CYCLICAL CHANGES OF THE BOVINE ENDOMETRIUM AND CERVICAL MUCOSA

by

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INTRODUCTION

The profit derived from the cattle industry is dependent on a high degree of reproductive efficiency. Normal reproduction is, in turn, dependent upon the production of viable sperm and ova and the maintenance of the genital tract of the female in an optimum condition for fertilization, implantation and fetal development. Temporary or permanent sterility, causes economic losses to the producer through reduction of the annual calf crop and lowered milk production. Fertility, then, is of major importance.

The reasons for reproductive inefficiency are numerous and poorly understood. Complete harmony between the anatomical components and the physiological processes of the reproductive system are essential if normal pregnancy is to occur. Any deviation in this unique and complicated process, caused by mechanical, nutritional, pathological or other factors, can produce unfavorable reproductive results.

Field and clinical problems dealing with the techniques and results of artificial insemination, the reasons for the often necessary repeat breeding and the causes of early embryonic losses must be investigated and solved. Sperm deposition occurs in the cervix of the bovine. It is the cervical epithelium that aids in the transmission and distribution of the spermatozoa at that time and functions as an anatomical and physiological barrier between the vaginal and uterine environments. The uterine changes occurring during the estrous cycle are repeated

preparations of the environment required by the fertilized ova. To gain an understanding of the reproductive adaptations, it is first necessary to establish the normal functions of the uterus and cervix, and to correlate these with cytological information obtained from the uterine surface epithelium, glandular tubules and cervical epithelium. Once this has been accomplished, the solutions to many reproductive problems may follow.

This study was undertaken in an effort to correlate histological, histochemical and ultrastructural findings in the bovine uterine and cervical epithelium. The characterization of the cyclic changes that take place in the normal cow are essential for a better understanding of the complex process of reproduction. Any contribution to the present-day knowledge may aid in completing the picture of bovine reproduction and thereby result in increased reproductive efficiency.

LITERATURE REVIEW

General

In the female embryo, the Mullerian ducts grow and develop into the female tract. Each of the paired ducts develop anteriorly into a uterine horn and oviduct while posterior to this area both ducts fuse together and form the uterine body, cervix and the anterior portion of the vagina (Arey, 1965). In cattle, the uterine horns are curved and in the heifer, each forms one complete spiral turn before joining the Fallopian tube.

The general uterine structure of the cow has been reported by Foley and Reece (1953). They stated that the uterus consists of three coats: a thin outer covering, the perimetrium; a thick middle layer, the myometrium; and an inner lining, the endometrium. The endometrium may be divided into caruncular and intercaruncular areas. Skjerven (1956b) described the endometrium as being divided into three layers. These layers were the basement membrane with pseudostratified columnar epithelium; the stratum compactum, a narrow zone consisting of densely cellular connective tissue; and the stratum spongiosum containing more loosely arranged collagenous connective tissue. The uterine glands were described as branched, coiled, and tubular and their ducts opened into the intercaruncular area. The stratum compactum of the caruncles did not contain any glands. The stratum spongiosum beneath the caruncles contained glands as did the stratum spongiosum of the intercaruncular area.

The literature review will follow this order:

1) Type of surface epithelium
2) Day-to-day changes during the cycle
3) Cyclic changes in the surface epithelium
4) Glandular epithelium
5) Histochemistry of uterine epithelium
6) Electron microscopy of uterine epithelium
7) Light microscopy of the cervical epithelium
8) Histochemistry of the cervical epithelium
9) Electron microscopy of the cervical epithelium

Type of Surface Epithelium

Several investigators have studied the bovine endometrium and have reported conflicting results. Most authors have described the surface epithelium as being pseudostratified columnar throughout the cycle with a variation in the size of cells occurring at various phases of the estrous cycle. Murphey (1924), Cole (1930), Weber et al. (1948a), Asdell et al. (1949), Roark and Herman (1950), Weeth and Herman (1952). Foley and Reece (1953) described the cells as being simple columnar of variable height. They thought that when the tissue sample was sectioned at an acute angle, the nuclei suggested a pseudostratified type of epithelium. Asdell et al. (1949) found the surface epithelium to be pseudostratified throughout the cycle but less so at two days postestrus. Weber et al. (1948a) reported the presence of ciliated cells in the bovine endometrium.

Day-to-day changes of the surface epithelium

Proestrus Weber et al. (1948a) found that the epithelium was tall columnar with elongated nuclei. A small portion of the surface was involved in cytoplasmic disintergration accompanied by nuclear pyknosis. Asdell et al. (1949) found the surface epithelial cells at one day proestrus to be extremely long, almost thread like with much elongated nuclei.

Estrus Weber et al. (1948a) found that during estrus the surface epithelium was at a maximum height while the glandular epithelium was at its lowest level. Cole (1930), Weber et al. (1948a), Asdell et al. (1949), Roark and Herman (1950), and Dziuk (1955) all reported that the histological picture of the uterus during estrus was similar to that seen during proestrus. Some destruction of the surface epithelium was observed by several investigators around and shortly after estrus: Weber et al. (1948a), Asdell et al. (1949), Roark and Herman (1950) and Weeth and Herman (1952).

Day one post estrus Weber et al. (1948a) reported that the epithelium was medium to low pseudostratified columnar. In some areas, the single layered epithelium appeared to be stretched so the nuclei were arranged obliquely.

Day two Most investigators agreed that at this time of the cycle, the surface epithelium was at the minimal height. Asdell et al. (1949), Sasser (1963), Cooper (1961), and Weber et al. (1948a) noted extensive erosion of the surface epithelium. Many cytoplasmic vacuoles

were present in the distal as well as basal part of the cells. These vacuoles sometimes contained leukocytes. Asdell et al. (1949) also noted the vacuolization of the cells and reported that the cell boundaries tended to be indistinct. The surface epithelium became normal pseudostratified columnar at day three. Weber et al. (1948a).

Early metestrus Weeth and Herman (1952) reported decreasing of the cell height and migration of the nuclei to the mid or distal cytoplasm, giving a vacuolated appearance to the proximal cytoplasm. Cooper (1961) reported that at seven to eight days, the surface epithelium had increased in height.

Diestrus Murphey (1924) reported that cells were found to be tall and columnar near the end of diestrus. Asdell et al. (1949) found the surface epithelium to be elongated and increased in size up to estrus. Cooper (1961) found the cells at 15 days as tall as at 8 days.

Cyclic changes in the surface epithelium

There is general agreement between reports of various research workers on the morphological changes that take place in the uterus during the estrous cycle. However, there is great disagreement and conflicting reports concerning the subtle cyclic changes and their quantitative aspects. Stockard and Papanicolaou (1917) were some of the first workers to correlate uterine histological changes with stages in the estrous cycle. In working with guinea pigs, they found the epithelium during estrus to consist of tall columnar cells filled with

mucus. Later, the epithelium tended to break away and regeneration followed. Marshall (1922) divided the estrous cycle into four periods: rest, growth, destruction, and recuperation. During growth, the stromal cells multiply and the mucosa increases slightly in thickness while vascularity also increases. Destruction causes some breaking of blood vessels, and epithelial cells sometimes were torn off. That was the time of estrus, which was followed by a period of recuperation. The epithelium was renewed at that time.

Murphey (1926) maintained that height and pseudostratification may be explained by volume changes in the mucosa due to congestion and edema. Dziuk (1958) reported that there was not a day-to-day pattern of changes in the surface epithelium but the changes were gradual. The overall activity of the uterus started to increase at estrus or a day or two after, and continued at a peak about 8-10 days after estrus when the activity gradually declined. Weeth and Herman (1952) concluded that the non-gravid uterus was altered relatively little during the estrous cycle. They reported no separate and distinct period of regression in the uterus during the cycle. Progestational proliferation continued throughout diestrus. Roark and Herman (1950) reported that cyclic changes in the uterus were more pronounced in the stroma and glands than in the epithelium. Asdell et al. (1949) reported that cells were tallest during proestrus and estrus. Due to increased secretory activity at the time of estrus and on the succeeding day, the cells were at their lowest two days

postestrus. After the second day, the cells increased in length throughout the remaining part of the cycle.

Dziuk (1955), Cooper (1961) and Sasser (1963) concluded that the surface epithelium varied greatly in height within the stages of the cycle making it impossible to accurately measure the changes. Casida and McKenzie (1932) studied the histological changes in the uterus of the ewe. They stated that the surface and glandular epithelium were similar and that they showed no definite variations in the average height in different individuals and from one area to another in the same animal.

Some other differences in results could have been due to the fact that both heifers and cows were used in the experiments. Weber et al. (1948a) used only heifers, Roark and Herman (1950) and Sasser (1963) used multiparous cows while Cole (1930), Weeth and Herman (1952), Dziuk (1955), Foley and Reece (1953) and Cooper (1961) had both heifers and multiparous cows. Weber et al. (1948a), Cole (1930), Roark and Herman (1950), and Weeth and Herman (1952) made it clear whether or not their investigations were concerned with the caruncular or intercaruncular areas. The other investigators did not specify this detail.

Changes in nuclei The condition of the nuclei during the cycle also changed. Asdell et al. (1949) reported that at estrus, the nuclei of the surface epithelium were round to irregular in shape and were rather granular. At two days, the nuclei became oval and more granular. At 12 days, the nuclei had assumed an elongated appearance that tended to increase up to the time of estrus. From four days postestrus onward, the nuclei

tended to be lifted from the base of the cell as basal vacuolization appeared. Murphey (1924) found that vacuolar changes in the epithelium increased gradually from day two to day four when this change was maximal.

Mitotic figures Mitotic figures were found by several investigators to be present mainly around the time of estrus. Weber et al. (1948a) reported mitotic figures at day one that increased in number on days two and three of the cycle. The same was reported by Murphey (1924), Casida and McKenzie (1932), and Skjerven (1956b).

Leukocytic infiltration Murphey (1924) noted that leukocytic infiltration started about the 19th day and reached its maximum on the 5th day and gradually subsided until the 14th day. Roark and Herman (1950) found Leukocytes in the epithelium throughout the cycle but in greatest numbers during proestrus and estrus. Skjerven (1956b) reported that variable numbers of leukocytes were present in the endometrium from area to area with the highest concentration in the stroma beneath the surface epithelium. The same results were reported by Weber et al. (1948a). Van Den Hoek (1959) did differential counting of leukocytes in the surface epithelium. He reported that neutrophils were always observed during the estrogenic phase of the cycle but absent in the progestational phase. Eosinophiles, lymphocytes, phagocytes and mast cells were found in both phases with different distributions through the uterus.

Secretory activity Sasser (1963) reported that the uterus was actively secreting from the surface epithelium at the time of estrus and

one day after estrus. Asdell et al. (1949) reported that due to the high secretory activity at estrus the cells were low in height at two days postestrus. After the second day, the cells began to increase in length. Sasser (1963) reported that the amount of secretion decreased after estrus until four days postestrus at which time the endometrium was in a very active stage of secretion. Asdell et al. (1949) also noted another wave of secretion that took place on the eighth day postestrus. Secretions were low about 12-13 days postestrus at which time the epithelium started to atrophy (Sasser 1963).

Vollmerhaus (1957) noted that during the early corpus luteal phase, the free surface of the epithelial cells was covered with a homogenous, grainy appearing film and the amount of secretion had decreased.

Glandular Epithelium

Skjerven (1956b) described the uterine glands as being branched, coiled, tubular glands which terminated near the myometrium. As they passed superficially, they became straight and their lumina enlarged. The cyclic changes in the epithelium of the uterine glands have been studied by several investigators. Cole (1930) reported that during estrus the epithelial cells became taller, the nuclei elongated and the lumen smaller. Weber et al. (1948a) and Cooper (1961) noted that during estrus, the epithelium reached its lowest measurements with a small lumen. Hammond (1927) observed that glandular hypertrophy was associated with the development of the corpus luteum, and although there were slight

time differences, the work of Cole (1930), Asdell et al. (1949), Roark and Herman (1950), Weber et al. (1948a), Weeth and Herman (1952), and Cooper (1961) confirmed this. Atrophy of the glands appears to be associated with the morphological regression of the corpus luteum as shown by Cole (1930) and Asdell et al. (1949). Weber et al. (1948a) reported massive disintegration and nuclear pyknosis during early proestrus. In later studies, Weber et al. (1948b) reported that the glandular epithelium did not regress until immediately before the following estrus. Vollmerhaus (1957) noted that the secretory activity of the uterine glands began at the gland orifice and proceeded downward; therefore, the cells of the middle and basilar parts of the glands had secretory functions at different times. He reported that during the late follicular phase, the epithelium was active at the glandular orifice. He reported secretory material in the lumen during the time of ovulation and the corpus luteal phase. Dziuk (1955) found that the size of the gland lumen and the amount of secretion and debris decreased from the first to the tenth day of the estrous cycle. The gland lumen was smallest at 8-12 days and largest at 16-20 days. Johnson (1965) reported that the glandular diameter was largest, 74.78μ, three days proestrus and decreased rapidly to 64.97μ at estrus. The gland then increased in size until the 19th day. The lumen also showed a definite cyclic pattern.

Weber et al. (1948a) reported the presence of mitotic figures in the glandular epithelium that continued from estrus until the third day.

Histochemistry of the Uterine Epithelium

Initial investigations on slaughter-house and biopsy material performed by Skjerven (1953a, 1953b) indicated that certain histochemical reactions revealed cyclic changes of the bovine endometrium. These observations on the occurrence of alkaline phosphatase and periodic-acid-Schiff (PAS) positive substances and neutral fats were confirmed by examination of biopsy material from normal animals done by Skjerven (1956a, 1956b).

Alkaline phosphatase

Weeth and Herman (1952) and Moss *et al.* (1954) worked on slaughter-house material and reported the wide distribution of alkaline phosphatase in the bovine endometrium and the cyclic variation in the enzyme activity, especially in the surface epithelium cells. Skjerven (1956b) reported that the activity of the alkaline phosphatase, as present in the distal cytoplasm of the surface epithelial cells, followed in conjunction with the development and regression of the corpus luteum and reached optimal values in the luteal phase. The same was found by Weeth and Herman (1952) and Larson *et al.* (1965). Moss *et al.* (1954) found the strongest activity of alkaline phosphatase occurred from the ninth through the thirteenth day. They saw distinct cyclic changes of the phosphatase activity only in the surface epithelium aside from the distal border. The distal border of the epithelium, stroma and the blood vessels had positive reactions throughout the cycle. Foley and Reece (1953) could not find any distinct cyclic changes in the amount of alkaline phosphatase in the glandular lumen.

Glycogen

Two methods have been used generally to study the presence of glycogen in the surface epithelium. Following the use of the Bauer-Feulgen technique, Weeth and Herman (1952) reported the presence of glycogen in large quantities in the surface epithelium around estrus, but they observed little or no glycogen from day eight till day fourteen of the cycle. Glandular glycogen distribution was largely limited to the superficial glands. Moss et al. (1954) used the PAS reaction to detect the presence of glycogen in the endometrium. Positive staining was seen from a few days before until at least five days after the beginning of estrus. No glycogen was present from days 8-13. The amount of glycogen in the superficial gland varied considerably but a correlation with the stage of the cycle could not be established. Skjerven's (1953a, 1956a, 1956b) results are generally in agreement with those of Moss et al. (1954). He reported a glycogen rich period in the surface epithelium lasting about fourteen days; the first six and the last eight of the cycle, while no glycogen was seen during the remaining seven days. The glycogen granules were fairly equally distributed over the entire epithelium. Moderate quantities of glycogen granules were always present in the glandular epithelium irrespective of the stage of the gland. Van Den Hoek (1959) reported that during estrus, the quantity of glycogen which could be observed in the surface epithelium, varied considerably. In one animal, it was almost nonexistent. The epithelium covering the caruncles was completely devoid of glycogen during estrus. In the progestational phase,

the surface epithelium was devoid of glycogen whereon it was always present in the caruncles. The glands contained varying amounts of glycogen irrespective of the phase of the cycle.

