections of Body and Right Horn)	T	T	T	T	T	T	T	T

A-N = active-normal pattern.
A-A = active-abnormal pattern.
T = transitional pattern.
PT = probable early transition.
* = specimen from right horn compatible with early transition or cyclic activity of diestrus.
** = specimen from uterine body compatible with cyclic activity of diestrus.

TABLE II

DEGREE OF REACTIVE EPITHELIUM SEEN IN CYTOLOGY SMEARS AND PARAFFIN SECTIONS

	Groups							
	A		B		C		D	
	Mare No.							
	1	2	3	4	5	6	7	8
Initial Collection								
Cytology Smears	--	--	--	--	--	--	--	--
Paraffin Sections								
Body	Mild	--	--	--	--	--	--	--
Right Horn	--	--	--	--	--	--	Mod.	--
Posttreatment (Five-Day) Collection								
Cytology Smears	Mild	Mild	Mrk.	Mrk.	Mod.	Mod.	Mrk.	Mod.
Paraffin Sections								
Body	Mild	--	--	Mrk.	Mild	Mild	Mild	Mild
Right Horn	Mod.	--	--	Mild	Mod.	Mild	Mod.	Mild
40-Day Collection								
Cytology Smears	Mild	--	--	--	--	--	--	--
Paraffin Sections								
Body	--	--	--	--	--	--	--	--
Right Horn	--	--	--	--	--	--	--	--

Mrk. = marked.
Mod. = moderate.

TABLE III

INFLAMMATION AND EPITHELIAL ABNORMALITIES IN INITIAL, POSTTREATMENT (FIVE-DAY), AND 40-DAY CYTOLOGY SMEARS

	Groups							
	A		B		C		D	
	Mare No.							
	1	2	3	4	5	6	7	8
Initial Collection								
Inflammation								
N	Mod.	Few	--	--	--	--	Mod.	Few
L	--	Few	--	--	--	--	Mod.	--
E	Few	--	--	--	--	--	--	--
Epithelium								
Reac.	--	--	--	--	--	--	--	--
Chloros.	--	--	--	--	--	--	--	--
Posttreatment (Five-Day) Collection								
Inflammation								
N	Few	Mod.	Many	Pur.	Mod.	Mod.	Pur.	Mod.
L	Mod.	--	--	--	--	--	--	--
E	(1)	--	--	Mod.	(1)	--	Many	Few
Epithelium								
Reac.	Mild	Mild	Mrk.	Mrk.	Mod.	Mod.	Mrk.	Mod.
Chloros.	Mod.	--	--	Few	Few	--	--	--

TABLE III (CONTINUED)

		Groups							
		A		B		C		D	
		Mare No.							
		1	2	3	4	5	6	7	8
40-Day Collection									
Inflammation									
N	Mod.	--	--	--	--	--	--	--	--
L	Mod.	--	--	--	--	--	--	--	--
E	Few	--	--	--	--	--	--	Few	--
Epithelium									
Reac.	Mild	--	--	--	--	--	--	--	--
Chloros.	Few	--	--	--	--	--	--	--	--
Other		--	--	--	(2)	(2)	--	--	--

N = neutrophils.
L = lymphocytes.
E = eosinophils.
Reac. = reactive epithelial cells.
Chloros. = chlorocytes.
Mrk. = marked.
Mod. = moderate.
Pur. = purulent.
(1) = single eosinophil seen.
(2) = abundant mucus plus cellular casts.

TABLE IV

INFLAMMATION IN CYTOLOGIC AND HISTOLOGIC SPECIMENS AT INITIAL, POSTTREATMENT (FIVE-DAY), AND 40-DAY COLLECTIONS

	Groups							
	A		B		C		D	
	Mare No.							
	1	2	3	4	5	6	7	8
Initial Collection								
Cytology Smears								
N	Mod.	Few	--	--	--	--	Mod.	Few
L	--	Few	--	--	--	--	Mod.	--
E	Few	--	--	--	--	--	--	--
Paraffin Sections								
Body								
N	Mod.	Few	--	Few	--	Few	Few	Few
L	Mod.	--	Mod.	Mod.	Few	Mod.	Few	Few
E	Few	Few	--	Few	--	--	--	--
Right Horn								
N	Few	Mod.	Few	Few	--	Few	Mod.	Mod.
L	Mod.	Mod.	Mod.	Mod.	Few	Mod.	Few	Few
E	Few	Few	Few	Few	--	--	--	--
Glycomethacrylate Sec.								
Body								
N	Few	Few	Few	--	--	Few	Few	Few
L	Few	Mod.	Few	Few	Few	Few	--	--
E	(1)	Few	--	Few	--	--	(1)	--

TABLE IV (CONTINUED)

	Groups							
	A			B		C		D
	Mare No.							
	1	2	3	4	5	6	7	8
Posttreatment (Five-Day) Collection								
Cytology Smears								
N	Few	Mod.	Many	Pur.	Mod.	Mod.	Pur.	Mod.
L	Mod.	--	--	--	--	--	--	--
E	(1)	--	--	Mod.	(1)	--	Many	Few
Paraffin Sections								
Body								
N	Few	Mod.	NS	Many	Many	Many	Many	Mod.
L	Mod.	Mod.	NS	Many	Many	--	--	Mod.
E	Mod.	Few	NS	--	--	Few	Few	Few
Right Horn								
N	Many	Many	Mod.	Many	Many	Mod.	Many	Mod.
L	Many	--	Mod.	Many	Many	Mod.	--	Many
E	Mod.	--	--	--	Few	Few	Many	Few
Glycomethacrylate Sec.								
Body								
N	NS	Mod.	Mod.	Mod.	Mild	Mod.	Mod.	Mod.
L	NS	--	--	Mod.	Mod.	--	--	Mod.
E	NS	Few	Few	Few	--	--	--	Few

TABLE IV (CONTINUED)

		Groups						
		A		B		C		D
		Mare No.						
		1	2	3	4	5	6	7	8
40-Day Collection									
Cytology Smears									
N	Mod.	--	--	--	--	--	--	--
L	Mod.	--	--	--	--	--	--	--
E	Few	--	--	--	--	--	Few	--
Paraffin Sections									
Body									
N	Mod.	Few	Few	Few	--	NS	Few	--
L	Mod.	Mod.	Mod.	Mod.	Few	NS	Mod.	Few
E	--	Few	--	Mod.	--	NS	--	--
Right Horn									
N	Few	Few	Few	Few	--	--	Mod.	Few
L	Mod.	Mod.	Mod.	Mod.	Few	Few	Few	Mod.
E	(1)	Few	--	Many	--	--	--	--

N = neutrophils.
L = lymphocytes.
E = eosinophils.
Mod. = moderate.
Pur. = purulent.
* = second section.
(1) = single eosinophil seen.
NS = specimen not satisfactory.

and medium-sized inspissated mucus casts were seen. The second control mare's smears contained a few neutrophils and a moderate number of lymphocytes. There was a small amount of degenerating pigmented fibrin and platelets indicative of previous hemorrhage.

Group B (Indwelling Catheter--Mares 3 and 4). In initial cytology smears, Mare 3 had a moderate increase in mucus with trapped epithelial cells. Significant inflammation was not present. The other mare's smears had a very few small inspissated glandular casts.

Group C (Catheter Plus Saline--Mares 5 and 6). Both mares had smears with a few inspissated casts. The casts were of medium size in Mare 5 and small in Mare 6.

Group D (Catheter Plus EDTA-TRIS Buffer--Mares 7 and 8). The cytology smears from Mare 7 had moderate numbers of neutrophils and lymphocytes. This specimen also had numerous superficial squamous epithelial cells, with and without keratin precursors, that were presumed to be the result of vaginal contamination during collection. In cytology smears from Mare 8, a slightly greater-than-normal number of neutrophils suggested the presence of very mild, acute inflammation.

Special Stains. In smears showing a transitional pattern (Mares 1, 2, 3, 5, 6, and 8), the epithelial cells were predominately a homogenous, pale blue when stained with AlcB-PAS-He. The long, slender orange-stained cells and ciliated cytoplasmic tufts stained a homogenous, dull pink (Figure 5).

In initial cytology smears from mares with an active pattern, there was a variety of staining reactions with AlcB-PAS-He. The apical

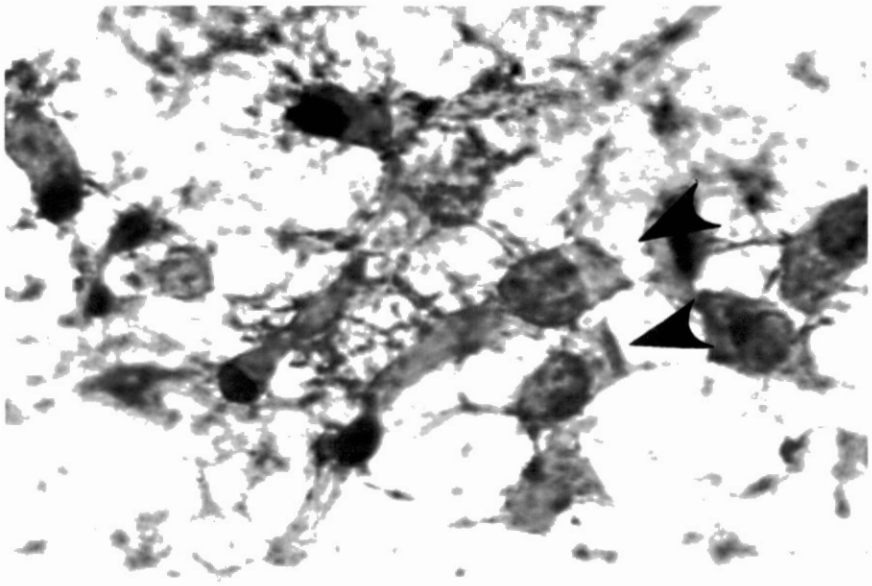


Figure 5. Pale cuboidal cells (arrows) and elongated dull pink cells. Compare with cuboidal cells and elongated orange-stained cell in Figure 3. AlcB-PAS-He X400.

portion of the cytoplasm of the nonciliated columnar epithelial cells varied from red to purple (Figure 6).

Cytology Preparations Examined with the Electron Microscope

In cytology specimens prepared for electron microscopic evaluation, initial specimens contained single epithelial cells as well as flat groups of epithelial cells. These corresponded to the single cells and "honeycomb" arrangements seen in the smears observed with the light microscope. Dark osmiophilic ciliated and nonciliated cells believed to correspond to the slender orangophilic cells in cytology smears were seen in mares with transitional smear patterns (Figure 7). Some of these cells appeared to be pinching off ciliated cytoplasmic tufts similar to those seen in smears. Examination of the cytoplasm in the constricted areas failed to indicate any structural difference predisposing to constriction in the areas where the apical ciliated cytoplasm separated from the basal portion of the cell. Free ciliated cytoplasmic tufts were numerous as were free groups of cilia that were matted and/or twisted (Figure 8). Fragments of cilia corresponding to those seen with the electron microscope were recognized in smears when these were reviewed (Figure 9). Their small size and lack of cellular attachment prevented precise identification, except in retrospect.

Electron microscopic preparation of cytology specimens from mares with transitional smear patterns also contained cuboidal cells. Some of the cuboidal cell nuclei were almost devoid of chromatin and were believed to correspond to the cells with hypochromatic nuclei and sparse cytoplasm seen with the light microscope (Figure 10). A few dark

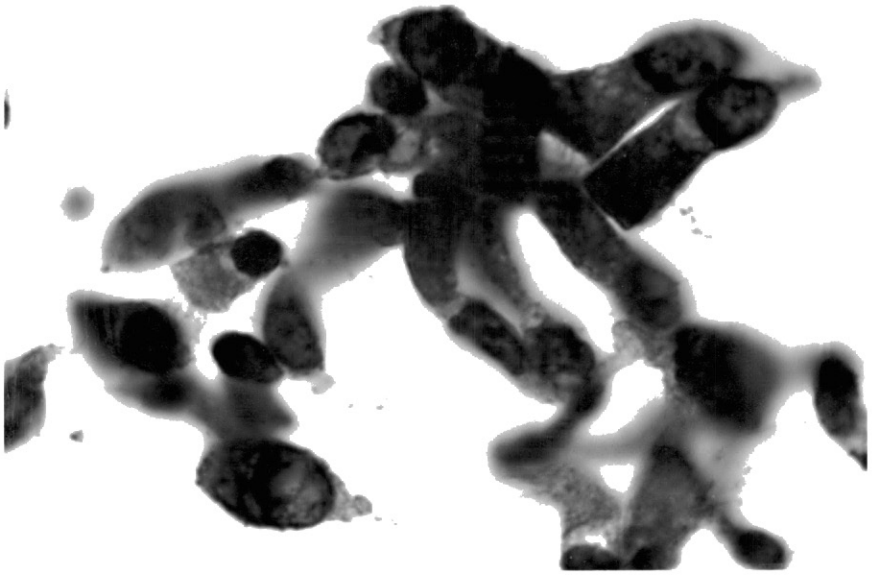


Figure 6. Tall columnar cells with purple apical cytoplasm. Compare with tall columnar cells in Figure 4. AlcB-PAS-He X400.

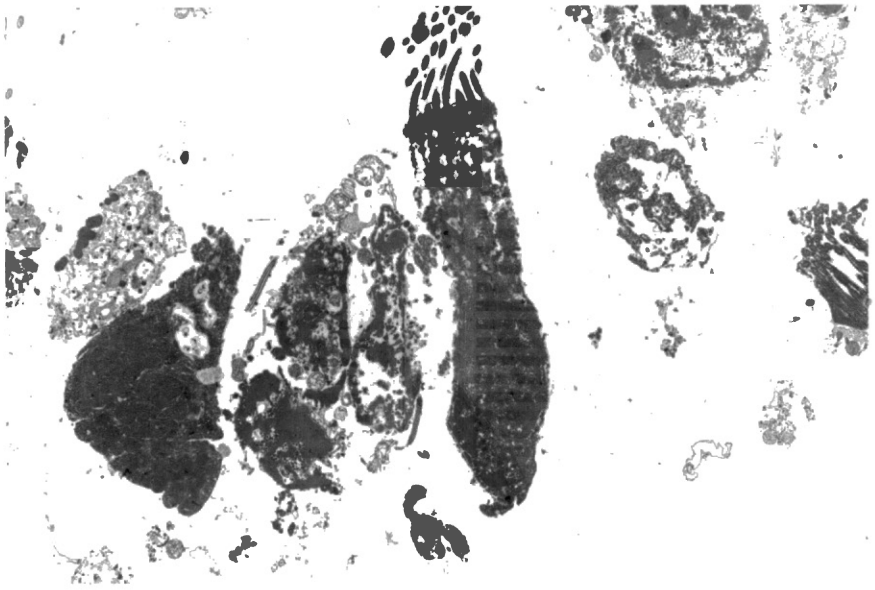


Figure 7. Dark osmiophilic columnar cell in electron microscopic preparation of cytologic specimen from mare with transitional pattern in cytologic smears. Compare with slender orange-stained cell in Figure 3. Uranyl acetate-lead citrate X3,600.

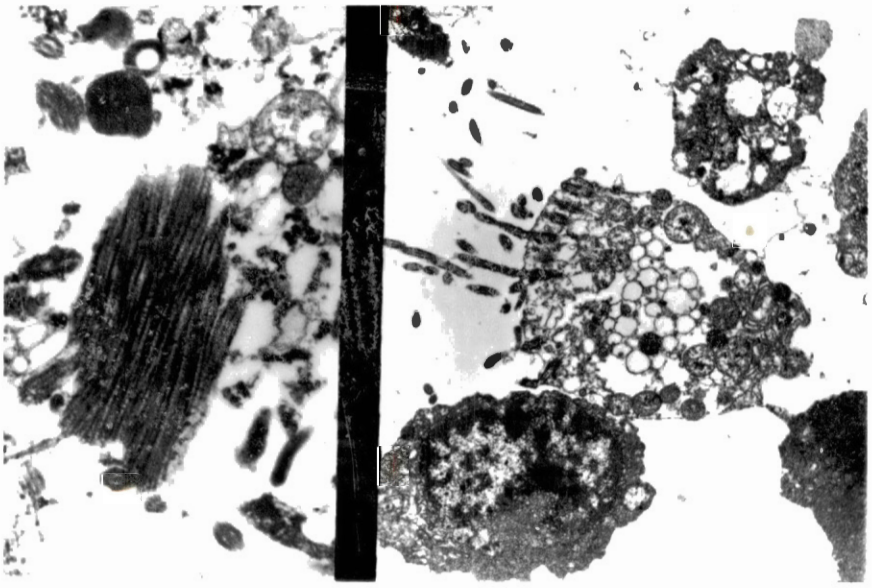


Figure 8. Ciliated cytoplasmic tuft (right) and free-matted cilia (left) in electron microscopic preparation of cytologic specimen. Compare with ciliated cytoplasmic tuft in Figure 3. Uranyl acetate-lead citrate, tuft X4,500, cilia X12,500.

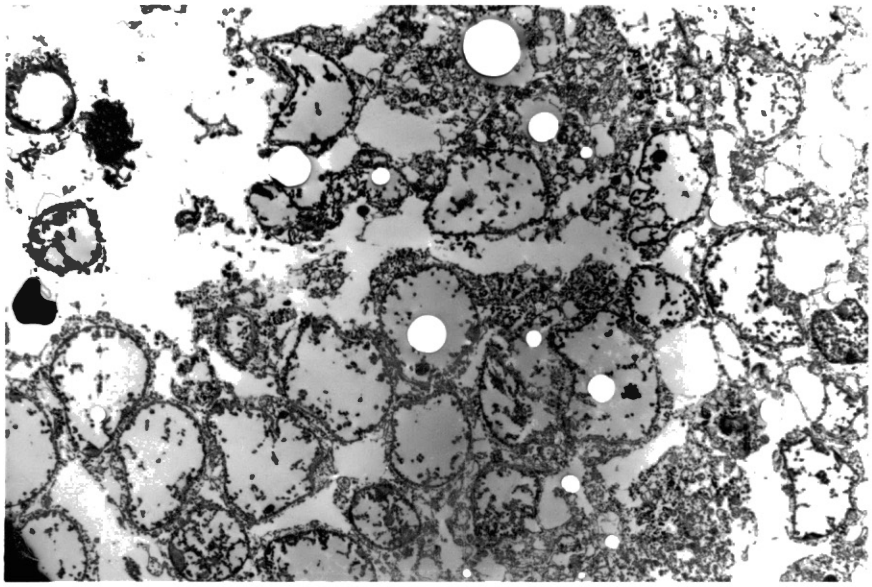


Figure 10. Flat sheet of epithelial cells with hypochromatic nuclei and sparse cytoplasm in electron microscopic preparation of cytologic specimen. Compare with cuboidal cells with hypochromatic nuclei in Figure 3. Uranyl acetate-lead citrate X1,390.

rounded cells were seen with microvilli and cytoplasmic vesicles containing flocculent material. Red blood cells were present as a layer at one end of the plug as a result of centrifugation in almost all speciemns, and white blood cells were scattered throughout the specimens. It was not possible to estimate the degree of inflammation in these specimens.

The electron microscopic cytology specimens from mares with active smear patterns (Mares 4 and 7) had numerous columnar cells, with and without cilia, corresponding to the columnar cells seen in smears. These cells had finely distributed chromatin and distinct nucleoli (Figure 11). Many cells with microvilli and cytoplasmimc vesicles were present. Very few ciliated cytoplasmic tufts were seen.

The electron microscopic examination of the cytology preparations from Mare 8 concurred with that of the light microscopic smears in that it had features suggestive of an early transitional pattern. There were flat cohesive groups of epithelial cells containing nuclei with distinct chromatin resembling that seen in cycling mares. There were some dark degenerated cells with cytoplasm that appeared to be pinching off.

Biopsy Specimens

Histologic Sections

Significant fibrosis was not present in any of the uterine sections from the mares used in this study. In initial specimens, luminal and glandular epithelia were without significant abnormalities, except for very mild focal reactive epithelium observed in the paraffin section from the uterine body of Mare 1 and moderate focal reactive epithelium observed in the paraffin section from the right uterine horn of Mare 7.

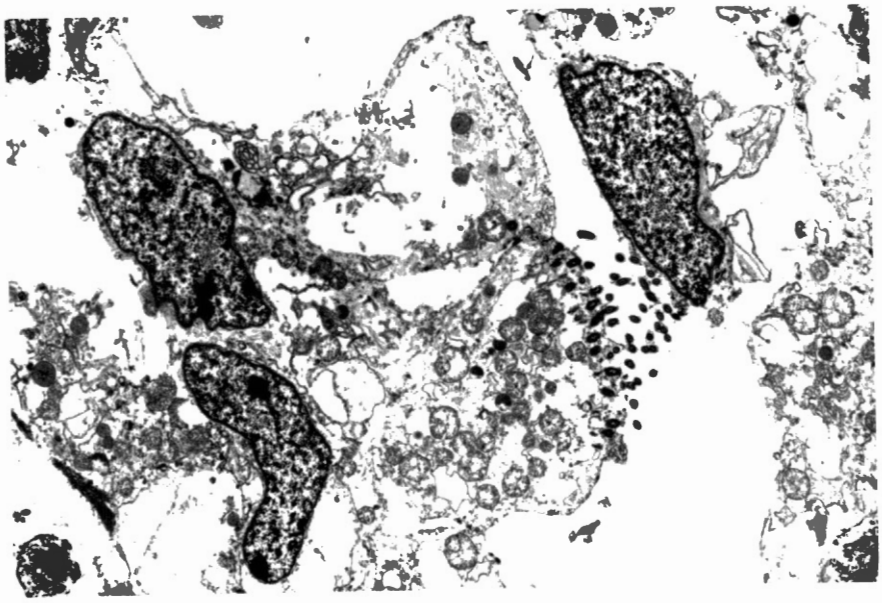


Figure 11. Tall columnar epithelial cells with finely distributed chromatin and distinct nucleoli. Electron microscopic preparation of cytologic specimen. Compare with tall columnar cells in Figure 4. Uranyl acetate-lead citrate X3,600.

The degrees of reactive epithelium seen in cytology smears and paraffin sections for initial, posttreatment, and 40-day collections are presented in Table II. Reproductive activity in all histologic sections was compatible with seasonal transition except for the paraffin section of the right uterine horn from Mare 4. This specimen was thought to represent either early transition or cyclic activity compatible with diestrus (Table I). In all mares, vesicles were present along the basement membranes of luminal epithelial cells. These ranged from few to many, were usually small in size, and were present in both paraffin and glycomethacrylate sections. Significant differences in numbers of vesicles were not seen in specimens from different areas of the uterus, but the number of vesicles differed greatly between mares. The vesicles were usually clear, but some contained small eosinophilic bodies and/or dark basophilic bodies resembling pieces of karyorrhectic debris. These vesicles and their contents were similar to those described and pictured as apoptosis by other investigators (47, 58, 59) (Figure 12). In many cases, significant differences in the degree of inflammation were observed in paraffin sections from the uterine body and right horn as well as between the paraffin and glycomethacrylate sections from the uterine body (Table IV).

Group A (Controls--Mares 1 and 2). The paraffin sections from Mare 1 both contained moderate numbers of lymphocytes and a few eosinophils. The numbers of neutrophils differed, with the body having moderate and the horn having mild neutrophilic inflammation. Fewer eosinophils were seen in the specimen from the right horn. The glycomethacrylate section contained mild neutrophilic and lymphocytic inflammation and a single eosinophil.

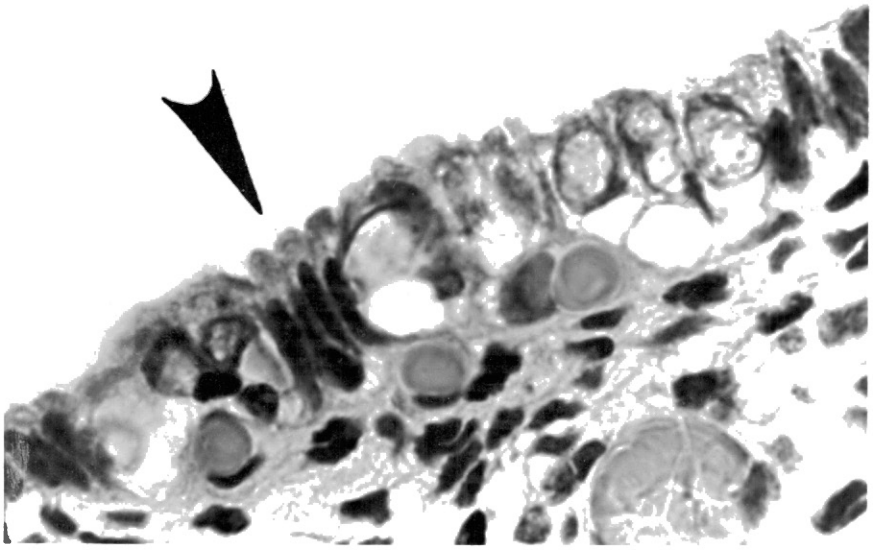


Figure 12. Vesicles in lining epithelium. Also, note cuboidal cells with hypochromatic nuclei and dark, slender cells crowded together (arrow). Paraffin section of uterine biopsy specimen. H&E X400.

In Mare 2, the paraffin sections both contained a few eosinophils although eosinophils were more numerous in the specimen from the right horn. The specimen from the uterine body contained a few neutrophils while the specimen from the right uterine horn contained moderate numbers of both neutrophils and lymphocytes. The glycomethacrylate section contained mild to moderate neutrophilic and lymphocytic inflammation as well as a few eosinophils.

Group B (Indwelling Catheter--Mares 3 and 4). Both paraffin sections from Mare 3 contained moderate numbers of lymphocytes, but the specimen from the right horn also contained a few neutrophils. The glycomethacrylate section contained a few neutrophils and lymphocytes.

Mare 4 had moderate lymphocytic inflammation in both specimens along with a few eosinophils. The specimen from the uterine body contained mild to moderate numbers of neutrophils while that from the right uterine horn contained mild neutrophilic inflammation. A few lymphocytes, eosinophils, and histiocytes were recognized in the glycomethacrylate section.

Group C (Catheter Plus Saline--Mares 5 and 6). Mare 5 had mild to moderate lymphocytic inflammation in both paraffin sections and one glycomethacrylate section. A second glycomethacrylate section contained a few neutrophils and many lymphocytes.

Both paraffin sections and the glycomethacrylate sections from Mare 6 contained mild neutrophilic and moderate lymphocytic inflammation.

Group D (Catheter Plus EDTA-TRIS Buffer--Mares 7 and 8). The paraffin sections of the specimen from the uterine body of Mare 7 contained

mild to moderate numbers of neutrophils and lymphocytes in contrast to moderate neutrophils and few lymphocytes in paraffin sections from the uterine horn. One glycomethacrylate section contained very mild neutrophilic inflammation and a single eosinophil. A second glycomethacrylate section contained moderate numbers of neutrophils and a few lymphocytes.

Mare 8 had a mild lymphocytic infiltrate in both paraffin sections with mild neutrophilic and moderate lymphocytic inflammation in specimens from the uterine body and right horn, respectively. One glycomethacrylate section contained mild neutrophilic inflammation, but a second glycomethacrylate section contained very few neutrophils with a few eosinophils.

Special Stains. Paraffin sections of biopsy specimens had predominately PAS-positive material in the apical portion of the majority of the luminal lining cells when stained with AlcB-PAS-He. The cytoplasm of the ciliated epithelial cells typically was clear. The glandular epithelial cells in superficial glands, probably corresponding to the neck of the glands, had some PAS-positive material in the apical portion of the cells. Epithelial cells in deeper glands had no detectable intracellular material. Luminal glandular secretions were granular to homogenously pink PAS-positive material.