Larson et al. (1965) reported that the concentration of the periodic-acid-Schiff material was greatest during estrus and lowest from 8 to 14 days. The superficial portion of the glandular epithelium followed the same cyclic changes as the surface epithelium but the basilar portion of the uterine glands did not exhibit any cyclic histochemical pattern.

Lipids

By means of the Sudan IV method and birefringent examination, Weeth and Herman (1952) observed only a slight reaction for lipids in the surface epithelium at one, four, and eighteen days postestrus. In the cyclic glands, epithelial lipids were maximal at four days postestrus and none was detected at 11, 14, or 15 days postestrus. Skjerven (1956b) used the Feltrat 7B method to study the fat content. Fat was observed in the surface epithelial cells in most biopsies obtained between day 10 and two days before the next estrus.

Electron Microscopy of the Uterine Epithelium

The electron microscope has been used to study the endometrium in several species. Burgos and Wislocki (1958) studied the endometrium of the guinea pig. At estrus, the surface end glandular epithelium consisted of tall columnar cells resting on a thin basement membrane with the nuclei almost filling the lower half of the cells. At metestrus, the cells

appeared shorter and terminal bars were more conspicous. The cells were smaller at diestrus with shrinkage of the nuclei and relative absence of cytoplasmic inclusions. Nilsson (1958a, 1958b, 1958c, 1959a, 1959b, 1959c) studied the ultrstructure of the mouse uterine epithelial surface under different estrogenic influences. He found that the differences in the appearance of the uterine epithelium from spayed mice and from estrous mice consisted of an increase in the cell height, a change in the amount of lipid material and a change in the appearance of the mitochondria. Uterine secretion was stimulated by injection of long acting estrogens. The secretion was characterized by the presence of long microvilli, larger Golgi region with many vesicles and vacuoles and an increase in cell size. Larsen (1962) studied the uterine epithelium in rabbits. The uterine epithelium in estrus was comprised of ciliated and non-ciliated cells. The cytoplasm of the ciliated cells was less electron opaque, the nuclei often irregular in shape and the mitochondria smaller and more varied in shape than that of the non-ciliated cells. Cyclic cytological changes occurring in the endometrial epithelium during the human menstrual cycle have been studied with the electron microscope by Borell et al. (1959), Gompell (1962) and Nilsson (1962). Borell et al. (1959) found that the glandular epithelial cells were short and possessed short microvilli in the inactive stage, and became taller and exhibited longer microvilli in the proliferation phase. Gompell's (1962) findings are in essential agreement with those of Borell and associates. Nilsson (1962) had added

to the above description by mentioning the occurrence of 1μ granulated bodies that were presumed to be lysosomes. Stinson et al. (1962) studied the ultrastructure of the bovine endometrium. They reported the surface epithelium to be pseudostratified columnar throughout most of the cycle, while at times, it appeared to be low and of a simple columnar type. The basement membrane was 0.1μ thick and microvilli were found on the distal part of the cells. Two types of mitochondria were found: a large spherical type and a long filamentous type. Glandular epithelial cells showed cilia on their distal border. The cyclic changes were described as consisting of a degenerative phase which began at the time of estrus and extended for the next six to seven days. A regenerative phase which began about the eighth day after estrus continued until the peak of the cellular activity occurred at the 19th day of the cycle. Fat granules were observed to accumulate in the cytoplasm at about the 13th day.

Light Microscopy of the Cervical Epithelium

Murphey (1924) described the typical loaded and secreting cells of the cervix as high columnar with a circular or laterally compressed, granular nucleus. He noted that the goblet cells were nearly always flanked by pyknotic cells. The maximal height of the loaded state occurred about the 17th or 18th day. Active secretion of mucus began at the tips of the primary and secondary folds of the cervix on the 19th or 20th day of the cycle. Hammond (1927) found the histological changes of the cervix to be similar to those occurring in the upper vagina: congestion and edema

during and just after heat, cuboidal cells in diestrus becoming columnar and full of mucus during heat, discharging and becoming ragged and cuboidal about 72 hours after heat. Cole (1930) reported that the cells of the cervical epithelium were tall and large during estrus with the long axis perpendicular to the basement membrane. At this time, the cells were actively secreting mucus which could be observed hanging in long strings from the vulva during heat. In proestrus, Cole (1930) reported that the nuclei were flattened and the cell size reduced. At diestrus, the cells no longer contained mucus and the epithelium was made up of a single layer of low columnar cells. Herrick (1951) gave a day-to-day description of the epithelial cells of the cervix during the cycle. He stated that all columnar epithelial cells of the cervix produced mucus and all may become goblet cells. The cells varied in size, the average measurements were 15μ in diestrus up to 19μ during heat. He reported the cells to be nonciliated.

Roark and Herman (1950) reported that the mucosa of the cervix was thrown up into many plica that had a central core of cell poor connective tissue. These plica were lined by a single layer of mucoid epithelium which formed simple sacculated and/or branched tubular glands. Evidence for secretory activity by the columnar epithelium was found at all stages of the cycle. During estrus, the cells were columnar with basally crowded and elongated nuclei. Two days postestrus, the height and form of the superficial epithelium was quite variable. In some areas, the epithelium was tall columnar, whereas in other regions, the cells had become cuboidal,

the nuclei being oval and less basally located. At 6-10 days postestrus, the epithelial cells were greatly reduced in size. In the deeper crypts however, some of the cells were columnar. During proestrus, the epithelium increased slightly in depth, the cells changing from cuboidal to columnar form. Secretion of mucus started at this time.

Histochemistry of the Cervical Epithelium

Wheeler and Danziger (1955) studied the histochemistry of the human cervix. They reported the presence of a maximum alkaline phosphatase reaction in the distal (luminal) cytoplasm of the cells, but the glandular secretions were also positive. Glycogen was locally present in the cytoplasm of the basal cells in some specimens and was present in the more superficial layers in all cases. They could not recognize the phase of the menstrual cycle in the cervix from the reaction of these substances. Gompell (1962), using a modified PAS staining procedure, reported the presence of a positive reaction in the apical area of the human cervical cell. He concluded that these materials were mucus. Herrick (1951) used Mayer's Muci-Carmine stain in his study of the bovine cervix to determine what cells in the mucous membrane were capable of producing mucus.

Electron Microscopy of the Cervical Epithelium

Chapman et al. (1964) studied the normal human cervical epithelium during pregnancy. They reported the presence of ciliated cells with poorly developed endoplasmic reticulum and very few secretory granules that were interspersed between the predominantly mucous cells. The apical

portion of each of these cells appeared to be occupied mainly by the secretory granules; however, Golgi zones, granular endoplasmic reticulum, and mitochondria were seen. No reports on the ultrastructure of the bovine cervical epithelium were found in the literature.

MATERIALS AND METHODS

Animals

Animals for this work were obtained from the beef herd of the Veterinary Medical Research Institute. Thirty two animals were used as follows:

5 animals were 2 years old
8 animals were 3 years old
3 animals were 4 years old
2 animals were 5 years old
10 animals were 6 years old
2 animals were 7 years old
2 animals were 8 years old

32 Total

Of these:

10 animals were heifers
7 animals had one calf
4 animals had two calves
8 animals had three calves
3 animals had four calves

32 Total

These females were selected from the herd because of one of the following reasons:

1. Mastitis
2. Poor gain of calves
3. Undesirable conformation and foot defects
4. Poor disposition (nervousness)
5. All mixed breeds, Holstein and Angus, were removed to establish a Hereford herd
6. Poor conception - animals #20, #21, #23 were cycling normally but showed poor conception rate

The animals were kept on pasture from May to November and on good quality alfalfa hay during the winter. Pregnant heifers were supplemented with grain. Animals were in good apparent health and were under supervision to

establish their estrous cycle for at least three complete cycles. The twelve animals that were used for biopsy samples conceived normally after the last operation.

Collection of Tissues

Tissues were collected by means of the following three methods:

1. post mortem
2. ovariohysterectomy
3. biopsy

The animal distribution for each method follows.

Post mortem

Cow no.	Breed	Age	No. of calves
3	Angus x Holstein	2	-
4	Angus x Holstein	2	-
7	Angus x Holstein	2	-
8	Angus x Holstein	2	-
9	Hereford	3	-
11	Angus	6	2
12	Hereford	3	-
18	Hereford	3	-
19	Hereford	3	-
20	Hereford	5	1
21	Hereford	5	-
23	Hereford	6	2

12 Total

Surgery

Cow no.	Breed	Age	No. of calves
28	Hereford	6	3
29	Hereford	6	3
30	Hereford	2	-
31	Hereford	6	3
32	Hereford	7	4

Cow no.	Breed	Age	No. of calves
33	Hereford	8	2
34	Hereford	6	3
35	Hereford	6	2
8 Total			

Biopsy

Cow no.	Breed	Age	No. of calves	No. of biopsies Uterus	Cervix
28x	Hereford	6	3	2	-
31x	Hereford	6	3	8	7
33x	Hereford	8	2	8	8
34x	Hereford	6	3	11	1
35x	Hereford	6	2	4	11
51	Hereford	3	1	1	1
53	Hereford	7	4	4	-
58	Hereford	3	1	1	-
60	Hereford	3	1	1	-
68	Hereford	8	4	1	-
75	Hereford	6	3	2	-
76	Hereford	6	3	2	-
82	Hereford	6	3	3	-
90	Hereford	4	3	4	4
92	Hereford	3	1	1	-
93	Hereford	4	1	2	1
94	Hereford	4	1	-	2
17				55	35

x-Biopsy was taken before surgery. No biopsies were taken from heifers.

Samples were taken in each month of the year and distributed as follows:

Number of samples	Month
12	January
2	February
4	March
5	April
7	May
8	June
10	July
9	August
7	September
14	October
11	November
2	December

or

16	Winter
16	Spring
27	Summer
32	Fall

Post mortem Tissues were collected from the uterus and cervix approximately fifteen minutes after slaughter. Samples of these tissues were used only for light microscopy and were placed in 10% formalin or 80% cold alcohol for the histochemistry and light microscopy work.

Hysterectomy The cows were prepared for hysterectomy by fasting for two days prior to surgery. Promazine hydrochloride* was given as an ataractic agent half an hour prior to surgery intravenously at a rate of 0.15 mg. per one pound of body weight.

The left paralumbar area was clipped with a #40 blade electric clippers. The skin was scrubbed three times with soap, rinsed with fresh water, dried and isopropyl alcohol applied. The skin was then painted

*Sparine: Wyeth Laboratories. Philadelphia, Pennsylvania

with tincture of Merthiolate*. Two per cent Xylocaine hydrochloride** was used for the paralumbar anesthesia.

The operation field was draped with sterile drapes leaving only enough space for the laparotomy incision. A perpendicular incision, 7 inches long, was made in the left paralumbar area. The external and internal abdominal oblique muscles were dissected, the transversus abdominus muscle separated and the peritoneum exposed and carefully incised. The abdominal visera was inspected and the uterus identified. The left ovary was palpated, the ovarian blood vessels were doubly ligated with #1 catgut and dissected in between. The broad ligament was bluntly separated from the uterus. The right ovarian vessels were then ligated and dissected in a similar manner. The os uteri was located and the uterus transected cranial to it thus allowing for the removal of the entire uterus and both ovaries at the same time.

The laparotomy incision was closed by suturing the peritoneum and the transversus abdominus muscle together with a continous suture using #2 catgut. The abdominal oblique muscles were sutured in a similar manner and the skin incision was closed with a continous suture using medium Vetafil***.

Biopsy A modified rectal biopsy instrument was used to obtain the biopsies (Stinson et al. (1962). The rectal-vaginal area was washed and

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*Corvel, Inc., 1124 Harney Street, Omaha, Nebraska.

**JenSal Laboratories, 1117 Grand Ave., Des Moines, Iowa.

***Vetafil: Begen, Begen and Co., Gmbh., 8112 Dreyer Street Hanover.

thoroughly cleaned. The cervix was fixed per rectum. The instrument, protected with a glass speculum when introduced into the vagina to prevent contamination, was passed into the uterus. The uterine wall was pressed into the jaws of the instrument and a sample approximately 3 x 5 mm. in size was snipped off. An attempt was made to take successive biopsies from various parts of both of the uterine horns. The same technique was used in obtaining the sample from the different cervical folds. The tissues were placed in the fixative within one minute after the operation.

Histological techniques

After obtaining the samples, they were divided into two parts. One part was fixed in a 10% neutral formalin for at least 24 hours. The tissue samples were then cut with a razor blade into 1 $cm.^2$ blocks and processed in the Technicon, embedded in paraffin blocks and cut into sections 6-10μ thick. Tissues fixed in formalin were stained with Hemotoxylin eosin and Periodic-Acid-Schiff stains. The second part was fixed in 80% alcohol at a temperature of 0° C for 24 hours and then processed in the Technicon. The sections were stained in Hematoxylin-Eosin and alkaline phosphatase stains.

Alkaline phosphatase For the demonstration of alkaline phosphatase the method of Gomori (1952, p. 184) was used. Sections were incubated for two hours in a solution containing sodium glycero-phosphate, calcium chloride, sodium barbital, and magnesiun sulfate

adjusted to pH 9.4. The tissues were then put in a cobalt nitrate solution, rinsed and then treated with ammonium sulfide solution. Brown cobalt sulfide was then visible in the tissue under the microscope.

Periodic Acid Schiff (PAS) The method of McManus (1946) was used to demonstrate the PAS positive reaction in the tissue. Control slides were incubated in diastase for two hours at 37° C to remove the glycogen.

Oil-red-O Tissues were fixed in 10% formalin for 24 hours and sectioning was then done on a freezing microtome. Sections of 10-15 micra were stained with oil-red-O (Mallory, 1942) for five minutes, counterstained with Harris hematoxylin for one minute and mounted in Farrants mounting media (Lillie, 1954).

Light microscopy Tissues were observed in the Leitz-Ortholux microscope. Only the intercaruncular area was examined and recorded. Measurements were done with an eyepiece micrometer using the high dry (42x) objectives. An effort was made to select a representative area of the section for measurements, but no averaging of the readings was done.

Black and white pictures were taken on the MP-3* camera and the color pictures were taken on a 35mm. camera. Estimation of the degree of reaction to alkaline phosphatase and PAS techniques was based on a scale ranging from 0-4 as recorded in Tables 1, 2, 3, 4 and 5.

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*Polaroid Corporation. Cambridge, Mass.

Electron microscopy Tissues were minced in 4.5% cold gluteraldehyde solution buffered with phosphate buffer to a pH of 7.4 They were then prefixed in that solution at 3° C for thirty minutes, washed in buffer solution and post fixed in cold 1% osmium tetroxide solution buffered with the same buffer (Millonig, 1962) for one hour. The blocks were then dehydrated in a series of ethyl alcohols of increasing concentration up to absolute alcohol. The tissues were then infiltrated and embedded in a 9:1 n-butyl-ethyl methacrylate to which 5% dibenzyl-benzene and 2% benzol peroxide were added (Kushida, 1961). The tissues were then placed in No. 0 gelatin capsules and polymerized in ultraviolet light. Tissues were also embedded in Araldite M*, placed in Beem** capsules and polymerized at 50° C for 48 hours. For the purposes of orientation, thick sections were cut with a razor blade and were examined by means of the phase microscope. The face of the block was trimmed to a .3x.4mm face that contained mainly the epithelial tissue. Sectioning was done with glass knives on LKB 8800 and Cambridge Ultramicrotomes. Thin sections showing grey or silver grey interference color were floated on water and picked up on copper grids that had been coated with parlodion. The sections were stained with lead acetate (Millonig, 1961) or lead citrate (Reynolds, 1963). The sections were examined by means of the Hitachi HS-6 and HU11A electron microscopes.