Orange-stained cells with red cilia were only recognized in trichrome-stained paraffin sections and may represent the *in situ* counterpart of the slender orangophilic cells seen in cytology smears (Figures 13 and 14). In a few cases, structures resembling ciliated cytoplasmic tufts seen in cytology smears were recognized, but their precise identification was in doubt due to the possibility of tangential sectioning producing artifacts in tissue specimens. Slender columnar

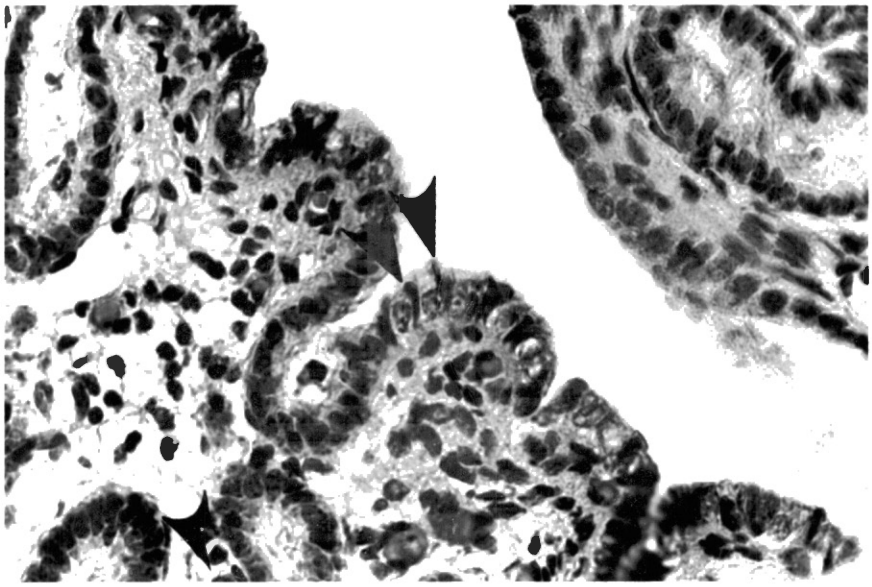


Figure 13. Low magnification of paraffin section of uterine biopsy specimen. Single orange-stained cells (arrows) present in lining epithelium. Compare with slender orange-stained cells in Figure 3. Sano Trichrome Stain X160.

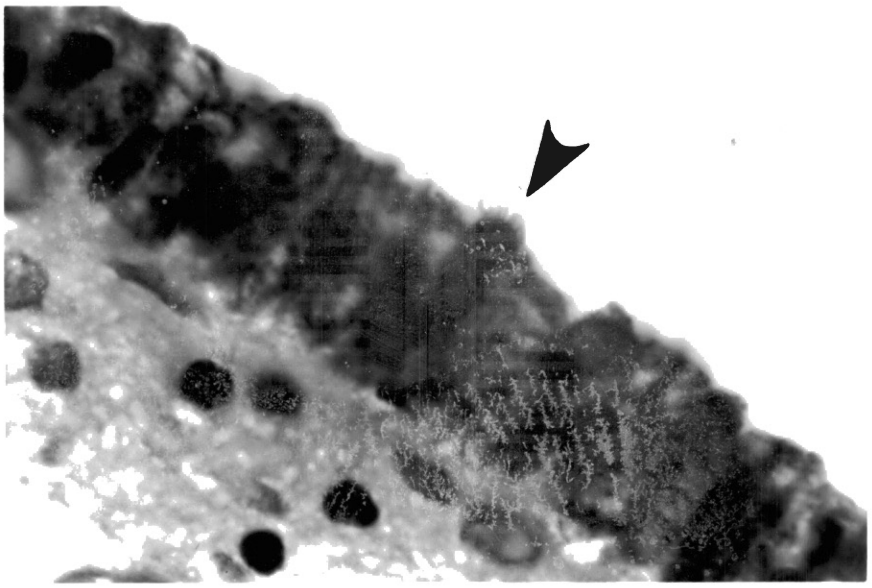


Figure 14. High magnification of uterine lining epithelium containing single orange-stained cells (arrow). Compare with slender orange-stained cells in Figures 3 and 13. Sano Trichrome Stain X400.

cells that were crowded together were present in some of the paraffin and glycomethacrylate sections and may represent precursors of the orange-stained cells seen in cytology smears and paraffin sections compatible with seasonal transition (Figure 12).

Glycomethacrylate Sections. The glycomethacrylate sections had features intermediate between the paraffin sections and electron microscopic preparations and matched closely the morphology of cellular and noncellular elements seen in cytology smears (Figure 15). Cilia were more easily recognized in glycomethacrylate sections than paraffin sections regardless of the stain used. Orange-stained cells were not seen in trichrome-stained glycomethacrylate sections.

In glycomethacrylate sections stained with AlcB-PAS-He, intracellular material was present in the same locations as in paraffin sections and was recognized as separate distinct granules (Figure 16). The range of colors was more easily appreciated and ranged from blue to red to purple. There was slightly less intracellular material in the epithelial cells of biopsies from mares with transitional patterns in cytology smears.

Biopsy Preparations Examined with the Electron Microscope

Mares with transitional patterns in cytology smears had low columnar to cuboidal surface epithelium with varying numbers of ciliated cells. Single ciliated cells were observed that appeared to be undergoing progressive loss of structure *in situ* (Figures 17, 18, and 19). Empty vesicles corresponding to those seen in light microscopic sections were seen (Figure 20). A single ciliated epithelial cell was seen in

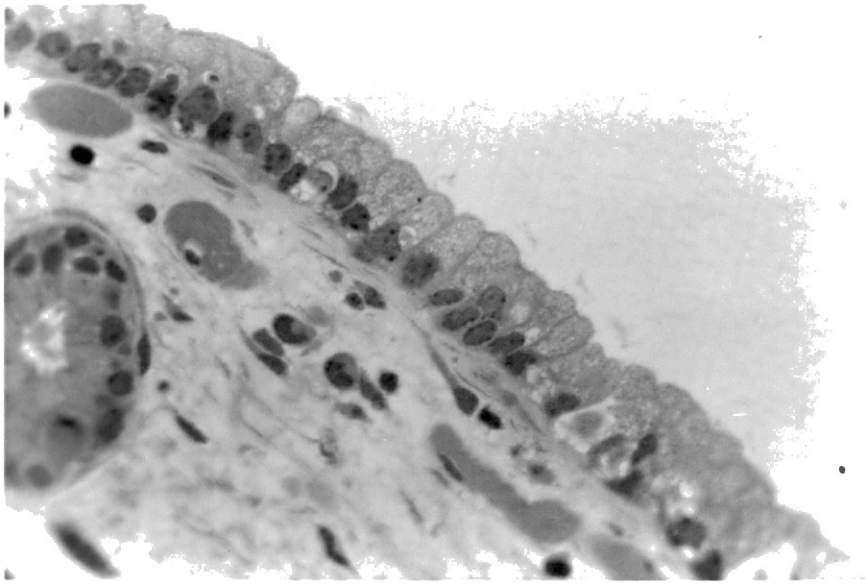


Figure 15. Glycomethacrylate section of uterine biopsy from same mare as in Figure 4. Note tall epithelial cells with foamy cytoplasm. H&E X160.

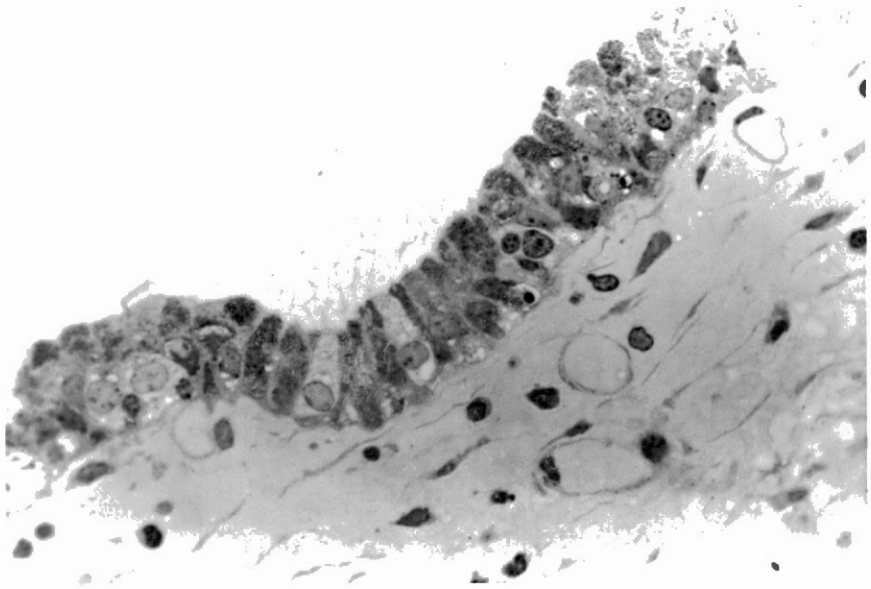


Figure 16. Variable cytoplasmic staining with distinct granules. Glycomethacrylate section of uterine biopsy specimen. AlcB-PAS-He X160.

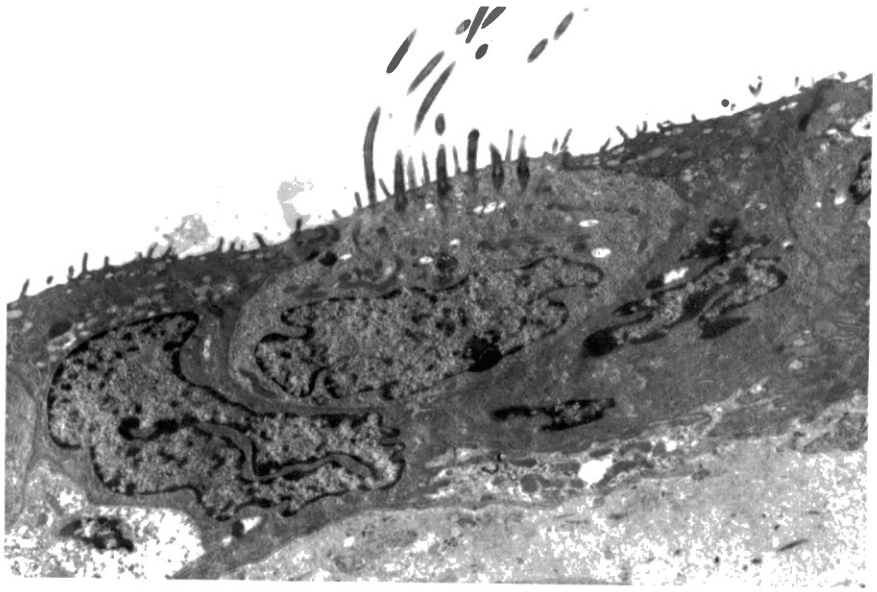


Figure 17. Healthy cuboidal ciliated epithelial cell in electron microscopic preparation of uterine biopsy specimen. Compare with cells from same specimen in Figures 18 and 19. Uranyl acetate-lead citrate X4,500.

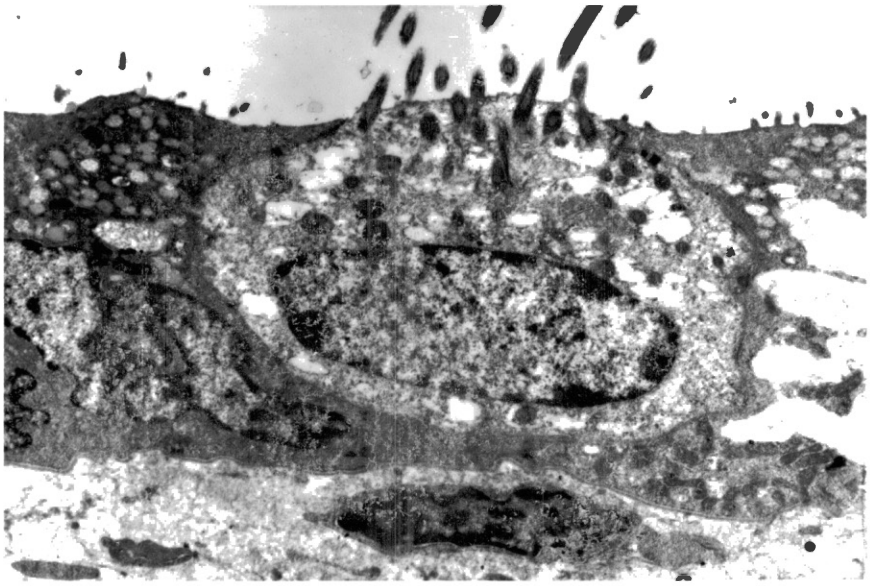


Figure 18. Moderate dissolution of cuboidal ciliated epithelial cell in electron microscopic preparation of uterine biopsy specimen. Compare with cells from same specimen in Figures 17 and 19. Uranyl acetate-lead citrate X5,900.

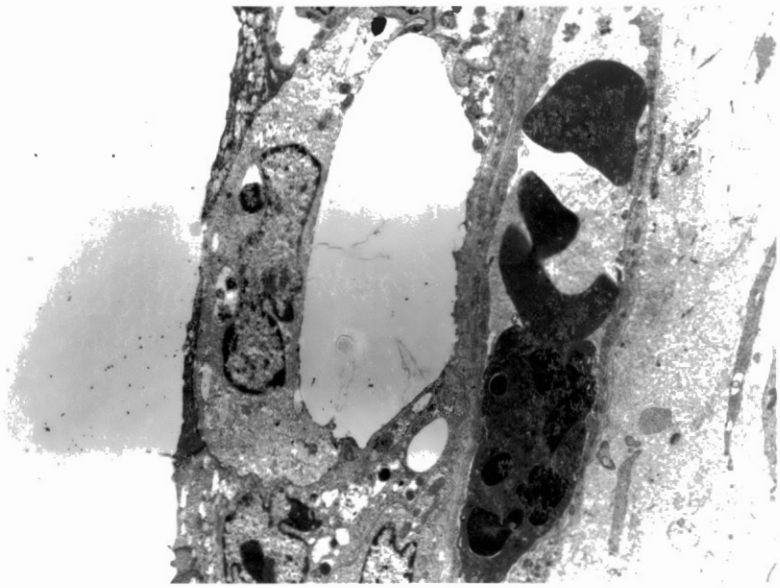


Figure 20. Vesicle in epithelial lining of uterine biopsy specimen examined with the electron microscope. Compare with vesicles in Figure 12. Uranyl acetate-lead citrate X3,600.

which the cilia were matted together to form a distinct club-like luminal projection (Figure 21). Similar cells were recognized in a glycomethacrylate specimen from the same mare (No. 6) when these were reviewed (Figure 22). The majority of lining cells were small, dark cells with dense cytoplasm. Some plump cells with prominent microvilli and cytoplasmic vesicles containing flocculent or globular material were seen (Figure 23). Ciliated cells were present in necks of glands seen opening onto the luminal surface. Few ciliated cells were seen in deeper glands. The epithelial cells in glands usually had prominent microvilli and apical secretory vacuoles. Material similar to that inside the vesicles was seen as extracellular material in the lumens of some of the glands (Figure 24).

Posttreatment (Five-Day) Collection

Cytology Specimens

Cytology Smears

All cytology smears at the five-day collection contained active-abnormal patterns with numerous groups of reactive epithelial cells (Figure 25). The degree of reactive epithelium was mild in the mares in Group A, marked in the mares in Group B, moderate in the mares in Group C, and marked in the mares in Group D (Table III). Differences in inflammatory response and other cellular and noncellular elements are detailed according to group in the following text.

Group A (Controls--Mares 1 and 2). There was moderate to marked necrosis consisting of karyorrhectic debris mixed with abundant mucus in the smears from Mare 1. A few inspissated glandular casts were present

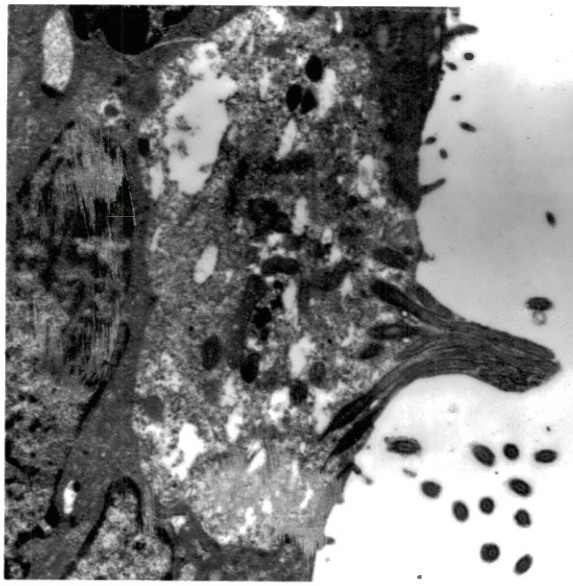


Figure 21. Cuboidal ciliated epithelial cell with matted, twisted cilia forming a club-like projection. Electron microscopic preparation of uterine biopsy specimen. Uranyl acetate-lead citrate X7,500.

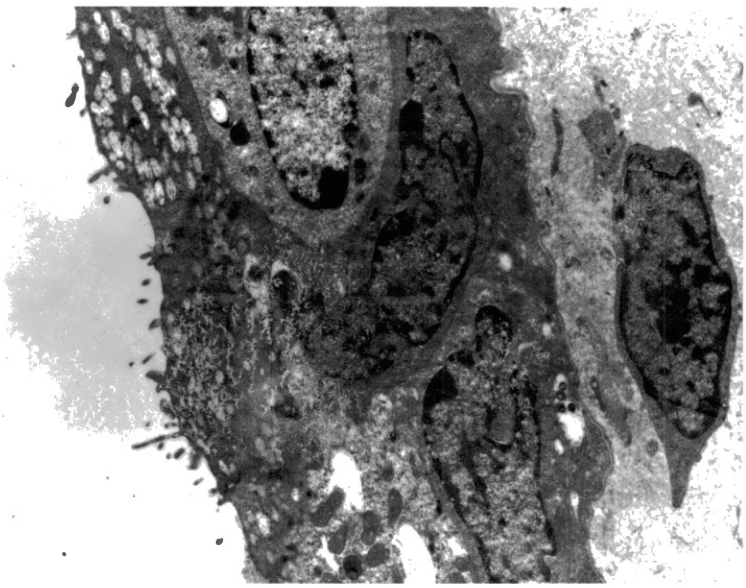


Figure 23. Dark cells with microvilli and cytoplasmic vesicles containing flocculent material. Electron microscopic preparation of uterine biopsy specimen. Uranyl acetate-lead citrate X5,900.

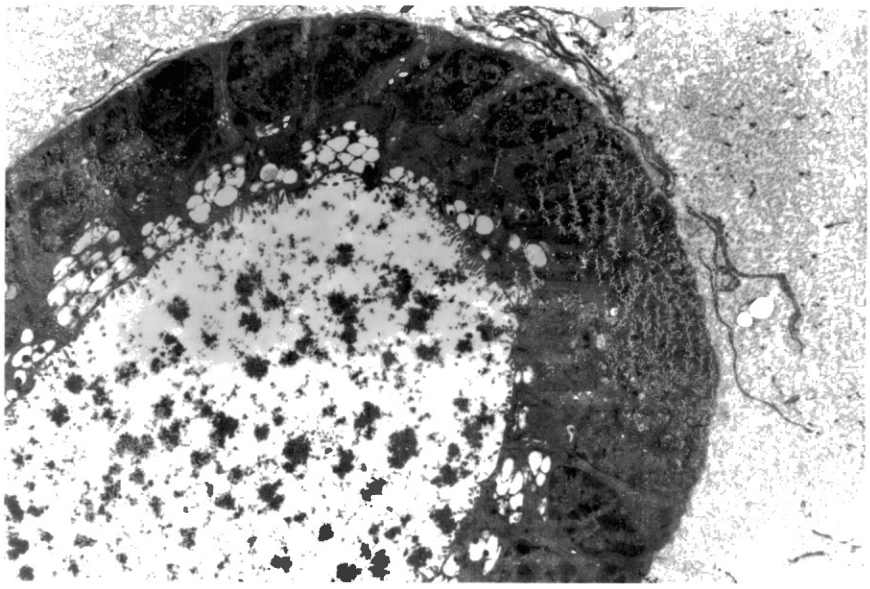


Figure 24. Uterine gland and contents. Uterine biopsy specimen examined with the electron microscope. Note similarity of material within cytoplasmic vesicles and in the lumen of the gland. Uranyl acetate-lead citrate X1,880.

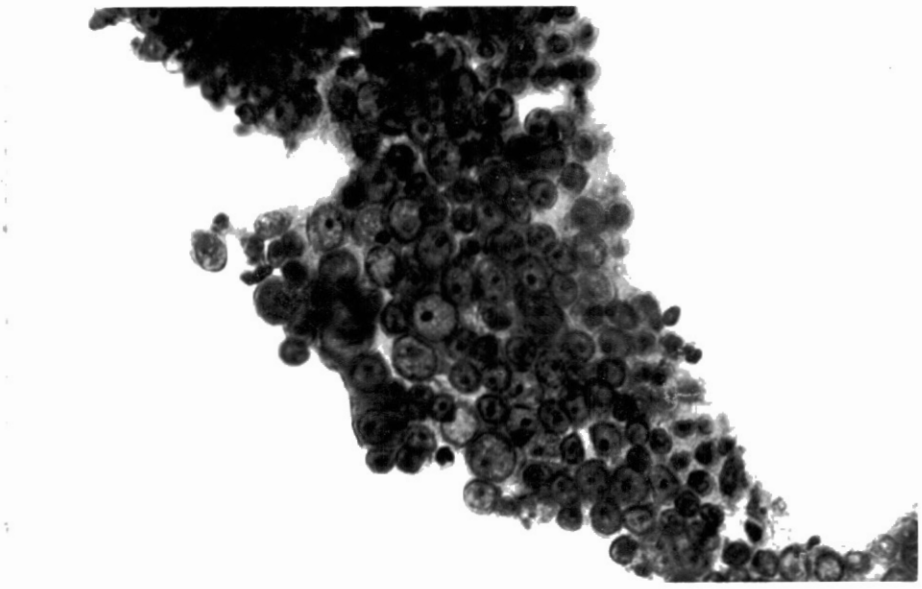


Figure 25. Large group of reactive epithelial cells with large nuclei and single prominent nucleoli. Posttreatment (five-day) cytologic smear from Mare 6. Sano Trichrome Stain X160.

along with a moderate number of neutrophils and a few eosinophils. Lymphocytes were moderately increased, and many erythrocytes were seen. There was a moderate number of small squamoid cells with dense, green cytoplasm known as chlorocytes. Some chlorocytes contained granular nuclear material surrounded by a halo that resembled nuclear inclusions (Figure 26). A few laminated concretions (Figure 27) resembling psammoma bodies found in gynecologic specimens from women (28) were seen. Similar structures have not been seen by the author in cytology smears from mares used in the study or from clinical cases. But, similar laminated concretions have been seen in the stroma of a uterine biopsy specimen from 1 of the 100 clinical cases included in this report and in the 40-day biopsy from Mare 3 (Figure 28).

In smears from Control Mare 2, neutrophils were moderately increased. A few inspissated casts were present along with a heavy protein background suggestive of the presence of blood (36).

Group B (Indwelling Catheter--Mares 3 and 4). Cytology smears from mares in Group B varied considerably. In smears from Mare 3, there were many normal and degenerating neutrophils which often occurred in groups. There were relatively few epithelial cells, but reactive epithelial changes were marked when epithelial cells were present. A few red ciliated cytoplasmic tufts were seen, and some enlarged stripped nuclei were present.

Mare 4 had a marked purulent reaction and many abnormal epithelial cells as well as a few reactive epithelial cells. A few casts were present. A moderate number of degranulated eosinophils, a few chlorocytes with intranuclear inclusions and marked necrosis, and cellular degeneration were present. Many degenerating or orange-stained cells

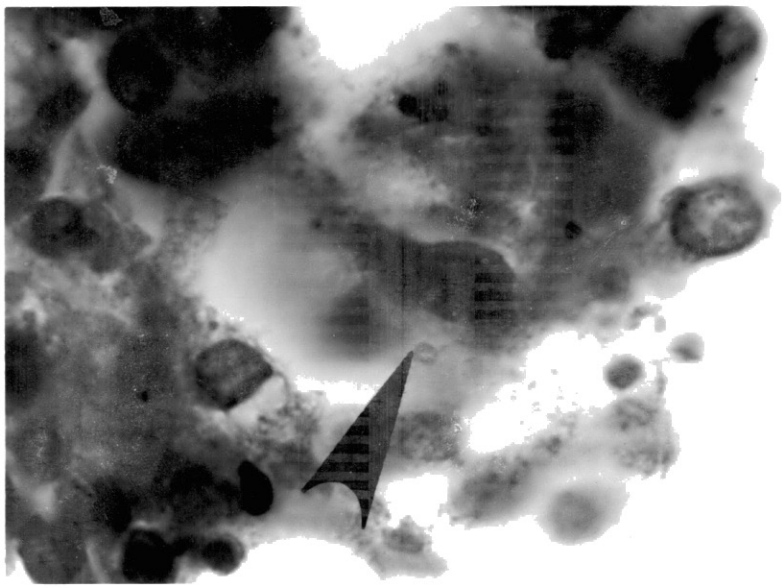


Figure 26. Single squamoid chlorocyte with dense, green cytoplasm and nuclear inclusion. Posttreatment (five-day) cytologic smear from Mare 1. Sano Trichrome Stain X400.

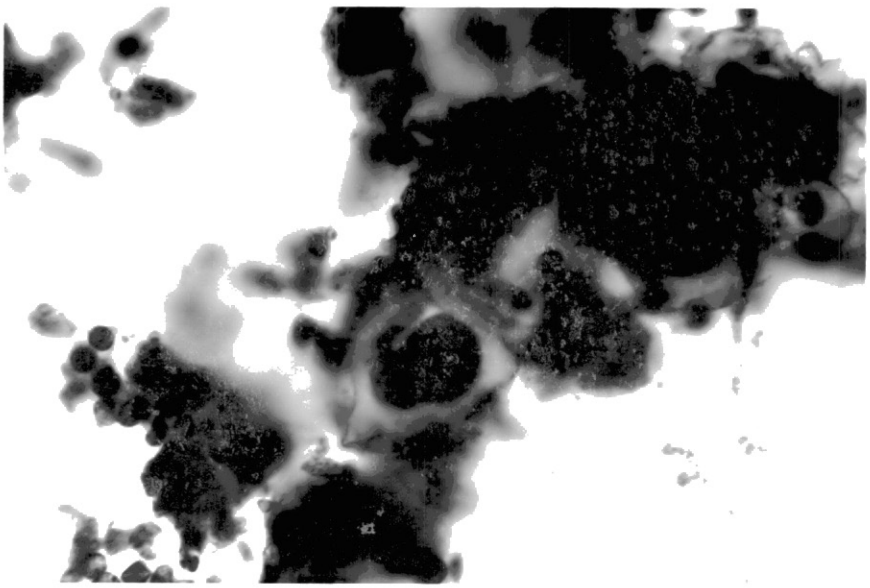


Figure 27. Laminated green concretions in posttreatment cytologic smear from Mare 1. Sano Trichrome Stain X160.