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OBSERVATIONS

Light Microscopy

Uterus (surface epithelium)

The observations made by means of the light microscope are summarized in Tables 1 and 2 and in Graph 1. Some differences were observed between the samples taken from older cows and heifers in this experiment. The surface epithelium obtained from the heifers showed fewer variations within the stage of the cycle and more definite changes correlated with the stage of the cycle than the samples from the cows. The size of the surface epithelium was lowest at estrus (Fig. 3) and increased in height from the fourth to the fifteenth day postestrus (Figs. 5, 11 and 13). After the fifteenth day, it reduced in size progressively until estrus when the epithelium was the lowest. Figs. 7 and 8 demonstrate the differences in the surface epithelium between samples taken from a heifer six days post estrus and a cow seven days post estrus. Some variations were found concerning the site of nuclei in the cells. During proestrus and estrus, the nuclei were found in all parts of the cells (Figs. 1 and 15) that is, some were located toward the luminal surface of the cells while others were located near the basement membrane. During midcycle the nuclei seemed to concentrate in the proximal half of the cells (Figs. 5 and 7). "Clear cells" and leucocytes were abundant during estrus, reduced in number in midcycle and increased again during proestrus. The samples taken from older cows did not show the typical cyclic

Table 1. Surface epithelium of heifers

Animal number	Collection method	Days in cycle	Size in micra	Nuclei site	Clear cells	WBC	PAS	AP	Cilia
21	P	1	12	1	4	4	4	3	-
18	P	4	17	1	4	4	2	2	-
12	P	5	28	1/2	2	2	2	2	-
3	P	6	32	1/2	2	2	0	4	Pr
7	P	12	34	1/2	2	2	0	4	-
30	S	13	32	3/4	3	1	1	3	Pr
19	P	13	32	1/2	2	1	0	4	-
4	P	15	35	1/2	1	1	1	1	-
8	P	16	34	1	3	1	2	4	-
9	P	18	17	1	3	2	3	2	-
10 Total									

P = Post mortem
S = Surgery
Pr = Present

variations that were observed in heifers. In the older cows, the height of the epithelium fluctuated throughout the cycle (Figs. 1, 9 and 15) as did the site of the nuclei, the number of clear cells and the leucocytes (Figs. 1, 8 and 9). There was no difference between samples taken by biopsy or surgery.

Cilia were found on the uterine surface epithelium of several cows in less than 1% of the cells. The percentage of the cells with cilia was much

Table 2. Surface epithelium of cows

Animal number	Collection method	Days in cycle	Size in micra	Nuclei site	Clear cells	WBC	PAS	AP	Oil-red-O	Cilia
34	S	1	28	1	1	3	4	1	0	Pr
34	B	1	30	1	2	3	4	1	-	-
29	S	2	20	3/4	1	4	3	2	0	-
34	B	2	30	1/2	2	2	2	-	-	-
34	B	3	32	3/4	2	2	0	-	-	-
11	P	3	30	1/2	3	3	2	2	-	-
35	S	5	20	1/2	2	3	0	4	0	-
90	B	5	25	1/2	2	3	0	-	-	-
28	S	7	27	1/2	2	1	1	4	0	Pr
31	B	7	20	3/4	1	3	0	-	-	-
34	B	8	30	1/2	1	2	1	-	-	-
31	S	10	34	3/4	3	2	0	4	1	-
23	P	10	34	1	4	4	4	4	-	-
31	B	10	32	3/4	3	4	0	-	-	-
33	B	10	30	1	1	2	0	-	-	-
35	B	11	30	1	2	1	0	-	-	-
31	B	12	28	3/4	1	4	-	-	-	-
34	B	13	23	3/4	3	3	1	-	-	-
33	B	14	26	1	2	2	1	-	-	-

Table 2 (Continued)

Animal number	Collection method	Days in cycle	Size in micra	Nuclei site	Clear cells	WBC	PAS	AP	Oil-red-O	Cilia
33	S	16	34	1	3	1	1	4	3	Pr
32	S	18	30	1	1	1	2	3	3	Pr
34	B	20	34	1/2	2	2	0	-	-	-
20	P	21	32	1/2	4	3	3	3	-	Pr

B = Biopsy
P = Post mortem
S = Surgery
Pr = Present

higher in the neck of the glands than on the surface epithelium (Figs. 17 and 18).

Uterus (glandular epithelium)

The glandular epithelium of the heifers showed marked differences from that of the cows. The results are summarized in Tables 3 and 4 and Graph 2. In the heifers, a cyclic change was observed in the size of the epithelium that was parallel, in general, to that found in the surface epithelium. It was low in estrus (Fig. 4) and started to increase from post estrus (Fig. 6) until day 13 (Fig. 12) when it started to decrease again. The size of the lumen and the overall size of the glands varied considerably and no pattern could be found, although in general, the lumen was much smaller during the midcycle than during estrus. Nuclei and

Table 3. Glandular epithelium of heifers

Animal number	Collection	Days in cycle	Epithelium size in micra	Lumen size in micra	Gland size in micra	Secretion	Cilia	Clear cells	WBC	PAS	AP	Oil-red-O
21	P	1	10	68	70x120	Pr	0	3	2	4	3	-
18	P	4	18	45	51x85	Pr	0	3	3	2	4	-
12	P	5	17	15	48x56	0	0	2	3	2	2	-
3	P	6	18	23	56x30	0	0	1	2	0	4	-
7	P	12	21	0	48x37	Pr	Pr	1	2	-	4	-
30	S	13	22	10	46x50	0	Pr	1	0	0	4	0
19	P	13	19	0	40x52	Pr	0	1	1	-	4	-
4	P	15	16	12	60x45	0	Pr	0	1	3	4	-
8	P	16	17	10	44x60	0	0	1	1	2	4	-
9	P	18	17	8	20x40	0	0	1	3	2	2	-

P = Post mortem
S = Surgery
Pr = Present

Table 4. Glandular epithelium of cows

Animal number	Collection method	Days in cycle	Epithelium size in micra	Lumen size in micra	Gland size in micra	Secretion	Clear cells	Cilia	WBC	PAS	AP	Oil-red-O
34	S	1	8	23	40x42	0	1	0	2	2	2	-
34	B	1	20	40	90x95	Pr	1	0	0	0	-	-
29	S	2	17	17	52x55	0	1	0	1	0	4	0
34	B	2	34	36	102x95	0	2	Pr	0	0	-	-
34	B	3	19	20	58x62	0	2	Pr	1	-	-	-
11	P	3	17	17	58x60	0	2	Pr	2	2	2	-
35	S	5	18	3	54x40	Pr	3	0	1	0	4	0
90	B	5	25	0	51x68	Pr	2	0	1	0	-	-
28	S	7	17	16	48x51	0	1	0	2	0	4	-
31	B	7	35	0	80x102	Pr	0	Pr	0	0	-	-
34	B	8	19	25	65x68	0	1	Pr	0	0	-	-
31	S	10	20	8	56x50	Pr	1	0	0	0	3	-
23	P	10	18	8	68x45	0	2	Pr	2	2	4	-
31	B	10	34	0	85x64	Pr	1	0	0	0	-	-
33	B	10	15	50	85x80	0	1	Pr	0	1	-	-
35	B	11	18	30	66x100	Pr	1	0	0	0	-	-
31	B	12	32	22	85x87	Pr	1	0	0	-	-	-
34	B	13	20	20	60x58	0	2	Pr	0	0	-	-
33	B	14	33	20	86x90	0	1	Pr	0	1	-	-
33	S	16	19	34	72x75	Pr	1	Pr	0	1	4	0
32	S	18	9	12	78x30	0	1	Pr	0	0	2	0
34	B	20	25	0	49x53	Pr	0	0	0	0	-	-
20	P	21	7	2	34x20	Pr	1	0	1	1	2	-

B = Biopsy
P = Post Mortem
S = Surgery
Pr = Present

cellular debris were found in the lumen in different stages of the cycle, with the greatest amount being found 10-15 days of the cycle (Fig. 10). Fluid secretion was difficult to observe with the light microscope. Cilia were observed whenever the lumen was free of secretion (Figs. 14 and 16). More "clear cells" were seen during estrus and postestrus and the same was true for the leucocytes. In mature cows, the glandular epithelial lumen and overall size showed no cyclic pattern. Variations within the same day of the cycle were even greater than between different stages (Figs. 2 and 6). There were fewer leucocytes seen around the glands than in the surface epithelium and some clear cells were present all of the time (Figs. 14 and 16).

Histochemistry of the uterine epithelium

Alkaline phosphatase A summary of the results can be found in Tables 1, 2, 3, and 4. Alkaline phosphatase was present in the samples of surface epithelium taken from heifers at all stages of the cycle. In general, higher concentrations of the enzyme were noticed during midcycle than during estrus (Figs. 21 and 22). The positive reaction for the enzyme was found on the luminal surface of the epithelial cells. The basal cytoplasm and nuclei were inactive. Glandular epithelium also showed positive reactions (Figs. 19 and 20) but, the results were not as consistent as in the surface epithelium. Glands located close to the surface contained more of the enzyme than glands located deep in the stratum compactum (Figs. 23 and 24). The glandular epithelium located

near the uterine surface of both cows and heifers showed the same results with slight increases in activity during the midcycle with only the distal border of the cells involved. The capillaries and the walls of the blood vessels reacted positively to the phosphatase technique with no relationship to the stage of the cycle. Connective tissue around the glands and in the stroma was also positive. The stratum compactum just under the surface epithelium showed a stronger positive reaction than did the stratum spongium (Figs. 22 and 23).

Periodic acid Schiff reaction A summary of the results can be found in Tables 1, 2, 3, and 4. In the surface epithelium, the PAS positive material showed distinct cyclic changes. There was, in most animals, more PAS positive material during estrus than any other stage but the material was also found during the first five days and the last seven days of the cycle (Figs. 25 and 26). In some cases, the stained granules were so dense that it was difficult to differentiate them from one another. The amount of the positive granules varied from cell to cell, but were mostly located in the supranuclear area. The granules disappeared following one hour of digestion in a 2% diastase solution but there was a very thin line of PAS reaction along the luminal surface of the epithelium that was saliva resistant (Fig. 27). Cow 23 showed marked reaction to the PAS staining in the midcycle. This cow did not conform to the pattern observed in the other animals studied in which PAS positive material was very scanty during midcycle. In the glands, only a few epithelial cells showed positive reactions. There was more PAS positive material during estrus than during

midcycle (Fig. 28) and more was found in the superficial than in deep glands. The amount of material in the glands never exceeded that of the surface epithelium. Mature cows had less PAS positive material observed in the glands than heifers.

Oil-red-O technique Only a limited number of specimens were available for this study. A positive reaction was found in the form of distinct droplets only in the surface epithelium. The droplets were found throughout the cells in variable sizes and locations. Most samples that were taken during proestrus showed positive reactions (Figs. 29 and 30). No droplets could be found in samples taken from cows during estrus or postestrus. Glandular epithelium did not show droplets of positive material but some weak general coloring of the gland was sometime observed.

Cervix-epithelium

The summary of the results of the 16 samples of cervical epithelium can be found in Table 5 and Graph 3. Two types of cells were found in the cervical epithelium; the mucoid cells and the ciliated cells. The mucoid cells contained mainly mucus; the quantity varying and depending upon the stage of the cycle. The cells were filled during estrus (Fig. 31), but were mainly reduced in size in the first few days of the cycle. On day 5, a slight increase in size was noticed that proceeded until a maximum was reached at 8 days (Figs. 32 and 33). A process of cell size reduction started at this stage and continued until day 16 of the cycle (Figs. 34 and 35). During proestrus, the cells started to increase in size once again

Table 5. Cervical epithelium

Animal number	Collection method	Days in cycle	Epithelium size in microns	Nuclei site	Cilia	AP	PAS
90	B	1	20	bottom	Pr	-	4
34	B	2	16	bottom	Pr	-	2
11	P	3	14	1/3	Pr	-	2
90	B	3	10	1/2	Pr	-	2
12	P	5	17	1/3	Pr	3	3
3	P	6	18	1/3	Pr	3	4
28	S	7	20	bottom	Pr	0	4
35	B	8	36	bottom	Pr	-	4
94	B	10	25	bottom	Pr	-	4
94	B	12	20	bottom	Pr	-	4
35	B	14	20	bottom	Pr	-	4
33	B	15	15	1/2	Pr	-	3
8	P	16	14	1/2	Pr	-	2
33	B	17	32	bottom	Pr	-	4
9	P	18	19	1/2	Pr	3	4

B = Biopsy
S = Surgery
P = Post mortem
Pr = Present
AP = Alkaline phosphatase

(Fig. 36). Nuclei were generally found in the basal part of the cells when the cells were tall and filled with mucus, but occupied one-half or more of the cell when the epithelium was low.

The second type of cell that was found in the epithelium was the ciliated cell. These cells contained little mucus and, when stained with Hematoxylin-Eosin and observed under oil immersion, were seen to have a bright color (Fig. 36) which made them stand out in distinct contrast to the mucous cells. The cilia could be seen extending toward the lumen (Fig. 42) and resembled the cilia found in the uterine glandular epithelium. The ciliated cells were found in all of the sections of the cervix that were studied. Approximately 10% of the cells in the cervical epithelium were estimated to be ciliated.

Histochemistry of the cervical epithelium

Alkaline phosphatase Only a limited number of specimens were available for this study. The results are summarized in Table 5. The enzyme was present in the capillaries and connective tissue of all of the sections in various quantities, (Figs. 37 and 38). At day 7, no reaction could be found in the surface epithelium (Fig. 37) but, in all the other samples studied, a positive staining for alkaline phosphatase was found (Fig. 38). The enzyme was located mainly as a line on the luminal surface of the epithelial cells and in the endothelium of the subepithelial capillaries.

Periodic-acid-Schiff PAS positive material was found in all the epithelilial cells of the cervix throughout the cycle. This material was present in granular form and occupied the same part that was taken by mucus in the cells. This was usually the luminal half or more of the cells. Quantities varied according to the location of the cells in the folds and to the stage of the cycle. Those located deep in the cervical folds (Fig. 39) usually had more P.A.S. positive material present. The reaction was strongly positive and at low power (100x) gave the impression that all of the cytoplasm of the cells was positive. Under high power, (420x) the luminal location of the granules could be determined. During midcycle, the positive reaction was not as strong (Fig. 40). At eighteen days post estrus, the amount of P.A.S. material started to increase again. No positive reaction was seen in the nuclei, the basement membrane or the connective tissue.

Those sections that were incubated in diatase to remove the glycogen before staining with Periodic-acid-Schiff stain did not show any marked difference from non-treated sections. This suggests that the material present was mainly mucopolysaccharide.

Electron Microscopy

Basic cytology of the endometrial epithelial cells

Surface epithelium The surface epithelium was found to be pseudostratified columnar during most stages of the cycle (Fig. 43) except during estrus when it was low columnar epithelium. Microvilli were present on the luminal surfaces at all stages of the cycle. They were extensions of the cell surface and were covered by the plasma membrane. Their number and length varied during the cycle. During estrus, there were only a few microvilli present (Fig. 44), but some were very long and branching was observed in some specimens (Fig. 45). Several sections photographed were without microvilli, but this was considered to be an artifact resulting from fixation or embedding problems (Fig. 47). During midcycle, the microvilli were numerous and resembled a brush border on the distal surface of the cell (Fig. 46). Some of the microvilli were covered by an outer coat adhering to the plasma membrane which gave a furry appearance. Small numbers of ciliated cells were found in the surface epithelium of several cows (Fig. 48). These cells were similar to the non-ciliated cells except for the presence of the cilia. Three types of attachments were observed that had the function of holding the cells together; terminal bars, desmosomes and cell membrane interdigitation. The terminal bars were found near the surface of the cells and were sometimes seen extending across the cell's distal part (Figs. 49 and 50). They extended around the entire circumference of the cells and consisted of an amorphous, dense substance. Desmosomes were found at different levels on the lateral cell membrane. The lateral cell surfaces were also found to have invaginations which interdigitated with similar processes

of adjacent cells. Some were very elaborate while others showed finger-like projections (Fig. 49).