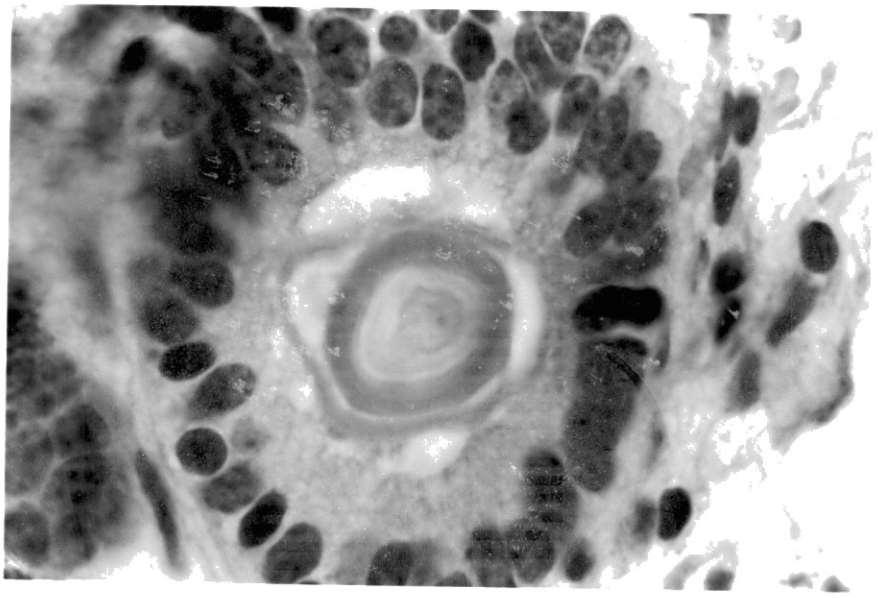


Figure 28. Laminated concretion within glandular lumen. Paraffin section of 40-day uterine biopsy specimen from Mare 3. Compare with Figure 27. H&E X400.

Cytology Preparations Examined with the Electron Microscope

The cytologic specimens contained epithelial cells primarily in cohesive groups (Figure 29). Disintegrating background material was present in the majority of specimens. In those specimens with a marked purulent response in cytology smears, numerous degenerate and non-degenerate inflammatory cells were seen. The columnar epithelial cells usually lacked cilia, and nuclear chromatin showed varying degrees of margination and karyolysis. In some cells, a single, large nucleolus was seen (Figure 29). Some of the cells had microvilli and numerous cytoplasmic vesicles containing flocculent to globoid material (Figure 30). In some cases, distinct structures were seen that were surrounded by a membrane enclosing multiple vesicles containing globoid material (Figure 31). These were similar to material seen in glandular epithelial cells and lumens from posttreatment biopsy specimens examined with the electron microscope (Figure 32). No bacteria were seen in posttreatment (five-day) cytologic preparations examined with the electron microscope.

Biopsy Specimens

Posttreatment (five-day) biopsy specimens contained varying degrees of epithelial abnormalities ranging from bizarre forms to subtle differences comparable to the reactive epithelial cells seen in cytology smears. The epithelial reaction was focal and varied between mares and between sites in the uterus. In general, the epithelial changes were relatively subtle and easily overlooked compared to the obvious changes recognized in cytology smears. The epithelial reaction was mild in

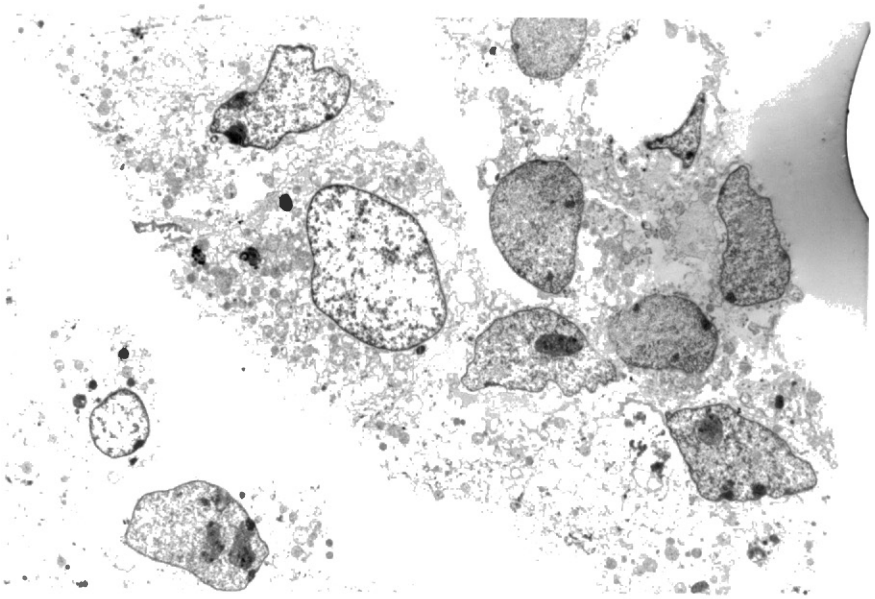


Figure 29. Group of reactive epithelial cells with large nuclei and several prominent nucleoli. Electron microscopic preparation of posttreatment (five-day) cytologic specimen. Compare with reactive epithelial cells in Figure 25. Uranyl acetate-lead citrate X1,800.

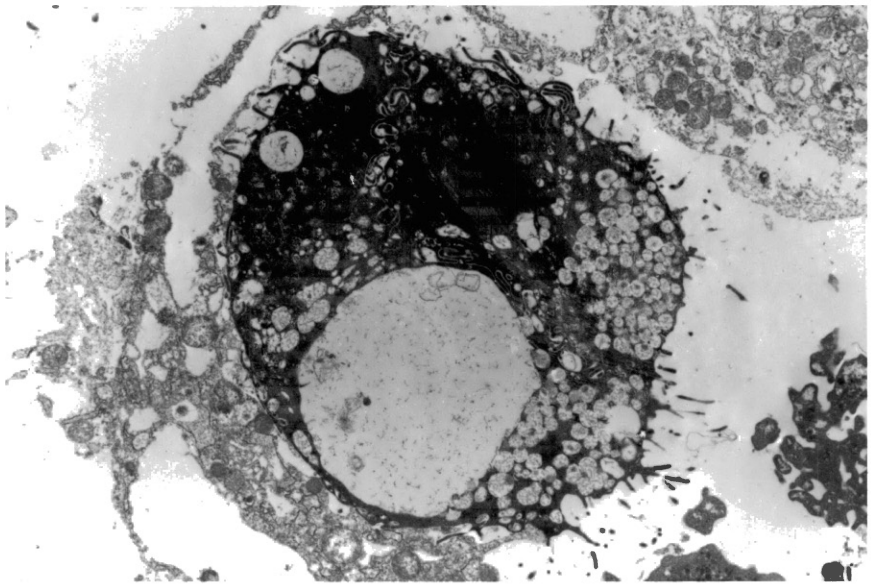


Figure 30. Secretory epithelial cell with microvilli and numerous cytoplasmic vacuoles. Electron microscopic preparation of posttreatment (five-day) cytologic specimen. Uranyl acetate-lead citrate X3,600.

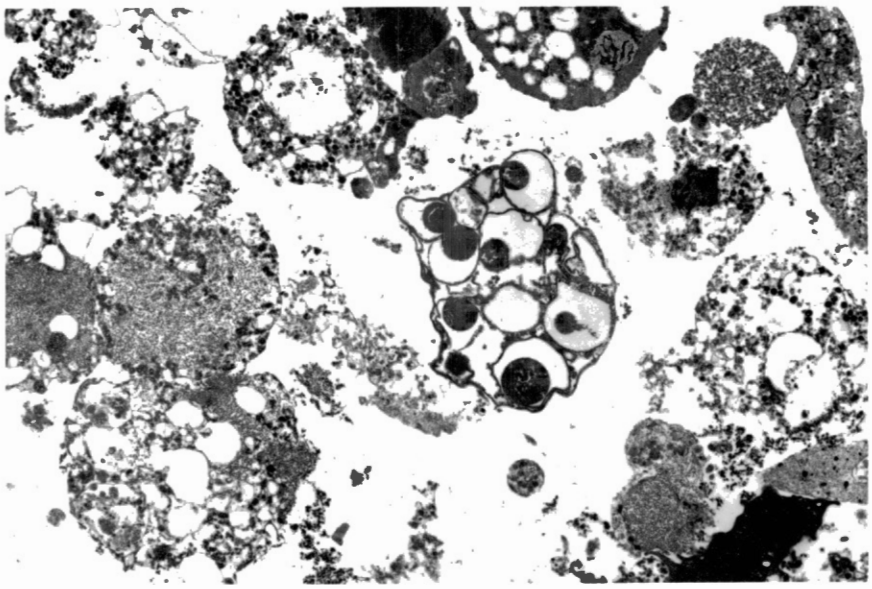


Figure 31. Membrane-bound structure containing vesicles enclosing globoid material. Posttreatment (five-day) cytologic specimen examined with the electron microscope. Uranyl acetate-lead citrate X3,600.

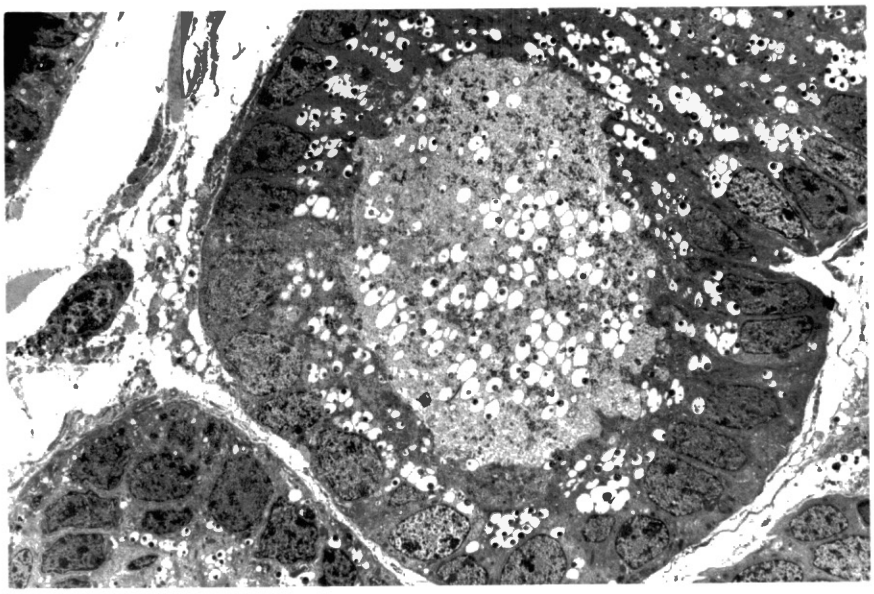


Figure 32. Uterine gland and contents. Electron microscopic preparation of posttreatment (five-day) biopsy specimen. Compare cytoplasmic vesicles and lumen contents with structure in Figure 31. Compare with gland and contents from initial biopsy specimen in Figure 24. Uranyl acetate-lead citrate X1,390.

paraffin sections from the bodies of Mares 1, 5, 6, 7, and 8 and in paraffin sections from the right horn of Mares 4, 6, and 8. Sections from the body of Mare 4 contained a marked epithelial reaction. Moderate epithelial reaction was seen in the sections of right horn from Mares 1, 5, and 7. No reactive epithelium was seen in either paraffin section from Mares 2 and 3 (Table II). Large areas of luminal epithelium with multiple layers of clear cells with large nuclei and clear cytoplasm were seen in many of the posttreatment specimens. Some of the luminal and glandular epithelium was very tall with homogenous or foamy cytoplasm and enlarged nuclei with prominent nucleoli. In some areas, all three types of epithelial cells were seen (Figure 33). Moderate to numerous vesicles along the basement membrane were present in the majority of histologic specimens. Some of these were similar to those in initial collections, but many contained degenerate or nondegenerate inflammatory cells. Reproductive activity in all posttreatment histologic specimens was compatible with seasonal transition except for the section from the uterine body of Mare 1, which was compatible with cyclic activity of diestrus (Table I).

Group A (Controls--Mares 1 and 2). Both paraffin sections from Mare 1 contained moderate numbers of eosinophils. The specimen from the uterine body contained mild neutrophilic and moderate lymphocytic inflammation. The specimen from the uterine horn had numerous neutrophils and lymphocytes. The glycomethacrylate section contained only muscle and connective tissue and was not suitable for interpretation. Mare 2 had moderate numbers of neutrophils and lymphocytes with a few eosinophils in the paraffin section of the uterine body and many neutrophils in the specimen from the right horn. The glycomethacrylate

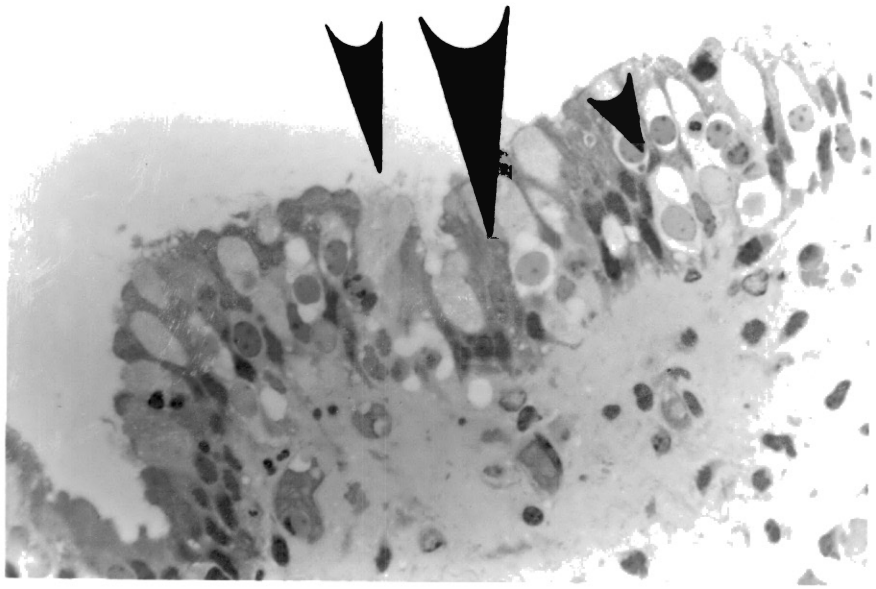


Figure 33. Cells with clear (small arrow), foamy (medium arrow), and homogenous (large arrow) cytoplasm in glycomethacrylate section of posttreatment (five-day) biopsy specimen. H&E X160.

section contained moderate numbers of neutrophils and a few eosinophils.

Group B (Indwelling Catheter--Mares 3 and 4). The paraffin section of the uterine body of Mare 3 contained moderate to severe neutrophilic and moderate lymphocytic inflammation. The paraffin section from the uterine body was lost in processing. The glycomethacrylate section had moderate to severe neutrophilic inflammation and a few eosinophils.