The nuclei of the cells were found in different positions in the cells at different stages of the cycle. Sometimes they were close to the surface while at other times they were very close to the basement membrane. The nucleoplasm was scattered with aggregations of chromatin material and was enveloped by a two unit membrane that consisted of two membranes with a space between them (Fig. 47). Two basic types of mitochondria were found in the cells; a round, small type and a large slender type (Fig. 51). They varied in number and size; however, the ultrastructure of the two types was identical. They were surrounded by a triple-layered membrane and showed typical cristae. The mitochondrial matrix was found between these cristae. Tubules of endoplasmic reticulum were observed in all cells as well as the Golgi complex. The latter varied in size, shape and number in different preparations. The Golgi complex was found to consist of pairs of paired membranes and small vesicles.

The epithelial cells rested on a basement membrane that was distinctly seen as a separation between the epithelium and the connective tissue (Fig. 52). Lipid granules were identified in some stages of the cycle. They were electron dense, irregular, large bodies. They were found in different parts of the cells but usually in close proximity to the nuclei. They were present only during diestrus and proestrus (Fig. 52). In some preparations (Figs. 49 and 50) an apocrine type of secretion was observed and fragments of cellular debris were seen next to the

cell surface.

Glandular epithelium The basic cytology of the glandular cells was found to be very similar to that of the surface epithelial cells (Fig. 53). The cells varied considerably in their length and width and showed a tendency for apocrine type secretion (Fig. 54). Many cells were observed to have cytoplasmic processes broken off into the lumen of the gland which was full of secretory material (Figs. 55 and 58). The big difference from the surface epithelial cells was the presence of a large number of ciliated cells (Fig. 53). These ciliated cells were very similar in structure to the non-ciliated cells that showed microvilli extending from their distal surface. The ciliated cells varied in number but were found much more frequently than the ciliated cells of the surface epithelium. Another type of cell that was found less frequently was an electron dense cell that seemed to have a smaller number of mitochondria and more extensive systems of endoplasmic reticulum (Fig. 56). Only a few of these cells were found and their origin and function are unknown. Both ciliated and non-ciliated cells exhibited a large number of long filamentous mitochondria that were found mainly in a supranuclear location. Endoplasmic reticulum and Golgi were similar to those found in the surface epithelium. Granular vacuoles were found in some cells and in the lumen of some glands (Fig. 57). The cells were found to be resting on a basement membrane, and the plasma membrane was observed to have a large number of desmosomes and terminal bars (Fig. 58).

Cyclic changes in the surface epithelium

Estrus The epithelium at this time was low columnar and was supported by a basement membrane. Vacuoles observed in some cells appeared

to be circumscribed pale areas in the epithelium near the surface and contained electron dense bodies (Fig. 44). The luminal surface of the cells had a small number of microvilli. Mitochondria were observed in the distal cytoplasm. Subterminal bars were observed. The cell membranes between adjacent cells were straight and not undulating. No lipid inclusions were observed in the cytoplasm.

Day two The nuclear membrane was observed to be undulating and only a few microvilli were present on the luminal surface of the cells. The mitochondria were small and there were not many spaces between adjacent cells (Fig. 59).

Day seven Channels between adjacent cells were observed near the luminal surface (Fig. 60). The intercellular spaces contained secretory material and were not continuous because the desmosomes at different levels retained the attachment between the adjacent cells. Microvilli were present. Two types of cells were noticed near the basement membrane; a clear cell and a leukocyte (Fig. 64).

Day ten Microvilli were present on the luminal margin of the cells. The cytoplasm contained long, filamentous mitochondria. The lateral cell membrane was undulating and interdigitating. The nuclei were located near the basement membrane. Intercellular spaces were found especially in the basal region. Few scanty lipid droplets were found in the cytoplasm.

Day thirteen Many microvilli were found on the luminal surface. The nuclei were located at different levels in various cells. Some were in contact with the basement membrane. A moderate number of lipid droplets were observed in the cytoplasm between the nuclei and the basement membrane. Large mitochondria were found in several specimens. Spaces

between cells were observed near the surface. Ciliated epithelial cells were observed, but did not show any marked differences from the other cells except for the presence of cilia that projected from the surface of the cell toward the lumen (Fig. 48).

Day eighteen Many long microvilli were seen at this stage extending from the luminal surface. Large numbers of mitochondria were observed (Fig. 61).

Day twenty Microvilli were present. Many irregularly shaped mitochondria were observed as well as endoplasmic reticulum and Golgi. Only a few lipid droplets were seen related to the mitochondria in the basal part of the cells. Desmosomes and subterminal bars were observed. There was a membrane bound vesicle of unknown origin near the surface of a cell (Fig. 62).

Cyclic changes in the glandular epithelium

Estrus The cells were of the simple columnar epithelial type. A large quantity of secretory material was found in the tubules that filled the lumen (Fig. 53). Different types of cells were noticed. An electron dense cell with well developed endoplasmic reticulum (Fig. 56) was observed between the regular epithelial cells in some preparations. Vacuolated intercellular spaces were found between the regular and the electron dense cells. Microvilli and cilia were extending from the distal surface of the regular cells. Some apocrine secretion was noticed in the lumen that appeared to be pinched off cell fragments. Large numbers of small mitochondria were found in these cells but also, filamentous types were seen. The Golgi apparatus was very well developed.

Day two The nuclei were found close to the lumen. The apocrine secretion continued and parts of the cells distal to the terminal bars were broken off in a manner similar to what was described at day 18 (Fig. 58). A large amount of secretory material was found in the lumen.

Day five Both ciliated and non-ciliated cells had large numbers of mitochondria (Fig. 65).

Day seven Secretory material was found in the lumen and cytoplasmic processes were pinched off near the terminal bars.

Day sixteen Large numbers of mitochondria were observed near the distal part of the cells. Dilated Golgi apparatus complexes were seen between the nucleus and the lumen. Cytoplasmic processes were noticed breaking off into the lumen near the cilia. In one specimen some cilia appeared to be surrounded by a free cytoplasmic fragment and were observed in cross section within the fragment (Fig. 55). Secretion granules and vacuoles were seen in the lumen.

Day eighteen The tubules were found to be filled with secretory material. Vacuoles of one specimen were morphologically similar in both the lumen and cytoplasm (Fig. 57). Golgi apparatus was distended and was found close to the surface (Fig. 58). Terminal bars were conspicuous at this stage of the cycle.

Basic cytology of the cervical epithelium

Two types of cells were found in the cervical epithelium; ciliated and non-ciliated (Fig. 66). The ciliated cells had considerable numbers of cilia projected from their apical border. The cilia originated from a basal body and were long and slender approximately 0.25 micra in diameter. Cross sections showed the typical organization of nine

microtubules or double filaments and a center pair all enclosed in the plasma membrane (Fig. 67). The ciliated cells contained large numbers of rounded and elongated mitochondria, very well developed Golgi apparatus and endoplasmic reticulum. The nucleus was found in the middle part of the cell (Fig. 66). The lateral plasma membrane was thrown into waves and was highly interdigitated with corresponding processes of adjacent cells. Very little mucus was found in these cells.

In the non-ciliated cell, the cytoplasm was distended with large, saccular spaces containing flocculent material of a low density (Fig. 68). Some of these granules had an electron dense center. The mucous granules took up the greater cytoplasmic space of the cells, and pushed the nucleus into the basal part (Fig. 69). Some of the granules after being released into the lumen, were enclosed by a limiting membrane (Fig. 70). Small mitochondria were sometimes present in these cells either interspersed between the mucous granules or around the nucleus (Fig. 66). The plasma membrane was usually complete over the granular mucus except in those areas where the membrane was broken in order to release the mucus into the cervical lumen (Figs. 71 and 72). After the cell was emptied, or partially so, the cell membrane was re-established over the cell surface. The endoplasmic reticulum and Golgi apparatus were easily observed (Fig. 73).

Numerous microvilli were found projecting from the apical border of the cells. Desmosomes were found on the lateral plasma membrane. Both ciliated and non-ciliated cells were found to be supported by a basement membrane (Fig. 74).

Cyclic changes in the cervical epithelium

Estrus Both types of cells, secretory and ciliated, were observed at this stage. The secretory cells were packed full with many round secretory granules. Some of the granules had an electron dense area in the center of a less dense area (Fig. 69). The cell membrane of these cells was intact but in some specimens, it was incomplete toward the lumen and secretory granules were observed to be passing out into the lumen. In the lumen, most of the granules lost their round shape but some retained the enveloping membrane (Fig. 70). Several desmosomes were observed between these cells. The cytoplasm of the secretory cells distal to the granules was more electron dense than the secretory material, and some mitochondria were observed between the luminal cell membrane and the secretory granules (Fig. 76). The nuclei were pushed down toward the basement membrane and were small and irregular. The ciliated cells had some secretory granules but not as many as the mucous cells. These granules were identical to those found in the mucous secreting cells. The cytoplasm of the ciliated cells contained large numbers of mitochondria and Golgi apparatus. The nuclei of the ciliated cells were round and were found close to the surface of the cells.

Day three The ciliated cells were found to be recessed back from the lumen. The nuclei of the mucous secreting cells were pushed to the basement membrane, and the organelles around the nuclei were not distinct. Some of the mitochondria were related to the invaginations of the nuclei. Both secretory and ciliated cells were present. Some of the secretory cells were already empty and had a well developed endoplasmic reticulum that was extended toward the luminal surface (Fig. 73).

Day five Many secretory cells were present, and the nuclei were crowded toward the basement membrane and had irregular membranes. Large numbers of microvilli were present and extended into the lumen from the complete cell membrane of both types of cells.

Day seven Channels between secretory cells were observed and in some instances contained some granular material (Fig. 75). The channels extended three quarters of the way to the surface. Mucous secretion was found in the lumen. The secretory granules in the cytoplasm were abundant; some with electron dense areas that resembled lysosomes. The nuclei were pushed to the basement membrane. The few mitochondria present were closely related to the indentations of the nucleus or were pushed to the edge of the cells by the secretory granules. The ciliated cells resembled those described for the other stages of the cycle.

Day ten Many secretory granules with electron dense spots were observed. The cell membrane became incomplete on the luminal surface and allowed secretions to spill out, however the lateral wall was thick and intact (Fig. 71). In some areas, the cytoplasm was more electron dense and no secretory granules were found. Golgi apparatus was located near the nucleus and was related to it. Fewer mitochondria were found in the secretory cells than were observed in the ciliated cells.

Day fourteen Not as many secretory granules were observed in this sample. The nuclei were located distally in the cells but channels between the cells were not apparent.

Day eighteen An increased amount of granules in the cells was noted.

DISCUSSION

General

The individual variation in the length of the cycle can be a source of confusion in trying to determine the exact cyclic changes in the endometrium. This problem could be overcome by taking several biopsies from the same cow during a cycle. The problem with this method was that by introducing the biopsy instrument every other day and keeping the cervix open, some changes were noted in the normal physiology of the uterus. In one individual from which eight biopsies were taken every other day, excessive amounts of mucus were found to be secreted through the cervix, and the cow exhibited signs of estrus 10 days after the previous estrus. It was impossible to determine the exact cause of this short cycle, and more work is needed to determine the side effects of repeated biopsies. Any other method that will use more than one animal for the study of cyclic changes will have to take into consideration the individual differences between animals especially in mature cows.

Methods of collection

Three methods of sample collection were used in this experiment: biopsy, surgery and postmortem. For light microscopy studies, one can use any one of the three; but for electron microscopy, it was found in this study that the hysterectomy procedure was the most satisfactory method. This method allows the investigator to obtain fresh tissue within a very short time after removal of the organ from the animal. It is also possible to determine the exact location of the area sampled, caruncular or intercaruncular, and it is easier to separate the endometrium from the connective tissue. Large samples are available for

use in histological methods for comparison purposes. Recovery after hysterectomy was uneventful in 5 of the 8 cows. One cow died of surgical shock, one of abdominal hemorrhage and peritonitis and one was sacrificed and posted after surgery. One attempt was made to remove two folds of the cervix during surgery. This procedure caused excessive intraperitoneal hemorrhage and was abandoned.

The biopsy technique was found to be difficult to perform on heifers and during midcycle. Excessive use of the operation on the same cow might have caused some side effects by keeping the cervix open. The sample obtained was small and sometimes not enough for histological techniques. In some biopsies, the surface epithelium was absent.

The postmortem method had the disadvantages of a long time elapsing from killing to fixation, and the inconvenience of working in the packing plant.

Surface epithelium

The interpretation of results obtained from studying uterine cyclic changes in the bovine is difficult because of the large number of factors that should be taken into consideration. The age of the animals studied and whether or not they were heifers or cows is very important. In this study, definite cyclic changes were found in samples taken from heifers. Several authors (Dziuk, 1955; Cooper, 1961; Sasser, 1963) reported that it was impossible to measure the changes in the surface epithelium of the bovine because of the great variation within each stage. It is interesting to note that these investigators used both heifers and cows in their studies. This fact could explain some of the differences in the results. Definite cyclic variations were found in

samples taken from heifers, but in mature cows, no cyclic changes in the surface epithelium could be observed. Although some of the measurements were difficult to interpret, this experiment suggests that the cycles in heifers varied less than in cows. In heifers, the surface epithelium was low at estrus and post estrus and began to increase in size until day fifteen of the cycle. Asdell (1946) reported that the individual variation in cycle length in unbred heifers was less than in cows. He noted a mean cycle length for heifers of 20.25 $\pm$ 0.05 days and a standard deviation of 2.33 days while the mean for cows was 21.28 $\pm$ 0.06 days and a standard deviation of 3.68 days.

Higaki and co-workers (1959) found differences in the quantity of blood estrogen between multiparous and primiparous animals. Peaks of estrogen levels were found in the bovine at estrus and days 9-10 post-estrus. In multiparous cows, the estrogen level was higher at days 9-10 postestrus than at estrus, while in primiparous animals, the level was higher at estrus than during the corpus luteal stage. Another factor that could explain the differences in results between different investigators could be explained by the work of Asdell (1946). He noted that sometimes an overlapping of cycles occurred and the corpus luteum did not begin to regress until proestrus. The genital tract showed both a luteal and a follicular influence during proestrus.

The intraorgan differences could also play a part in creating the differences. The surface epithelium of the caruncular area was found to be different from the intercaruncular area, but only a few investigators made it clear which of these areas were referred to in their observations. When a sample is taken by biopsy, it is almost impossible to define the

area. Another source of confusion might be the terminology used. It was not always clear when the term "day one" was used if it was referring to estrus or day one postestrus. It is also difficult to define the term "estrus". A sample taken from a cow in the first hours of a six hour estrus period will be quite different from a sample taken during the last hour of a 24 hour estrus period. In this experiment, the term "day one" refers to the period when the animal was ready to accept the male. The exact length of the estrus period or the time of ovulation was not determined.

These factors have to be taken into consideration when evaluating the results. A better way to do it would be to determine, by repeated palpation at frequent intervals, the exact length of the estrus period and the time of ovulation. By taking samples before and after ovulation, one could hope to get a more exact picture of the type of endometrium present in the cow at estrus.