Both paraffin sections from Mare 4 contained many neutrophils and lymphocytes. The glycomethacrylate section had moderate to severe neutrophilic and lymphocytic inflammation and a few eosinophils.

Group C (Catheter Plus Saline--Mares 5 and 6). Both paraffin sections from Mare 5 contained many neutrophils and lymphocytes although the section of the specimen from the right horn also contained a few eosinophils. The glycomethacrylate section contained mild neutrophilic and moderate lymphocytic inflammation.

Mare 6 had severe neutrophilic inflammation and a few eosinophils in the paraffin section from the uterine body. A few eosinophils were also present in the section from the right horn along with moderate numbers of neutrophils and lymphocytes. Only moderate numbers of neutrophils were seen in the glycomethacrylate section.

Group D (Catheter Plus EDTA-TRIS Buffer--Mares 7 and 8). Severe neutrophilic inflammation was present in both paraffin sections from Mare 7 although a few eosinophils were present in the section from the uterine body. The glycomethacrylate section contained moderate numbers of neutrophils.

A few eosinophils were present in both the paraffin sections and the glycomethacrylate section of specimens from Mare 8. The paraffin

section of the uterine body contained moderate numbers of neutrophils and lymphocytes in contrast to moderate numbers of neutrophils and many lymphocytes in the section from the right uterine horn. The glycomethacrylate section contained moderate to many neutrophils and moderate numbers of lymphocytes.

Special Stains. The epithelium in biopsies from all mares was similar regardless of treatment group. The luminal epithelium typically contained dense, blue blebs at the apex of the cell when stained with AlcB-PAS-He (Figure 34). The superficial glands were dilated and contained abundant material similar to that seen in the luminal epithelium (Figure 34). Deep glands did not differ significantly from those seen in initial specimens.

Glycomethacrylate Sections. The staining in these sections, as in initial specimens, was more delicate and had greater variation in tinctorial properties than recognized in paraffin sections. In most sections stained with AlcB-PAS-He, dark blue apical blebs were present. Focal areas of reactive epithelial cells similar to those seen in cytology smears were more easily recognized in glycomethacrylate than paraffin sections. These changes were still very subtle compared to the cytology smears in which many of the epithelial cells were abnormal. In one case (Mare 7), the luminal epithelium had distinct cytoplasmic projections that did not contain stained material. These were similar to cytoplasmic projections seen in the specimens from this mare examined with the electron microscope (Figure 35). Similar structures have been described in electron microscopic studies of uterine epithelium of women following ovulation and associated with intraluminal secretion (62).

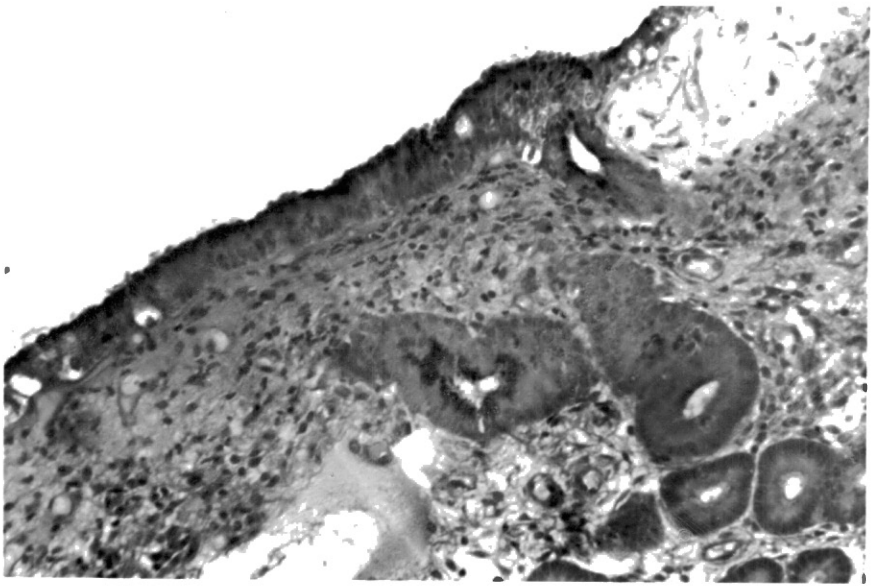


Figure 34. Paraffin section of posttreatment (five-day) biopsy specimen. Dark blue material in the epithelium of the endometrial lining superficial glands. AlcB-PAS-He X64.

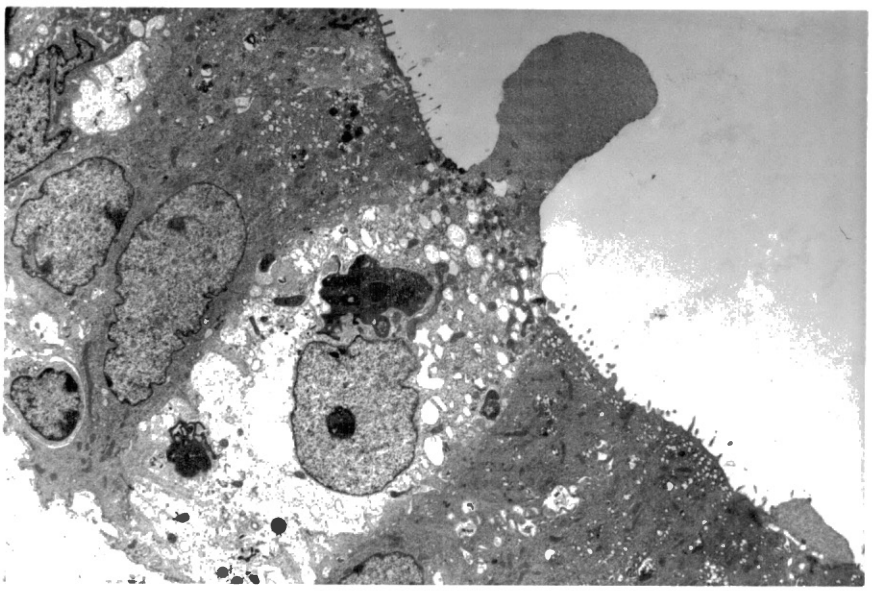
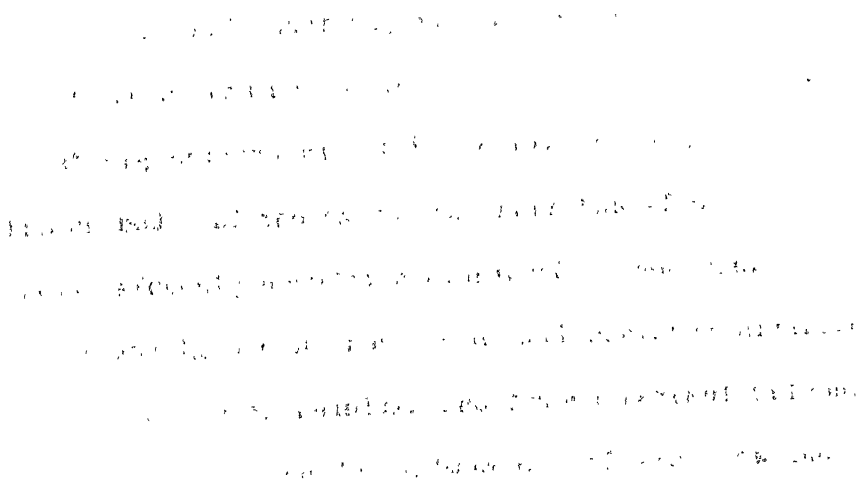


Figure 35. Cytoplasmic bleb projecting from an endometrial epithelial lining cell. Electron microscopic preparation of posttreatment (five-day) biopsy specimen. Uranyl acetate-lead citrate X1,880.



Biopsy Preparations Examined with the Electron Microscope

Biopsy specimens from mares following treatment contained varying numbers of inflammatory cells and primarily secretory epithelial cells. In some cases, cilia were seen projecting from the surface of cells with apical vacuoles containing flocculent or globoid material, suggesting conversion of ciliated cells to secretory ones (Figure 36). A few cells were seen with apical cytoplasmic projections similar to those described in the postovulatory endometrium of women and associated with intraluminal secretion (35, 59) (Figure 35). In uterine glands, separations between adjacent cells and numerous subnuclear dark bodies and laminated myelin whorls were seen. Separations between glandular cells were not seen in paraffin sections but were seen in one glycomethacrylate section. Glandular luminal secretions differed from those seen in initial specimens and were similar to membrane-bound structures seen in posttreatment cytology specimens examined with the electron microscope (Figure 32). A few bacteria within vesicles in the luminal epithelium were seen in the posttreatment biopsy specimen from Mare 1.

40-Day Collection

Cytology Specimens

Cytology Smears

Cytology smears from seven of the eight mares taken 40 days after the start of the study were similar in that they contained some single epithelial cells and epithelial cells in tight groups that showed only subtle features suggesting reactive epithelial changes. Nucleoli were

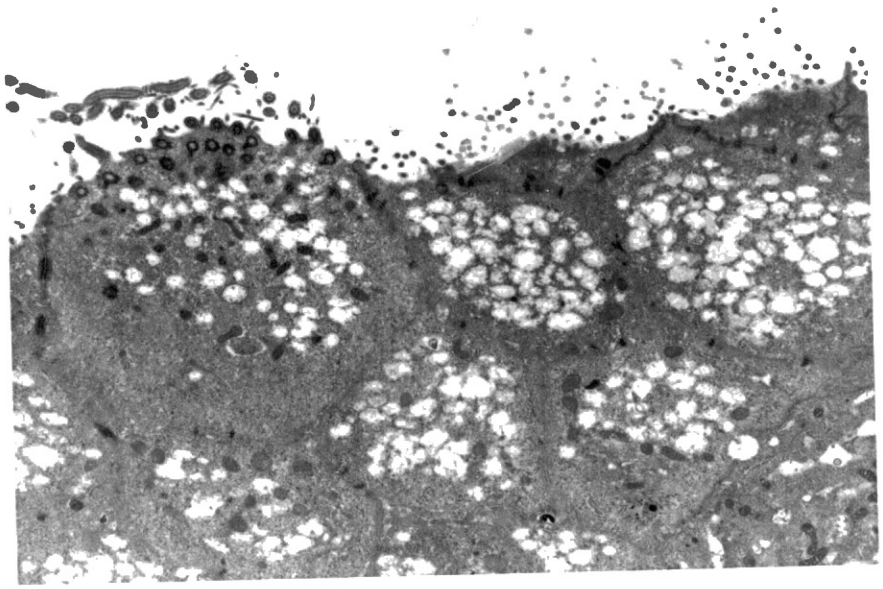


Figure 36. Endometrial lining cell containing both cilia and secretory cytoplasmic vesicles. Post-treatment (five-day) biopsy specimen examined with the electron microscope. Uranyl acetate-lead citrate X5,900.

not as prominent as those seen in specimens collected immediately following treatment. In many of the single cells, as well as the cells in groups, the nuclei were bland and hypochromatic (Figure 37). In several cases, the nuclear borders appeared to be folded. The smears were virtually free of inflammation with only a few neutrophils. A few to moderate number of stripped nuclei were present. A few to moderate numbers of slender orange-stained and red ciliated cytoplasmic tufts were seen. In Mare 7 in Group D (Catheter Plus EDTA-TRIS Buffer), a few degranulated eosinophils were seen.

Mare 4 in Group B (Indwelling Catheter) and Mare 5 from Group C (Catheter Plus Saline) also had moderate to large amounts of loose mucus, both free and in loose casts containing stripped nuclei and debris (Figure 38). Cellular, worm-like casts were seen in moderate numbers in both mares (Figure 39). These smear patterns were interpreted to represent progression of the seasonal transition to a relatively inactive state, with minimal inflammation and a variable catarrhal response.

The one mare that significantly differed from this pattern was Mare 1 in Group A (Controls). This mare's smears had an active-abnormal pattern with a large number of single epithelial cells and a moderate number of reactive epithelial cells in groups. Nuclei and nucleoli were not as enlarged as in posttreatment (five-day) smears. Chromatin stippling was prominent. A moderate number of hypochromatic stripped nuclei were present along with very few slender orange-stained cells. A moderate number of green ciliated cytoplasmic tufts were seen. Very few eosinophils, a few histiocytes, and a moderate number of neutrophils and lymphocytes were seen. A small number of chlorocytes and a few small

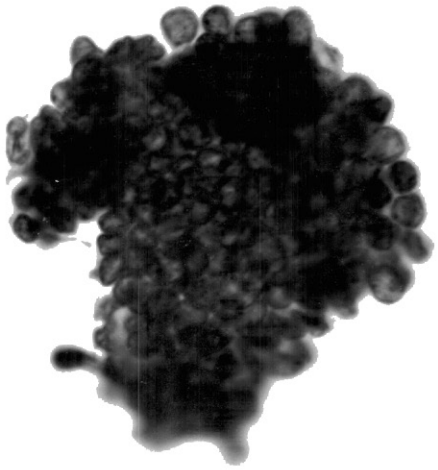


Figure 37. Tight group of epithelial cells with hypochromatic nuclei in cytologic smear 40 days after the start of the study. Sano Trichrome Stain X160.

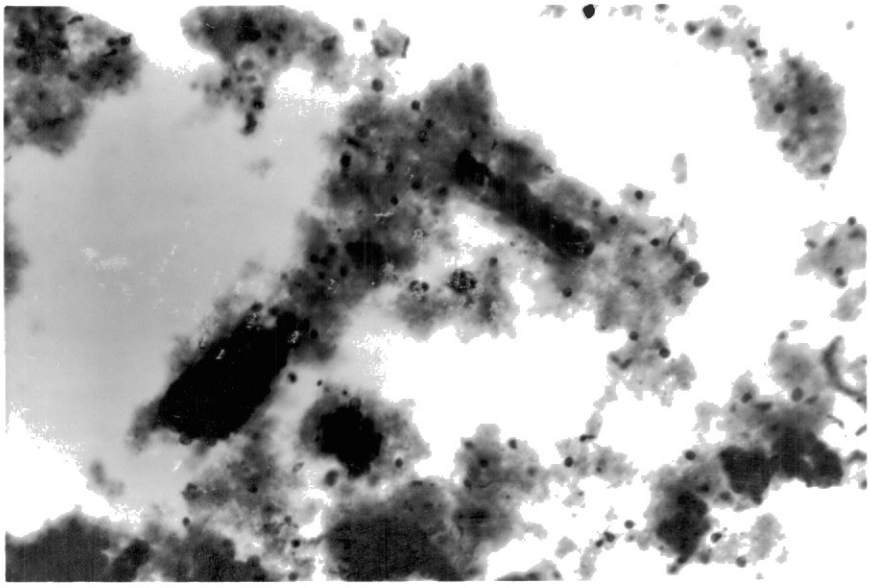


Figure 38. Abundant mucus mixed with cells and debris. Cytologic smear from Mare 4. 40-day collection. Sano Trichrome Stain X64.