Clear cells Cells with clear cytoplasm were found in the surface epithelium. These cells could be either epithelial cells undergoing mitosis and could be called mitotic figures or white blood cells that were penetrating into the epithelium. Some difficulties were encountered in the interpretation of these cells under the light microscope and it was decided to call them "clear cells". Although a large number of specimens were studied in the electron microscope, only one clear cell was observed (Fig. 63). The cell was found close to the basement membrane with a centrally located nucleus and a low density material or cytoplasm.

In this experiment, ciliated cells in the surface epithelium were

observed under the light as well as the electron microscope. These cells were definitely part of the surface epithelium rather than the neck of the glands. Only a small number of these cells were found and only in some samples. Ciliated cells were reported to be present in the surface epithelium by Weber (1948b). It is interesting to note that ciliated cells were reported before in the oviduct and glandular epithelium and are reported here in the cervical epithelium. Lovell* (1966), in a preliminary study, reports the presence of ciliated cells in the surface epithelium of sows in certain stages of the cycle. Ciliated cells were also reported by Larsen (1962) in the rabbit. The significance of these ciliated cells is not known. They might have persisted from ciliated cells of the Mullerian ducts, but because they are present in such small numbers, it is doubtful that they are of great significance.

Glandular epithelium

No cyclic changes could be found in the glandular epithelium of mature cows. The height of the epithelium fluctuated throughout the cycle as did the size of the lumen and the overall size of the gland. It was difficult to make accurate measurements because of these great variations. The results are not in agreement with those of Cooper (1961), Sasser (1963) and Johnson (1965) who found definite cyclic changes in the gland size. In heifers, the epithelium size followed cyclic changes throughout the cycle. Another factor that can contribute

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*J. E. Lovell, Department of Veterinary Clinical Sciences, Iowa State University of Science and Technology, Ames, Iowa. Uterine Epithelium of the Sow. Private communication. 1966.

to the large variation in results can be the difficulties in differentiating the gland from the glandular neck when taking the measurements.

Alkaline phosphatase The results of this experiment do not agree with the results of other investigators (Weeth and Herman 1952, Moss et al. 1954 and Skjerven 1956b). They found the alkaline phosphatase reaction to be present mainly during midcycle. With some variation, it was found that alkaline phosphatase was present at all stages of the cycle although in larger quantities at midcycle. The alkaline phosphatase activity followed the development and regression of the corpus luteum and appeared to be related to the progesterone level in the organism. The precise physiological function of alkaline phosphatase in the endometrium remains unknown. In general, the phosphatases catalyze the hydrolysis of phosphate esters. Changes in the phosphatase activity could reflect changes in the metabolic activity induced by the sex hormones. The fact that the enzyme was found to be confined to the distal part of the surface and glandular epithelium might suggest that it is involved in the secretory mechanism of the cell.

Periodic acid Schiff P.A.S. positive material was found in the uterus during estrus and proestrus. These results are in agreement with the other investigators (Weeth and Herman 1952, Moss et al. 1954, Skjerven 1956b and Van Den Hoek 1959). P.A.S. positive material decreases in quantity after ovulation and is plentiful in the cells during the follicle development. This could show a connection between the estrogen level and P.A.S. positive material. According to the reports of Higaki and co-workers (1959), large quantities of estrogen were found in the blood during the midcycle. Only limited amounts of P.A.S.

positive material were found during this period except in cow No. 23 which, at ten days, exhibited large numbers of P.A.S. granules. This did not follow the pattern observed in other cows.

The function of these P.A.S. positive materials is open to speculation. It is possible that these materials serve as a source of readily utilizable energy at implantation. This does not explain the fact that most of it was found during estrus and none was present in the surface epithelium or glands at the theoretical time of attachment.

Oil red O Oil red O staining droplets were found during late diestrus and proestrus. This corresponds to the stage of the cycle during which lipid inclusions were observed in the surface epithelial cytoplasm with the electron microscope. Skjerven (1956b) reported the presence of fat droplets from day ten to two days prior to estrus. The functions of these substances are not known, but it seems reasonable to suggest that they are used for the nutrition of the early embryo.

Histochemical cyclic changes in the glands were not as apparent as in the surface epithelium. Great variations were observed between the glands close to the surface and those situated deep in the stroma. Skjerven (1956b) suggested that the changes in the glands appeared to be decided by the individual rhythm of the cells rather than the stage of the cycle. Variations in alkaline phosphatase activity and glycogen content from gland to gland and from cell to cell in the same gland in material examined in this study tend to confirm this observation.

Cervical epithelium

The results of this experiment suggested a two phasic cycle in

height of the bovine cervical epithelium that could be explained by the following hypothesis. The first phase begins with estrus and the secretion of large quantities of mucus, and the reduction in cell size. This process could be controlled by the estrogen that is secreted at that time. At day five post estrus with the increase in progesterone secretion, the mucous cells increase in size and mucus accumulates in the cells. The peak of this stage takes place at day eight when the first wave of follicles developing in the ovaries are secreting estrogen. With this estrogen secretion, the cells reduce in size again until day sixteen of the cycle. At that stage under the influence of progesterone, the cells increase in size. This suggests that progesterone is responsible for the cell size increase and estrogen for the secretion of mucus. This hypothesis is contrary to Hartman's (1962) report on the endocervix cycle of the monkey. He reported that the peaks in cell height were caused by the peaks in estrogen levels rather than progesterone. Herrick (1951) found that cells became more distended with mucus only during proestrus and estrus. The question of whether estrogen or progesterone effects the increase in cell size and the interrelationship between these hormones and the cervical epithelium will need more investigation before a definite answer to the question can be given.

Asdell et al. (1949) and Cooper (1961) reported that the administration of progesterone to ovariectomized cows caused the uterine surface epithelium to become tall pseudostratified similar to that found in intact animals on day fifteen postestrus. Sasser (1963) reported that progesterone administration increased branching of the uterine glands but reduced the size of the epithelial cells and the secretory phase of

the gland followed.

In estrogen treated ovariectomized cows Cooper (1961) reported that the surface epithelium was similar to that of cows shortly after estrus. Asdell et al. (1949) found that estrogen produced large epithelial cells with more rounded, irregularly shaped nuclei.

Rajakoski (1960) studied the variation in the ovariefollicular system during the estrous cycle. He found that the corpus luteum reached the peak of its development between the ninth and the twelfth days of the cycle, and then regressed rapidly after the seventeenth day of the cycle. Two waves of small follicles were also reported by Rajakoski (1960). The first of these occurred between the third and the fourth days and the second, between the twelfth and fourteenth days. Both resulted in a follicle of preovulatory size. The large follicle of the first wave underwent atresia from the twelfth day. The large follicle of the second wave was ovulated. If estrogen is produced by the follicle of the first wave (and from the work of Higaki et al. 1959, it seems reasonable to believe so,) the period of 9-12 days is characterized by a high secretion of both estrogen by the follicle and progesterone by the corpus luteum. The impact of the simultaneous secretions of estrogen and progesterone on the uterus at this stage of the cycle is probably essential for the preparation of the organ for implantation.

Variations in the ability of the corpus luteum to secrete progesterone or the follicles to secrete estrogen at this stage of the cycle could be the cause of obtaining variable results from different cows.

The changes in the endometrium during the estrous cycle (induced by the ovarian hormones) could be outlined in the following way:

Day 18 to day 1 (Little progesterone activity and increased estrogen secretion)

1) The surface epithelium is low.
2) Large amounts of glycogen are present in the cells.
3) Microvilli are present but are reduced in numbers toward the end of the period.

Day 2 to day 8 (Reduced estrogen and increased progesterone secretion)

1) Epithelium starts to increase in size.
2) Glycogen granules are less numerous.

Day 9 to day 12 (Simultaneous secretion of estrogen and progesterone)

1) Cell size continues to increase.
2) Secretion of the surface epithelial cells begins.
3) Channels are seen between adjacent cells.
4) Mitochondria are large and filamentous.
5) Alkaline phosphatase is plentiful.

Day 13 to day 17 (Decreased estrogen activity and continous progesterone secretion)

1) Lipid droplets in the cytoplasm.
2) Cell size starts to decrease toward the end of the period.
3) Glandular epithelium starts to decrease in size.
4) Large channels are found between adjacent cells.

SUMMARY

1. Uterine and cervical samples were obtained from 32 animals by post mortem, surgery and biopsy techniques.

2. The samples were studied by light microscopy, electron microscopy and histochemistry.

3. The uterine surface epithelium that was obtained from heifers was low (15u) at estrus and increased in height (30u) at 6 days after heat remaining at this level until the 16th day of the cycle. It then started to decrease again to the level observed at estrus.

4. The surface epithelium of mature cows varied considerably in height within the stages studied and did not have a definite cyclic sequence of changes.

5. Ciliated cells were found in the surface epithelium in small number (less than 1% of the cells). The presence of cilia was not associated with any particular stage of the cycle. Except for the presence of cilia the ciliated cells were similar to the non ciliated cells.

6. Microvilli were present in greater numbers on the luminal surface of all epithelial cells during midcycle compared to what was observed during estrus.

7. The mitochondria were small during estrus and became elongated and larger during midcycle.

8. Apocrine type of secretion was observed in the surface epithelial cells 5 days after estrus.

9. Intercellular channels were noticed between surface epithelial cells. These channels were interupted by desmosomes and occurred both

in the basal and distal part of the cell. These channels were not observed earlier than 7 days following estrus.

10. Alkaline phosphatase was found on the luminal surface of the epithelium at all stages of the cycle with greater concentration during midcycle.

11. Oil red O positive granules were found in the surface epithelium from 13-18 days. Lipid inclusions were found during the same stage by means of the electron microscope.

12. The glandular epithelial cells of heifers reached their lowest level at estrus 10u and increased up to 20u at midcycle.

13. No cyclic changes could be found in the size of the lumen, the overall size of the glands or the amount of secretion in the glands.

14. No cyclic changes could be observed in glandular epithelium of mature cows.

15. An electron dense type of cell was found in the glandular epithelium during heat. This cell had a more conspicuous endoplasmic reticulum than the regular glandular cells.

16. The cervical epithelium showed a biphasic cycle in height. The cells were low following estrus (15u) increased in size from day 3 to day 8 (35u) reduced in size again to day 16 (15u) and started to increase in size again prior to heat (30u).

17. Two cell types were found in the cervical epithelium: a secretory and a ciliated type. The secretory cells were packed with membrane bound granules that sometimes had an electron dense center. Some of these granules retained their membrane after release into the lumen. The ciliated cells were found to have very few or no secretory

granules and more mitochondria and endoplasmic reticulum than secreting cells. Approximately 10% of the epithelial cells of the cervix were ciliated.

18. The cilia of the cervix were similar to the cilia found in the glandular epithelium.

19. Mucus was released from cervical cells into the lumen through a break in the plasma membrane of the luminal surface of the cells.

20. Some mucus was always present in the cells. The amounts varied according to the stage of the cycle and the height of the epithelium.

21. Intercellular spaces were found in the cervical epithelium. These spaces were restricted by desmosome attachments between adjacent cells. The channels were most extensive in the basal part of the cells and were larger than those found in the surface epithelium.

22. Alkaline phosphatase positive reaction was observed in the luminal margin of the cervical epithelium during most parts of the cycle.

23. Periodic acid Schiff positive reaction was found in the cervical cells at all stages of the cycle. Only the mucus was stained.

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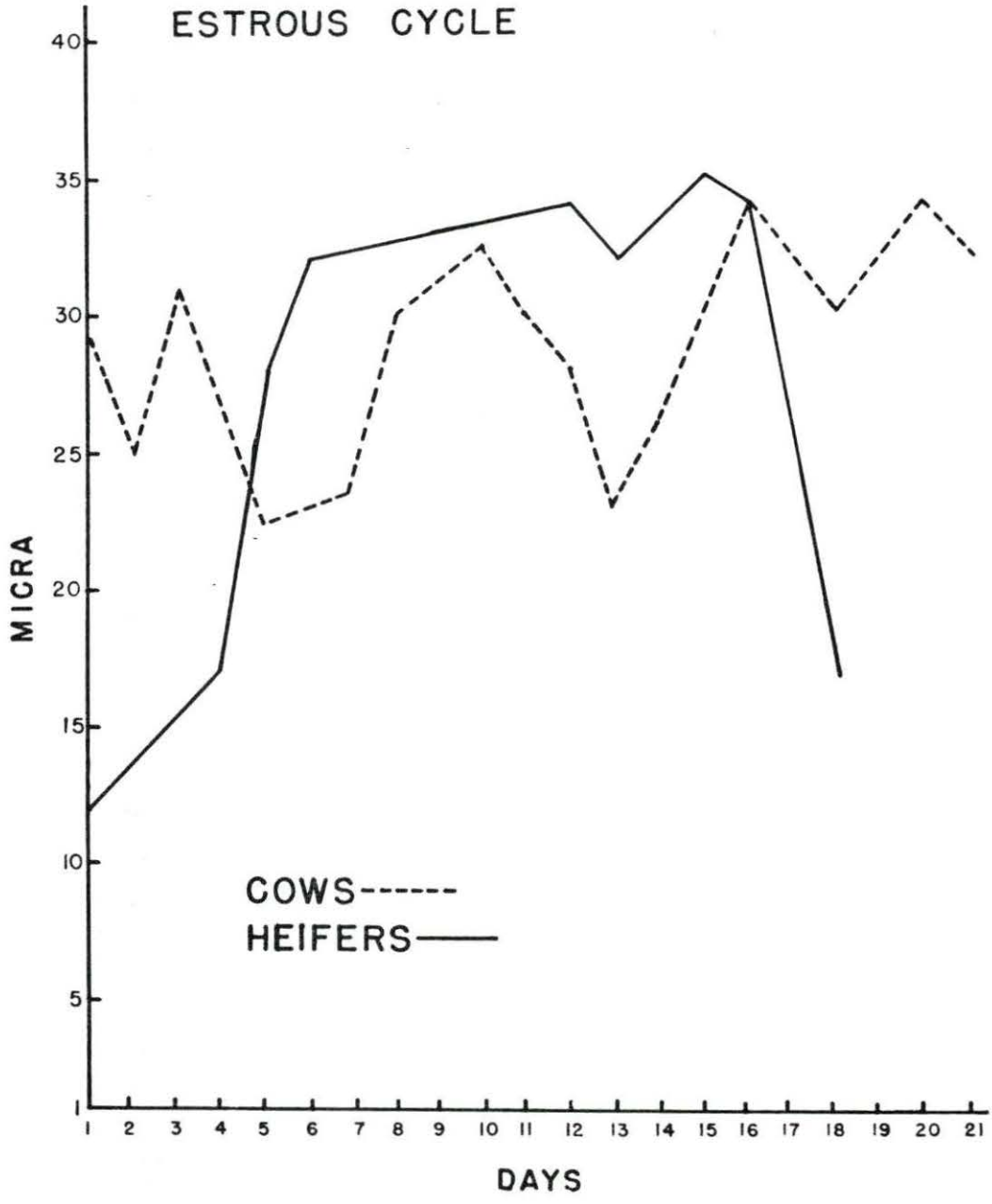
APPENDIX

Illustrations

Graph 1. Comparison of the uterine surface epithelium between heifers and cows during the estrous cycle.