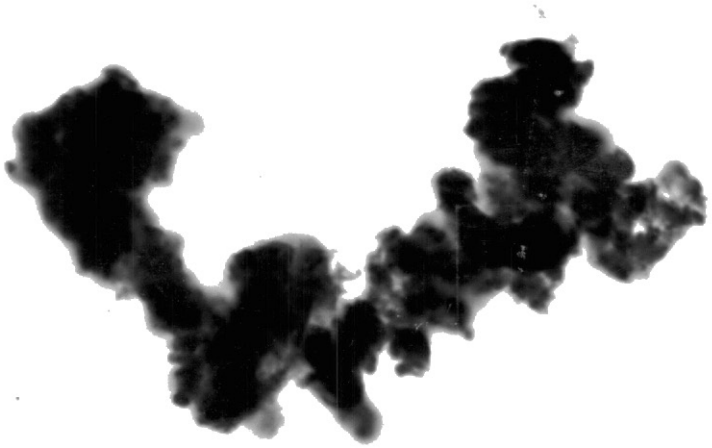


Figure 39. Cellular, worm-like cast in cytologic smear from Mare 5. 40-day collection. Sano Trichrome Stain X400.

inspissated mucus casts were seen.

Biopsy Specimens

Glycomethacrylate sections on biopsy specimens taken 40 days following the start of the study were not done. All paraffin sections from all mares were compatible with seasonal transition (Table I). In 40-day biopsy specimens, there were focal areas of multilayered luminal epithelial cells with clear cytoplasm, similar to that seen in posttreatment specimens. Focal patches of cells with large, hypochromatic nuclei also were seen. These were similar to but larger than the cells with homogenous cytoplasm and hypochromatic nuclei seen in initial biopsy specimens (Figure 40). The differences in inflammation were as follows.

Group A (Controls--Mares 1 and 2). The section of the uterine body of Mare 1 contained moderate numbers of neutrophils and lymphocytes while that from the right horn contained a few neutrophils, a moderate number of lymphocytes, and a single eosinophil. Both sections from Mare 2 contained a few eosinophils and moderate numbers of lymphocytes. Neutrophils were few in the section from the uterine body and moderate in the section from the right uterine horn.

Group B (Indwelling Catheter--Mares 3 and 4). Both sections from Mare 3 contained mild neutrophilic and moderate lymphocytic inflammation. Some areas of the section from the right horn were relatively free of inflammation.

Moderate numbers of lymphocytes were present in both paraffin sections from Mare 4. The section of the uterine body also contained a moderate number of eosinophils and several foci of neutrophils and

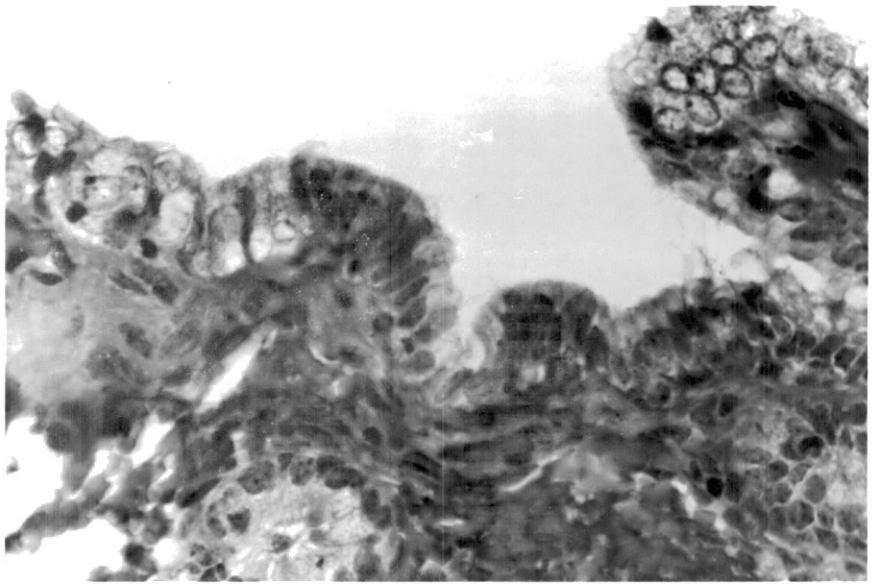


Figure 40. Paraffin section of 40-day biopsy specimen. Focal areas of cells with large hypochromatic nuclei. Sano Trichrome Stain X160.

lymphocytes. The section from the right uterine horn contained a few neutrophils and many eosinophils.

Group C (Catheter Plus Saline--Mares 5 and 6). The sections from Mare 5 had a few lymphocytes in the uterine body and a moderate number of lymphocytes in the right uterine horn. Mare 6 had a few lymphocytes in the section from the right uterine horn. The paraffin section of the specimen from the body of the uterus was lost in processing.

Group D (Catheter Plus EDTA-TRIS Buffer--Mares 7 and 8). In Mare 7, a few neutrophils and a moderate number of lymphocytes were present in the sections of the uterine body, and the right horn contained a moderate number of neutrophils and a few lymphocytes.

Mare 8 had a moderate number of lymphocytes in both sections and a few neutrophils in the section from the right uterine horn.

Special Stains. Trichrome-stained sections did not have features that differed significantly from those seen in H&E-stained specimens. The AlcB-PAS-He-stained sections had variable staining of the apical aspect of the luminal epithelium. There was less distinct concentration at the apex of the cell than in the posttreatment (five-day) specimens. Pink PAS-positive material was present as well as blue material similar to that seen in paraffin sections following treatment. The clear cells did not contain intracellular material. The epithelium of the superficial glands (necks) was not uniformly involved. Some areas were similar to the staining observed in the luminal epithelium while others were similar to superficial glands seen in initial specimens (Figure 41).

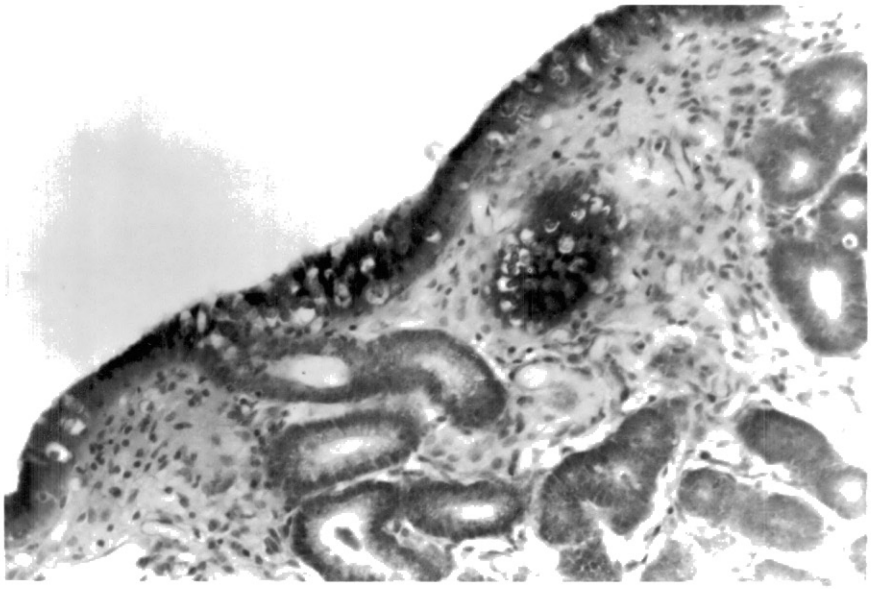


Figure 41. Paraffin section of 40-day biopsy specimen. Dark blue and pink staining of surface epithelium with focal areas of clear cells with pale blue nuclei. Variable involvement of superficial glands. Compare with staining reaction in Figure 34. AlcB-PAS-He X64.

Clinical Cases

Pre- and posttreatment equine uterine cytology and biopsy specimens were available from 32 clinical cases. The pretreatment cytology smears and histologic sections indicated a variety of conditions, including urine pooling, pyometra, fungal infection, and varying degrees of acute and chronic inflammation. They were collected at varying times of the year, and active, inactive, and transitional states were recognized in some of the specimens. A general pattern was apparent, however, in response to treatment with intrauterine infusions of various saline-diluted antibiotics, with and without EDTA-TRIS buffer. The posttreatment cytology smears from these mares invariably contained numerous reactive epithelial groups similar to those seen in this study. In some cases, however, a squamous epithelial component was part of these groups (Figure 42). A larger number of single tall columnar epithelial cells with foamy to granular cytoplasm and a large nucleus with a single, large nucleolus were seen in smears from clinical cases (Figure 43). Although inflammation was always present, the severe purulent response following treatment seen in two mares in this study was not seen in clinical cases except for one mare in which pyometra was present prior to treatment. The epithelial component in clinical cases usually involved a much larger proportion of the smear, and the inflammatory component was relatively less apparent than in the smears from mares in this study. Severe epithelial abnormalities similar to those seen in posttreatment (five-day) specimens from Mare 4 in Group B were rarely seen in smears from clinical cases. Similar cells have been seen prior to treatment in one mare with a clinical history of infertility and cytologic evidence of urine pooling and following treatment in a

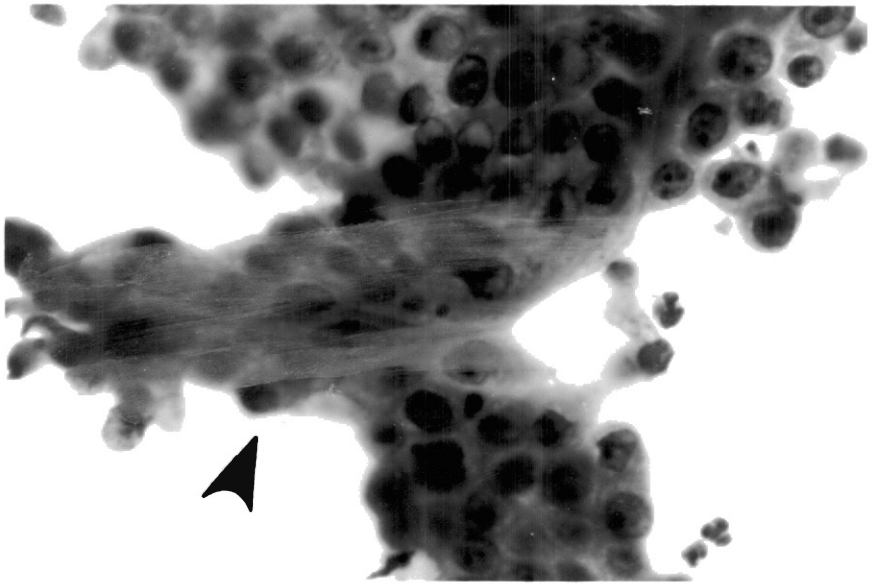


Figure 42. Group of reactive epithelial cells with squamoid features (arrow). Cytologic specimen from a clinical case following five days of treatment. Sano Trichrome Stain X320.

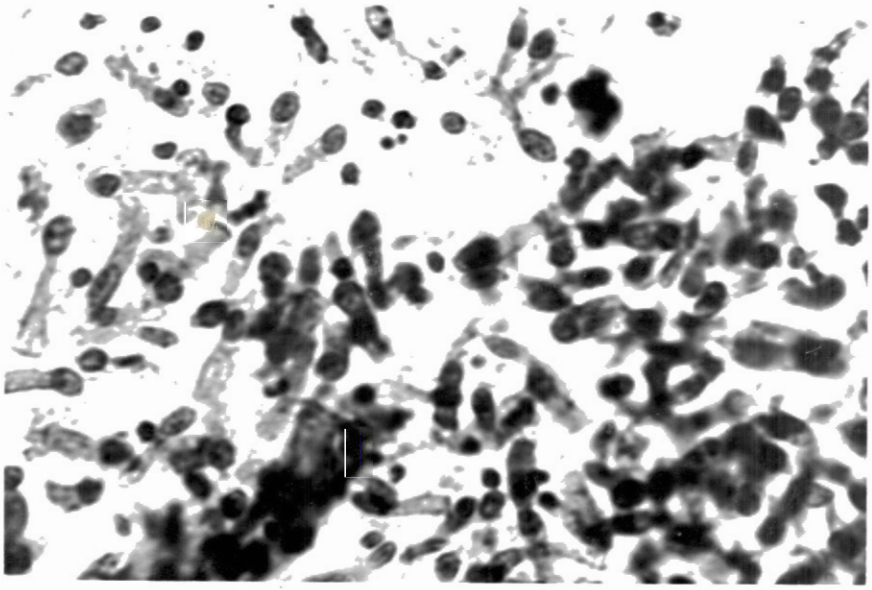


Figure 43. Large number of single tall columnar cells with foamy cytoplasm characteristic of smears from clinical cases following treatment. Sano Trichrome Stain X160.

mare with a clinical history of infertility and mild acute inflammation in uterine cytology and biopsy specimens. Chlorocytes were seen in many smears from clinical cases both prior to and following treatment.

Endometrial biopsy specimens from clinical cases prior to and following treatment also varied considerably. But, epithelial changes other than increased height and foaminess of surface-lining cells were difficult to detect. Focal patches of lining epithelium and epithelium extending into the superficial glands contained nuclei in which large nucleoli were present. In some cases, disorganized epithelium with loss of nuclear polarity was seen. Routine paraffin sections of a single biopsy specimen stained with H&E were used for evaluation of clinical cases. Glycomethacrylate embedding and special stains were not routinely done.