GRAPH I

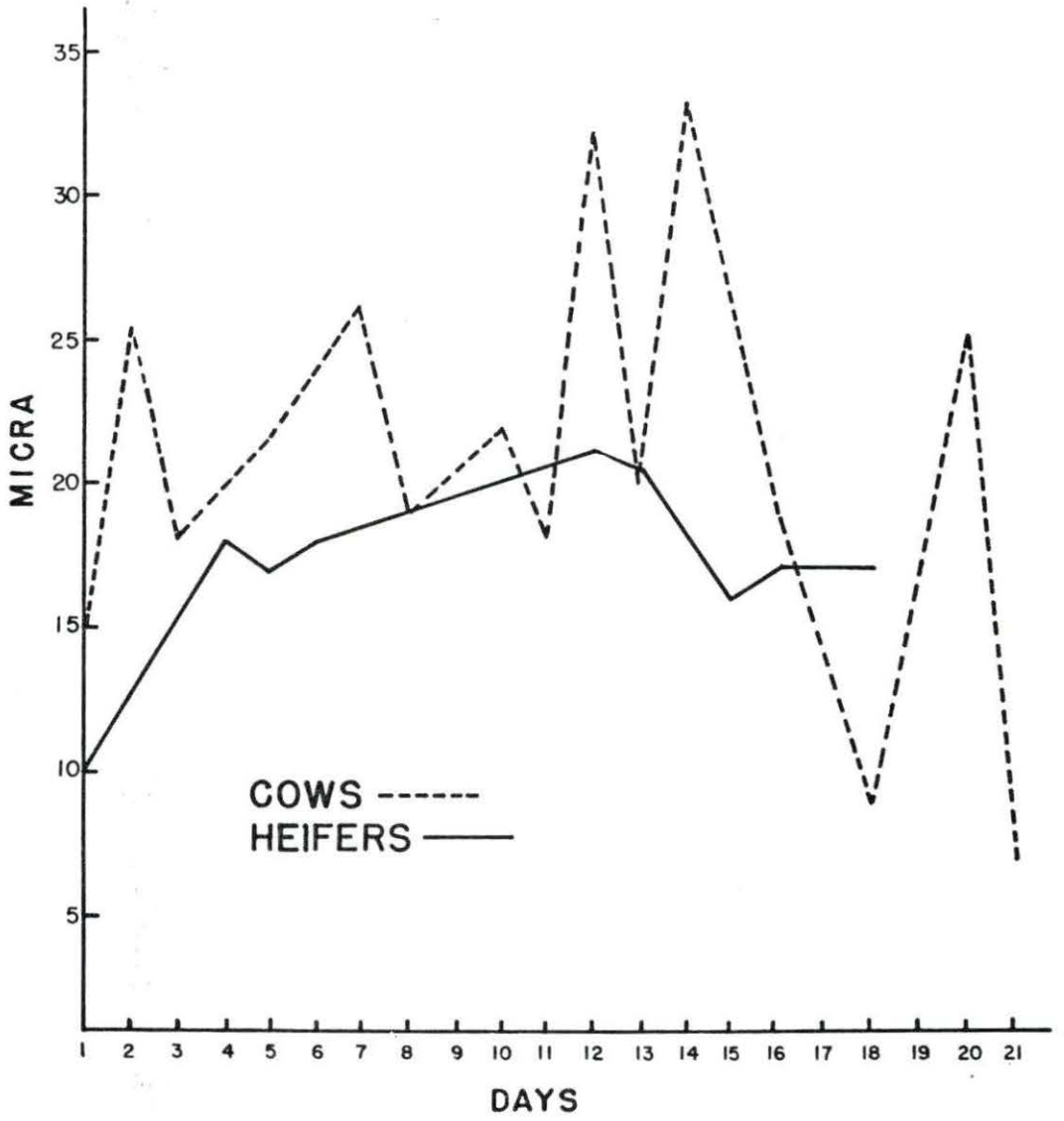
COMPARISON OF THE UTERINE SURFACE EPITHELIUM BETWEEN HEIFERS AND COWS DURING THE ESTROUS CYCLE



Graph 2. Comparison of the uterine glandular epithelium between heifers and cows during the estrous cycle.

GRAPH 2

COMPARISON OF THE UTERINE GLANDULAR EPITHELIUM BETWEEN HEIFERS AND COWS DURING THE ESTROUS CYCLE



Graph 3. Bovine cervical epithelium during the estrous cycle.

GRAPH 3

BOVINE CERVICAL EPITHELIUM DURING ESTROUS CYCLE

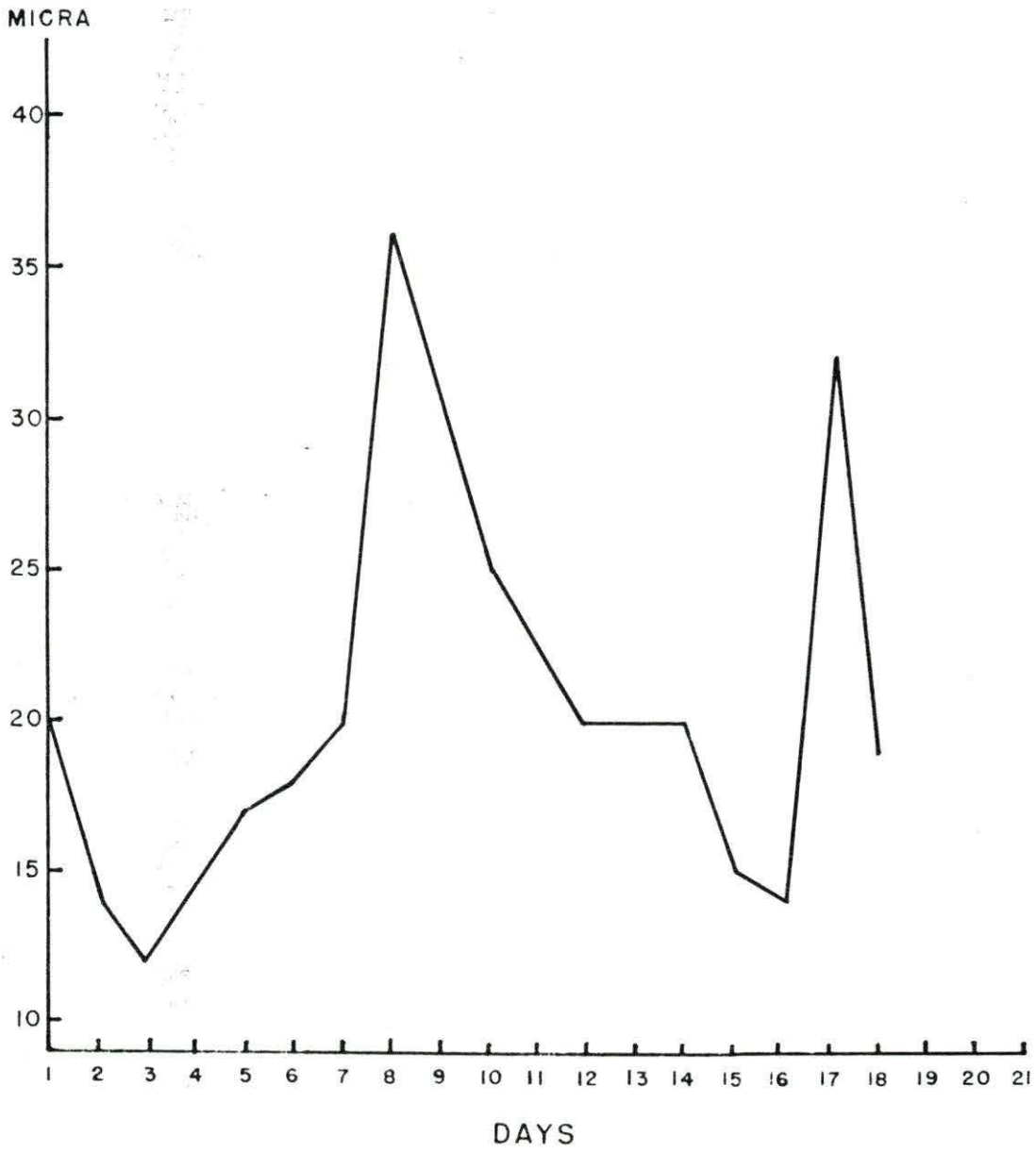
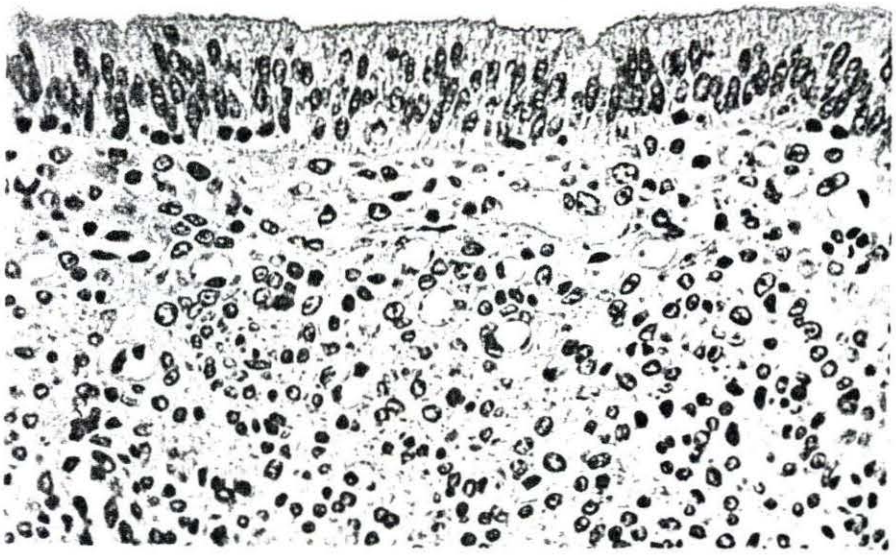


Fig. 1. Uterine surface epithelium during estrus. Cow 34, age 6 years. Stained with Hematoxylin-Eosin. 420x magnification. Several "clear cells" may be observed near the basement membrane in the epithelium.

Fig. 2. Uterine glandular epithelium during estrus. Cow 34, age 6 years. Stained with Hematoxylin-Eosin. 420x magnification.



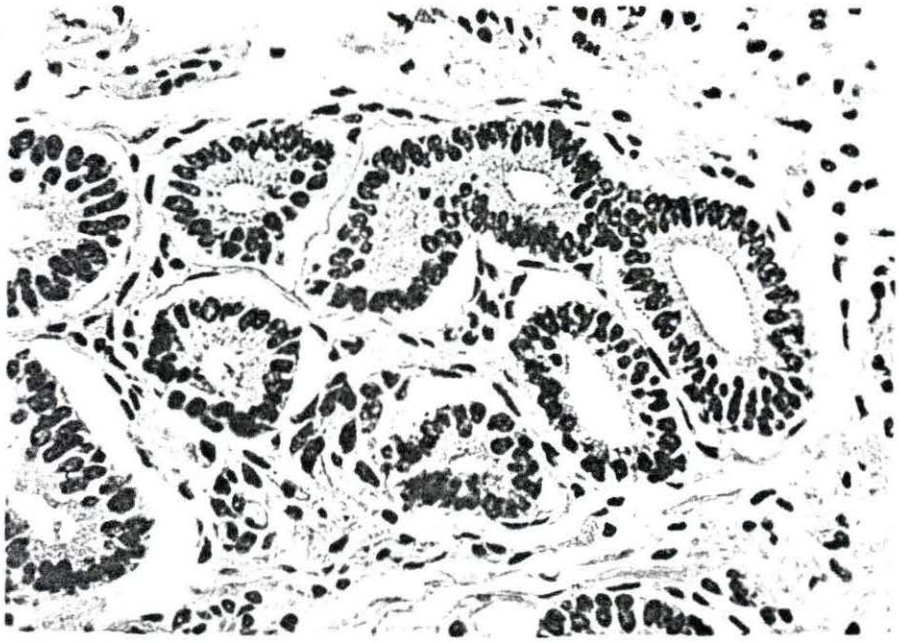
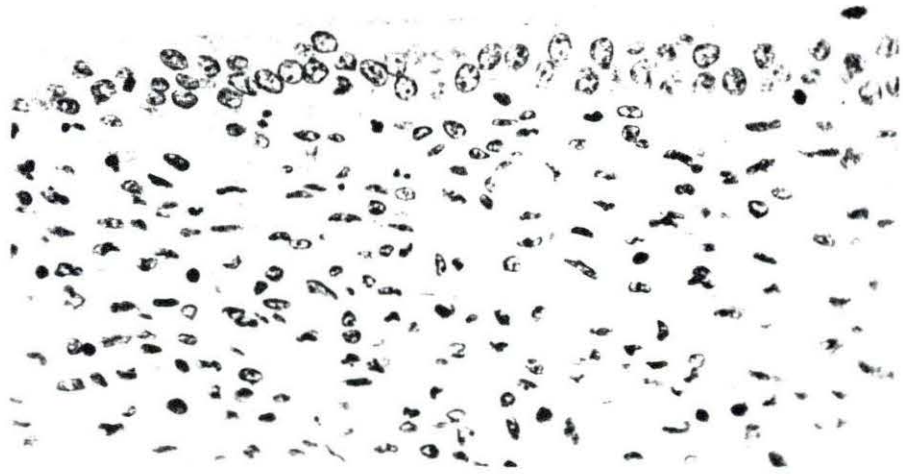


Fig. 3. Uterine surface epithelium during estrus. Cow 21, age 5 years. Stained with Hematoxylin-Eosin. 420x magnification. Two clear cells may be observed near the basement membrane.

Fig. 4. Uterine glandular epithelium during estrus. Cow 21, age 5 years. Stained with Hematoxylin-Eosin. 420x magnification.



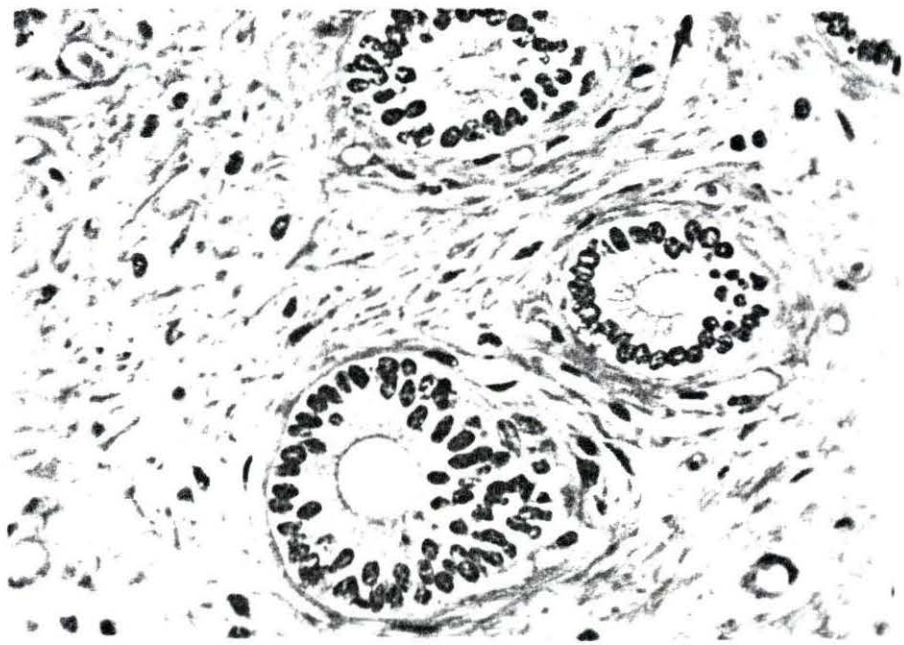
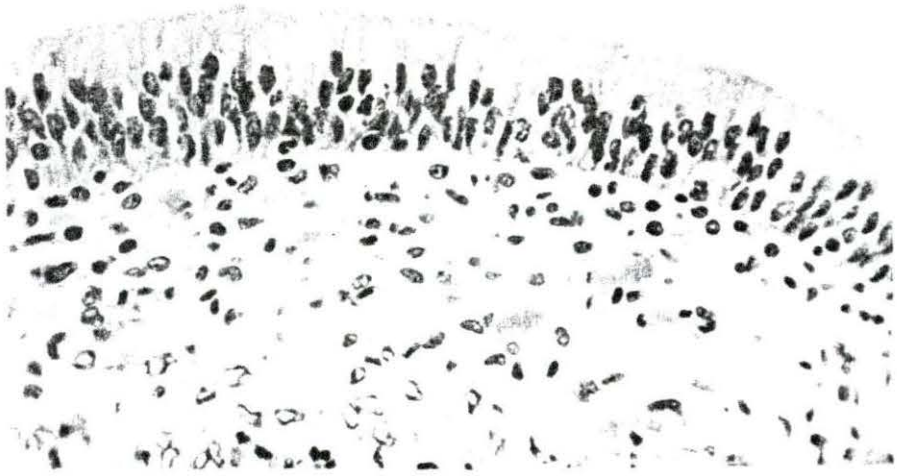


Fig. 5. Uterine surface epithelium day five post estrus. Cow 12, age 3 years. Stained with Hematoxylin-Eosin. 420x magnification. Note the "clear" cells among the surface epithelial cells.

Fig. 6. Uterine glandular epithelium day 5 post estrus. Cow 12, 3 years old. Stained with Hematoxylin-Eosin. 420x magnification.



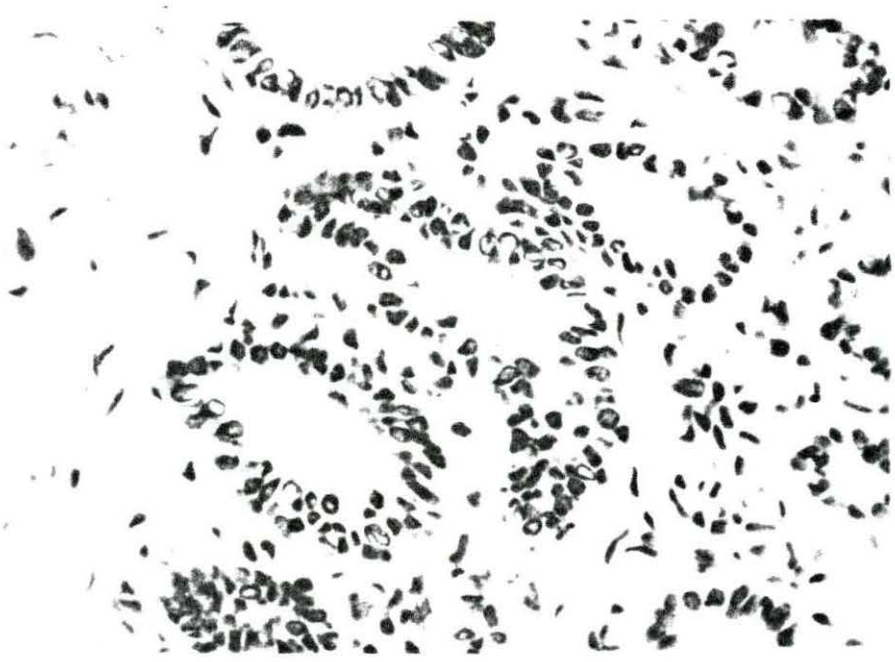


Fig. 7. Uterine surface epithelium six days postestrus. Cow 3, 2 years old. Stained with Hematoxylin-Eosin. 420x magnification. Several clear cells may be observed near the basement membrane.

Fig. 8. Uterine surface epithelium seven days postestrus. Cow 28, 6 years old. Stained with Hematoxylin-Eosin. 420x magnification. Note the difference in epithelium height from that of Fig. 7.

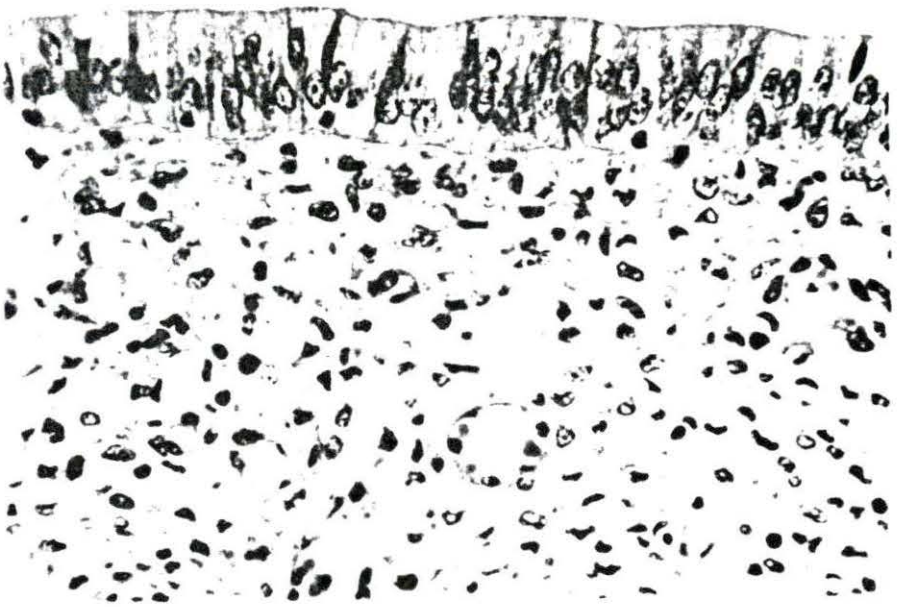
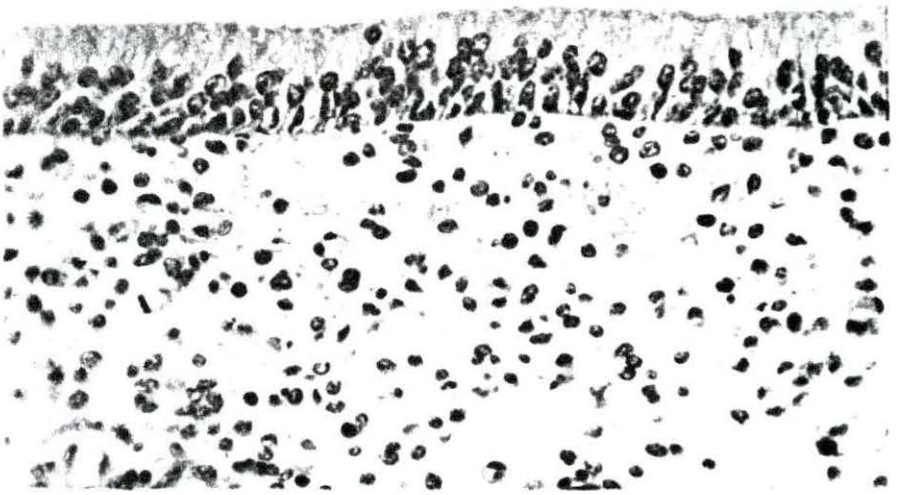


Fig. 9. Uterine surface epithelium 10 days postestrus. Cow 31, 6 years old. Stained with Hematoxylin and Eosin. 420x magnification.

Fig. 10. Uterine glandular epithelium 10 days postestrus. Cow 31, 6 years old. Stained with Hematoxylin and Eosin. 420x magnification.



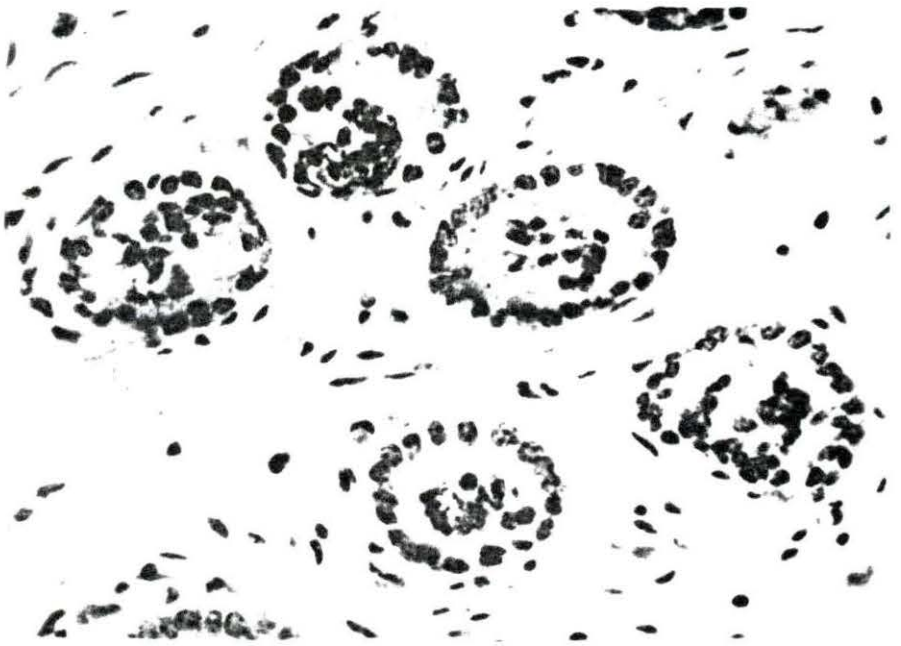
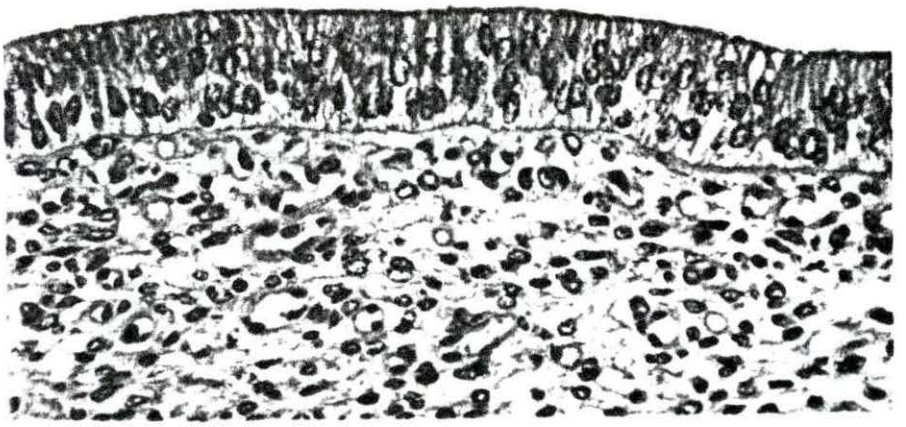


Fig. 11. Uterine surface epithelium 13 days postestrus. Cow 30, 2 years old. Stained with Hematoxylin and Eosin 420x magnification.

Fig. 12. Uterine glandular epithelium 13 days postestrus. Cow 30, 2 years old. Stained with Hematoxylin and Eosin. 420x magnification.



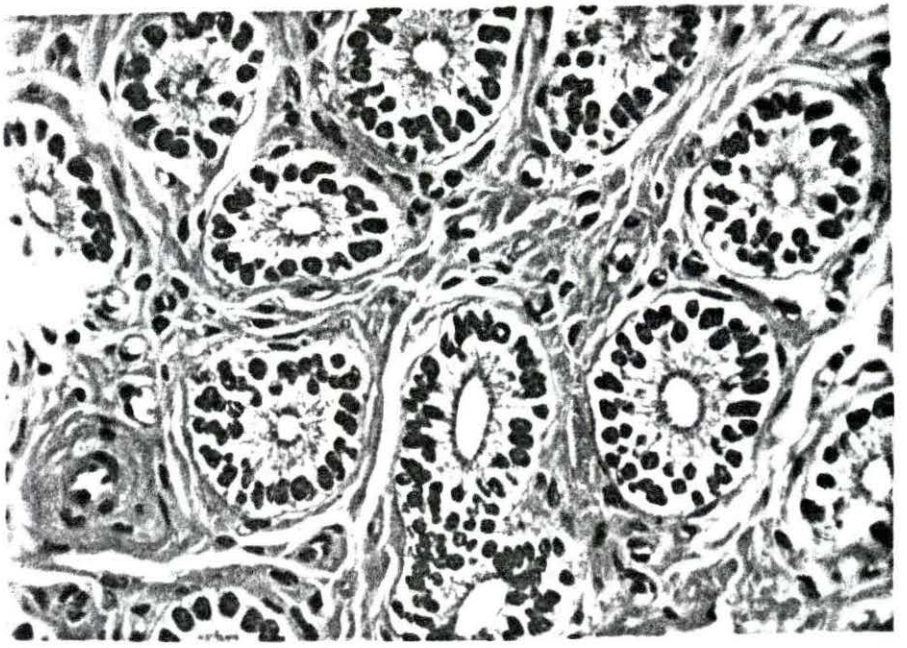
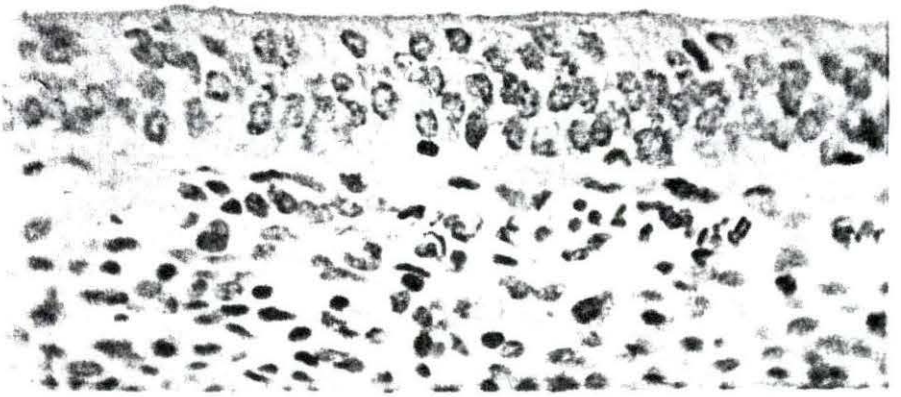


Fig. 13. Uterine surface epithelium 15 days postestrus. Cow 4, 2 years old. Stained with Hematoxylin-Eosin. 420x magnification.

Fig. 14. Uterine glandular epithelium 15 days postestrus. Cow 4, 2 years old. Stained with Hematoxylin-Eosin. 420x magnification. Clear cells may be noted among the epithelial cells of the tubules.



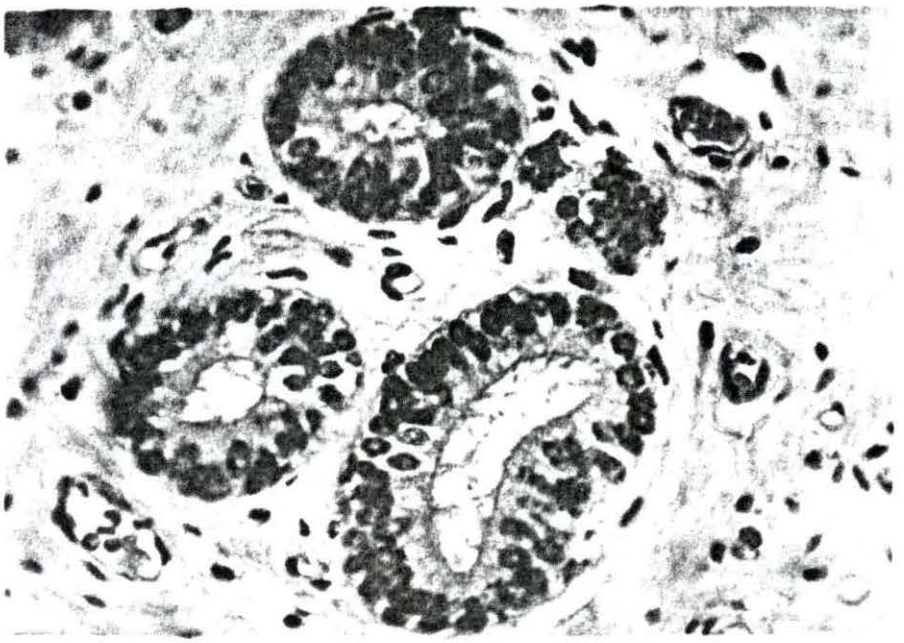
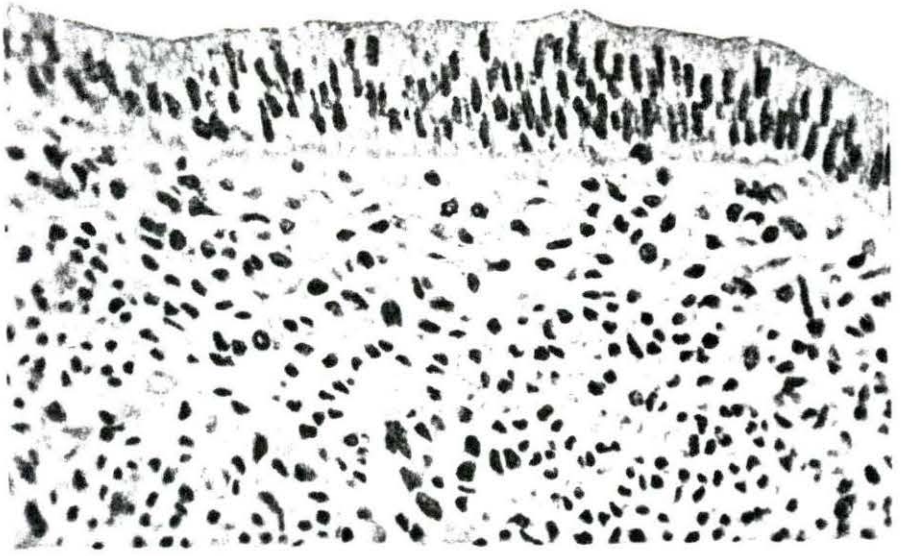


Fig. 15. Uterine surface epithelium 18 days post estrus. Cow 32, 7 years old. Stained with Hematoxylin-Eosin. 420x magnification.