In seven cases, subsequent cytology and biopsy specimens collected 20 to 60 days following treatment were also available. The smears from these cases usually continued to have an active pattern with return of epithelial morphology toward normal and decreasing inflammation. In several cases, biopsy specimens did not differ significantly from previous specimens although cytology specimens reflected dramatic improvement. Recommendations for breeding of problem mares upon the return of cytology smears to normal resulted in conception, even though the interpretation of the corresponding biopsy may not have warranted such a favorable prognosis due to the persistence of inflammation.

CHAPTER IV

DISCUSSION

Light microscopic studies of equine uterine cytology specimens and light and electron microscopic studies of equine uterine histology specimens (1, 3, 5, 6, 7, 17, 18, 19, 21, 24, 29, 32, 33, 34, 38, 39, 40, 41, 43, 46, 47, 48, 49, 52, 53) are limited when compared to the volume of material from similar studies conducted on human specimens (4, 10, 11, 13, 15, 16, 22, 28, 30, 50, 57, 58, 59). Previous studies on equine cytology specimens have primarily been concerned with quantification of the inflammatory response (29, 33, 46, 47, 48, 52, 53, 54). There are conflicting reports on cytologic patterns associated with phases of the equine reproductive cycle (6, 29). Specific patterns were not recognized in association with proestrus, estrus, or diestrus in the mares in this study or in clinical specimens. The results of this study were similar to those reported by another investigator (6). The features in endometrial washes associated with active cycling, winter anestrus, and seasonal transition have not been reported previously.

Reports on the histologic appearance of the equine uterus have dealt with recognition of inflammation, fibrosis, phases of the reproductive cycle, seasonal transition, anestrus, and involution following parturition (3, 5, 19, 21, 24, 32, 43, 46, 47, 48). Other studies have reported histologic changes associated with infectious agents (1, 7, 33, 34). Electron microscopic studies of the equine uterus have been

limited to specific etiologic agents, the cycling mare, and the equine placenta (7, 33, 38, 39, 40, 41, 49). No electron microscopic studies of equine uterine cytology specimens were found in the English literature.

The interpretation of reproductive activity in cytology smears was shown to depend on representative epithelial morphology, without interfering inflammation and/or treatment. The interpretation of a single active-normal pattern compatible with normal cyclic activity in the initial cytology smear from Mare 4 did not match the interpretation of the biopsy specimens from the same mare (Table I). The cytologic interpretation was influenced by the lack of inflammatory cells in the initial smear. The interpretation of the initial smears from Mare 7 as active-abnormal reflects the possible influence of inflammation on epithelial morphology. The interpretation of reproductive activity in the biopsy specimens relied primarily on stromal characteristics and tortuosity and dilation of the glands, features which are not represented in cytology smears. Although inflammation was present in the biopsy specimens from Mare 4, the results of this study suggest that false interpretations of cyclic activity may occur when inflammation is not present in cytology smears. Therefore, caution in interpretation of reproductive activity in cytology smears is urged since the collection of a single cytology and biopsy sample was sufficient to cause marked changes in the cytology specimens collected five days later from control mares.

The evaluation of distribution of inflammation and recognition of epithelial changes were not uniform between locations in the uterus (body vs. right horn) nor between methods of specimen preparation

paraffin sections vs. glycomethacrylate sections) (Tables II and IV). In many cases, the degree of inflammation in glycomethacrylate sections was estimated to be less than in the corresponding paraffin section of tissue from the same location (uterine body). This may be due to less shrinkage in the glycomethacrylate sections with relatively fewer inflammatory cells in a given amount of stroma. Glycomethacrylate sections were helpful in locating reactive epithelium not readily apparent in corresponding paraffin sections and in providing a different perspective for evaluation of standard biopsy specimens. Each type of specimen and preparation of these specimens (cytology smears, paraffin sections, glycomethacrylate sections, special stains, and electron microscopic preparations) offered unique perspectives on the evaluation of normal and abnormal processes. The value of the use of multiple techniques lies in their confirmation and support of future interpretations of standard cytology and bioipsy specimens from clinical cases.

Although the inflammation observed in paraffin sections varied with location of the sample, the prognostic grade assigned to such a biopsy would not have differed. These findings support those of other investigators regarding the representativeness of the single uterine biopsy in evaluation of inflammation and assignation of a prognostic grade (3). Significant fibrosis was not present in any of the biopsy specimens from the mares in this study, so its distribution cannot be evaluated. This work also supports the previous hypothesis that eosinophils may not occur in an even distribution throughout the uterus and that a combination of both cytology and biopsy specimens may increase the likelihood of detection (46) (Table IV).

The recognition of abnormal epithelium in biopsy specimens may

depend greatly on the skill of the pathologist, diligence in examination of the tissue, and the knowledge of its significance. In this study, reactive epithelial changes were much more obvious in cytology smears than histology specimens. Therefore, cytology smears are recommended over biopsy for their detection.

The response to the uterine treatments varied between and within groups. The occurrence of a purulent response in cytology smears did not appear to depend on the presence of significant inflammation in previous smears nor on the presence of a particular cytologic pattern of activity (active vs. transitional) in previous specimens. Although a subjective evaluation, the degree of epithelial reaction in cytology smears was less in control mares than in the treated mares. The epithelial and inflammatory responses were similar in single mares from the groups treated with indwelling catheters (Group B), catheter plus saline infusion (Group C), and catheter plus EDTA-TRIS buffer infusion (Group D) (Mares 3, 6, and 8). One mare from the group treated with only the indwelling catheter had the most significant epithelial abnormalities, consisting of severe epithelial atypia in addition to markedly reactive epithelial cells.

The relatively more-pronounced epithelial reaction consisting of many single cells, cells in typical groups, and the relatively decreased inflammatory responses seen in posttreatment smears from clinical cases following treatment may be due to the inclusion of antibiotics as part of these treatments. The presence of inflammation and/or various bacterial agents prior to treatment may also have influenced the patterns seen following treatment. However, the presence of inflammation in pretreatment cytology and biopsy specimens in the mares used in this

study did not result in posttreatment specimens with features similar to those seen from clinical cases.

Sodium chloride solution, in volumes approximately equal to those used in this study, is a common diluent for antibiotics placed in the uterus of the mare to insure adequate coverage of the endometrial surface (9). EDTA-TRIS buffer also has been used in conjunction with intrauterine antibiotic therapy for resistant organisms, particularly Pseudomonas and Klebsiella (25, 60). Results from this work may provide the basis for future studies designed to separate the effects of antibiotics from concurrently used fluid vehicles and the physical trauma of catheterization and cytology and biopsy collections.

The endometrial epithelium of women consists of nonciliated secretory and ciliated nonsecretory epithelial cells (11, 22, 59). This study indicates that the uterus of the mare is similar. Scanning electron microscopic studies of uteri from women (22) and mares (41) indicate that ciliated cells are more numerous surrounding openings of glands. The light and transmission microscopic specimens from the mares in this study had many ciliated cells associated with openings of glands onto the lumen of the uterus. The concentration of ciliated epithelial cells in women varies between patients and between regions of the uterus, being more abundant close to the cornua and the endocervix (11, 59). The surface epithelium of the uterus of women typically contains more ciliated cells than the glands (11). Our observations indicate that the surface epithelium has more ciliated cells in the mare. Ciliated cells in women (11), as in the mares in this study, are typically "clear" cells with abundant translucent cytoplasm. Other "clear" cells in the uterus of women have been identified as cells in

early prophase and as degenerating cells with karyorrhexis (11). In addition, ciliated cells in women may undergo deciliation by aprocrine mechanisms, reciliation or necrosis in situ, as well as having the ability to develop secretory capacity (11, 15, 59).

Biopsies from the mares in this study and from clinical cases suggest that equine endometrial ciliated cells also undergo deciliation in situ. The presence of green ciliated cytoplasmic tufts in smears from mares with active cytologic patterns suggests that ciliated cells may shed their ciliary borders and transform into typical tall, foamy columnar cells associated with secretory activity. The presence of cilia on cells with other features associated with secretory activity in electron microscopic preparations supports this hypothesis (Figure 36).

The number of ciliated cells in the uteri of women increases and decreases in direct proportion with the levels of estrogen (11, 59). A similar situation may exist in the mare since fewer ciliated cells were observed in light and electron microscopic specimens from mares with a transitional smear pattern, when ovarian activity is expected to decline versus an active smear pattern compatible with cyclic activity and more elevated levels of estrogen (17). However, concurrent hormone profiles would be necessary to confirm this hypothesis.

The presence of clear cells in equine endometrial biopsy specimens examined with the electron microscope and similar to those seen in posttreatment samples in this study has been reported in association with contagious equine metritis infections (33). It was hypothesized that they represent a proliferative response accompanying degenerative changes. The current electron microscopic study indicates that these clear cells may reflect a response to injury rather than cellular

proliferation. Features associated with epithelial regeneration in the uterus of women and rabbits include flattened to cuboidal epithelium with abundant organelles and prominent nuclei and nucleoli. Features consistent with active cell metabolism and nuclear DNA synthesis such as abundant ribosomes, endoplasmic reticulum, mitochondria and Golgi, and nuclei with prominent nucleoli have been reported to represent cell growth and epithelial regeneration (13, 14). Such features are lacking in the epithelial cells seen in posttreatment specimens in this study. This hypothesis also is supported by a report on cytologic specimens from women (16). In gynecologic cytology specimens from over 1,000 women, epithelial cells similar to the reactive epithelial cells described in this report were classified according to the degree of morphologic alteration. Several categories were used based on retention of features suggestive of a specific epithelial cell line of origin and degree of nuclear and nucleolar deviation from normal. The reactive epithelial cells seen in mares in this study most closely resemble those in the human study with the fewest deviations from normal and were suggestive of glandular and/or squamous origin. The appearance of groups of reactive epithelial cells in mares which had large nuclei and prominent nucleoli in essentially every nucleus also was characteristic of the features seen in women (16). It was hypothesized that these cells, rather than representing regeneration of epithelium following prior destruction, represented epithelial cells which were in the process of reacting or responding to one of many noxious stimuli (16). In their study, inflammation, ionizing radiation, and chemical and physical trauma were implicated in producing such a response.

Previously, investigators interpreted these cellular features as

representative of epithelial regeneration based on the association of nuclear and nucleolar enlargement with cellular proliferation (4, 10, 28, 30, 50). In cytology specimens from women, the similarity of these reactive epithelial cells to neoplastic cells, particularly when they occur singly, has been acknowledged (16, 50). Reactive responses may result in alterations in cellular metabolic activity leading to increased nuclear and nucleolar enlargement. Robbins states, "... the evolution of a cancer involves more than loss of growth controls and rapid proliferation; it may also represent a disorder of differentiation, maturation, and death of (cancer) cells" (36). It may be that the reflections of these metabolic and maturational differences are common to basic biochemical processes in both reactive and neoplastic cells and makes their differentiation difficult.

Recently, there have been attempts to differentiate types of cell death and better define the process of normal cell death in physiologic and pathologic processes (44, 55, 56). The term "apoptosis" has been suggested to designate a particular type of physiologic cell death that may occur in association with various pathologic and nonpathologic conditions. This subject has been extensively reviewed by several authors (44, 55, 56). Some of the changes observed in light and electron microscopic specimens in this study were compatible with apoptosis.

Apoptosis in isolated cells results in the deletion of large numbers of cells without disturbance of the overall organization of the tissue. Apoptosis may occur as a result of decreased hormonal stimulation (44, 55, 56). It is characterized by condensation and vacuolation of the cell cytoplasm with maintenance of the integrity of organelles and progression to dispersal as apoptotic bodies. It differs from

necrosis, in part, by involvement of single cells rather than contiguous groups and by lack of accompanying inflammation. Certain features, such as pyknosis and karyorrhexis, are common to some stages of both necrosis and apoptosis when observed with the light microscope. The involvement of single cells, the small size of most apoptotic bodies, and rapid digestion of the bodies in adjacent cells without concomitant inflammation has been proposed as the reason this process is difficult to recognize in tissues (55, 56). Electron microscopic examination may be required to confirm its presence (55, 56).

The presence of single degenerating cells with well-preserved organelles and without accompanying inflammation in electron microscopic preparations of biopsy specimens in this study is compatible with the description of apoptosis (Figures 17, 18, 19, and 20). Typical apoptotic bodies and cytoplasmic vacuoles, similar to those pictured in reviews on the subject, were present in light microscopic specimens (Figures 12 and 20).

Reports of apoptosis in cytologic preparations could not be found. The orangophilic cells and red ciliated cytoplasmic tufts observed in cytology smears have electron microscopic features of apoptosis. The nature of cytology smears, in which cellular features are more clearly seen, may contribute to the recognition of this process more readily in cytology smears. Apoptosis is derived from the Greek word meaning to "drop off" (44). This is appropriate since it seems these cells are more readily recognized in cytology smears when they drop off in significant numbers. Apoptosis has been reported to occur in the uterine epithelium of hamsters during periods of decreased estrogen stimulation (42). This may allow the simultaneous elimination of large numbers of

cells from the uterus of the mare during the transitional period and may account for the depletion of ciliated cells proposed to occur in this study as estrogen levels decreased.

Cilia in the endometrium of women have been hypothesized to function in the movement of endometrial secretions (11, 22, 59). The presence of matted and twisted cilia in equine biopsy specimens examined with the electron microscope may be significant in that ciliary function would undoubtedly be impaired in such a state. Matted cilia have been reported but not pictured by other investigators (33). Whether the cilia were similar to those seen in this report is not known.

The presence of chlorocytes only in posttreatment smears, in the 40-day smear from one mare with persisting inflammation, and in smears from clinical cases suggests that these cells are not associated with normal processes. These cells often resemble metaplastic squamous cells. The exact significance of chlorocytes in equine endometrial cytology smears is not known but deserves further investigation.

The present study involved a limited number of mares but offers information that should be useful in future evaluation of normal and abnormal equine uterine specimens and the response of the equine uterus to treatment.

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VITA 2

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