Fig. 16. Uterine glandular epithelium 18 days post estrus. Cow 32, 7 years old. Stained with Hematoxylin-Eosin. 420x magnification. One large clear cell may be observed in the tubule to the left.



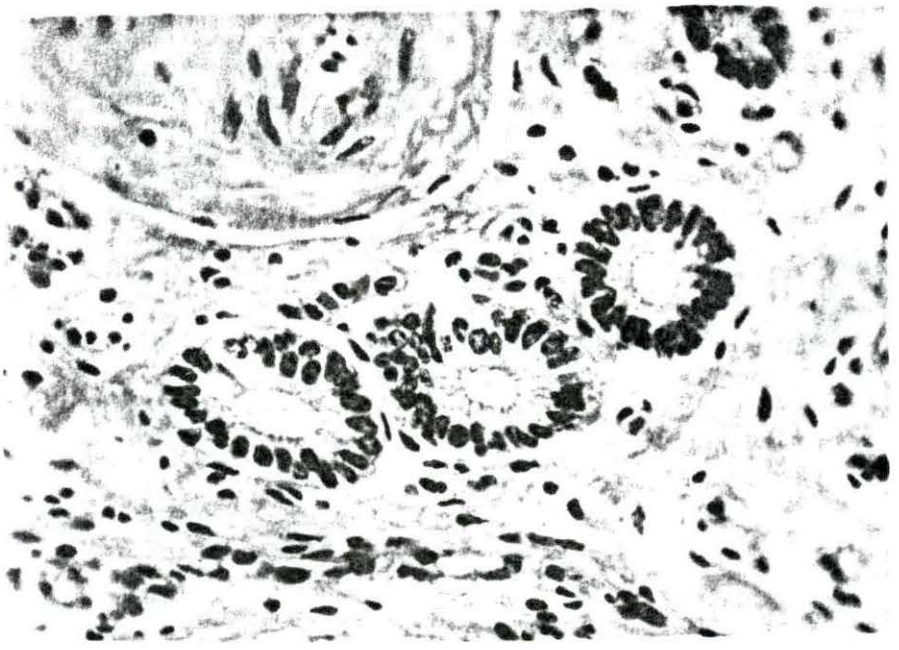
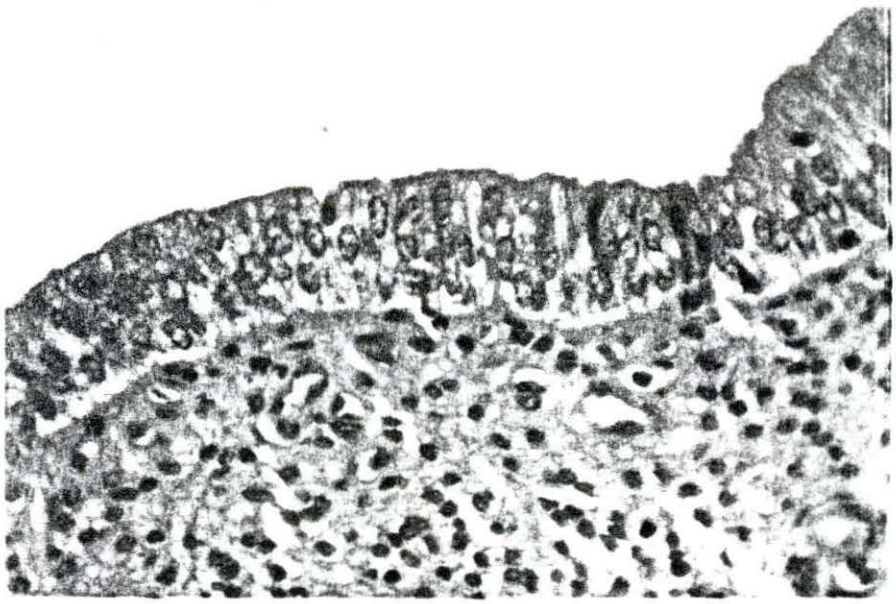


Fig. 17. Uterine surface epithelium 13 days postestrus. Cow 13, 2 years old. Stained with Hematoxylin and Eosin. 420x magnification. Note the ciliated cell.

Fig. 18. Same area as Fig. 17, magnified to x950. Note the presence of ciliated cells.



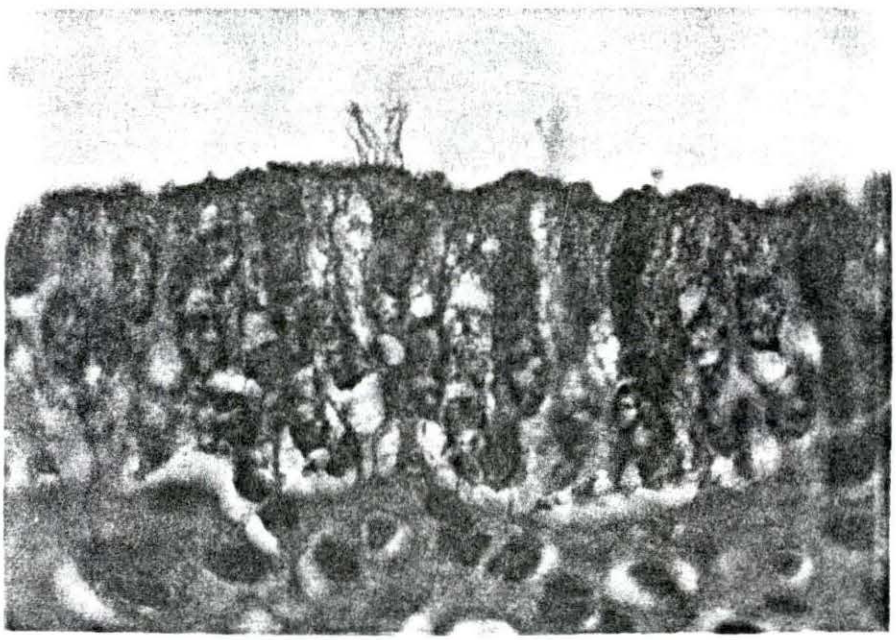
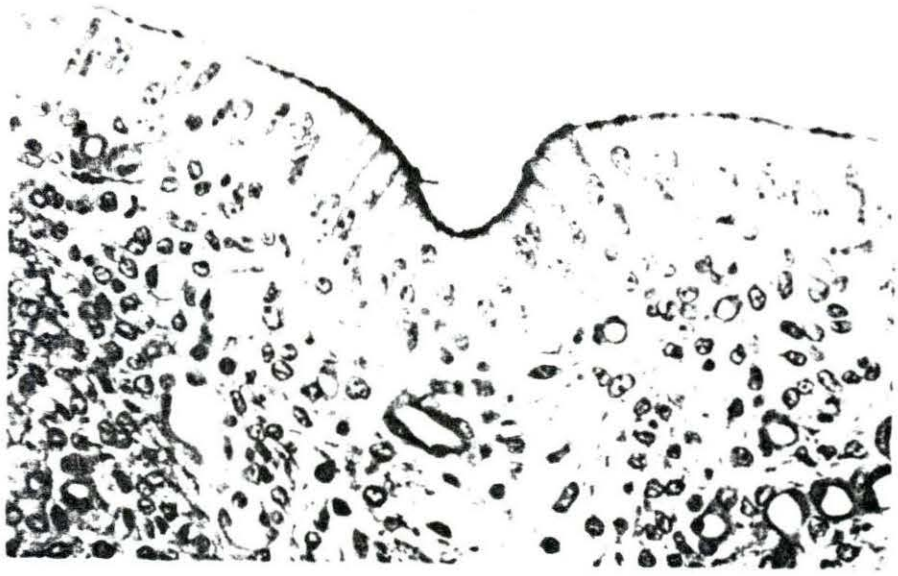


Fig. 19. Alkaline phosphatase in the uterine surface epithelium of Cow 29, 6 years old. Day two of the cycle. 420x magnification. Evidence of the presence of alkaline phosphatase may be observed in the apical portion of the cells with increasing amounts in the gland orfice.

Fig. 20. Alkaline phosphatase in the uterine glandular epithelium of Cow 29, 6 years old. Day two of the cycle. 420x magnification. Note the presence of alkaline phosphatase in the distal part of the cells and in the connective tissues around the glands.



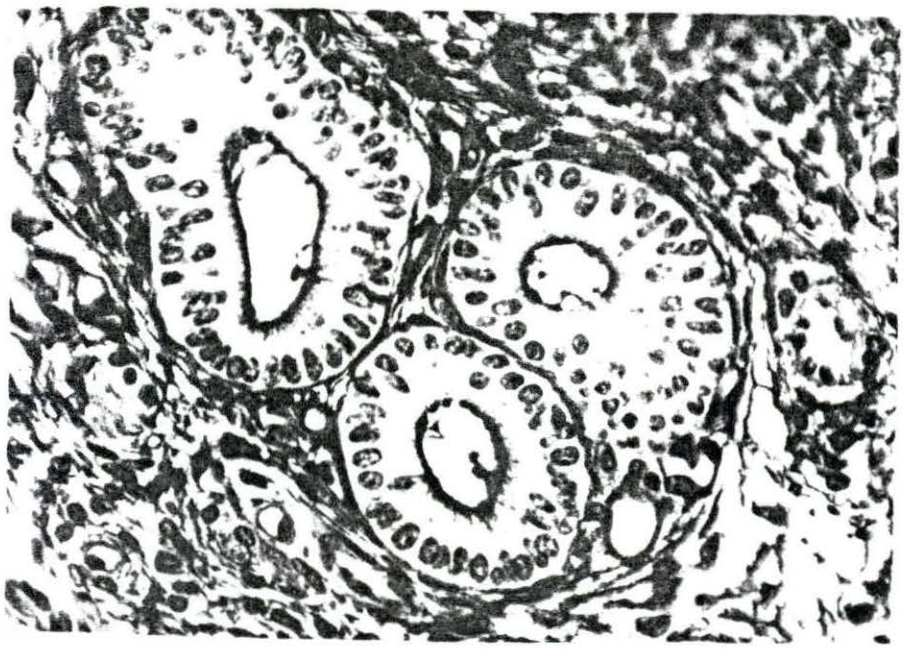


Fig. 21. Alkaline phosphatase in uterine surface epithelium at estrus. Cow 34, 6 years old. Alkaline phosphatase may be observed in the distal part of the cells but the nuclei are not stained.

Fig. 22. Alkaline phosphatase in uterine surface epithelium 5 days post estrus. Cow 12, 3 years old. Note the increased amount of alkaline phosphatase in the distal part of the epithelial cells and in the stroma.

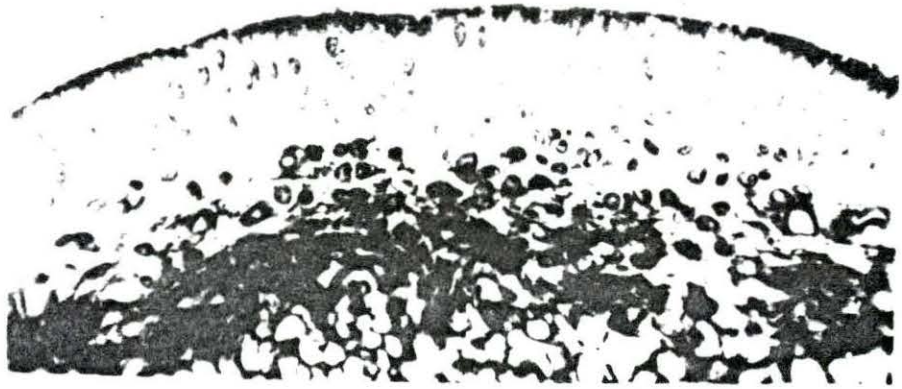
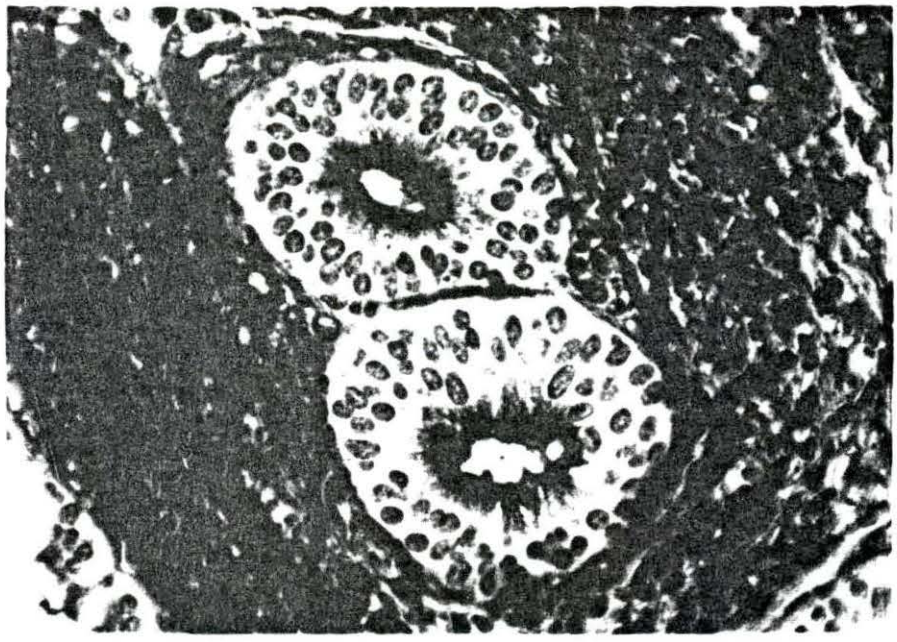


Fig. 23. Uterine glandular epithelium 13 days post estrus. Cow 30, 2 years old. Alkaline phosphatase positive reaction in the distal parts of cells and in connective tissue. Sample taken from area next to surface epithelium. 420x magnification.

Fig. 24. Uterine glandular epithelium 13 days post estrus. Cow 30, 2 years old. Alkaline phosphatase reaction is minimal. Taken from the same slide as Fig. 23 but from the stratum spongium.



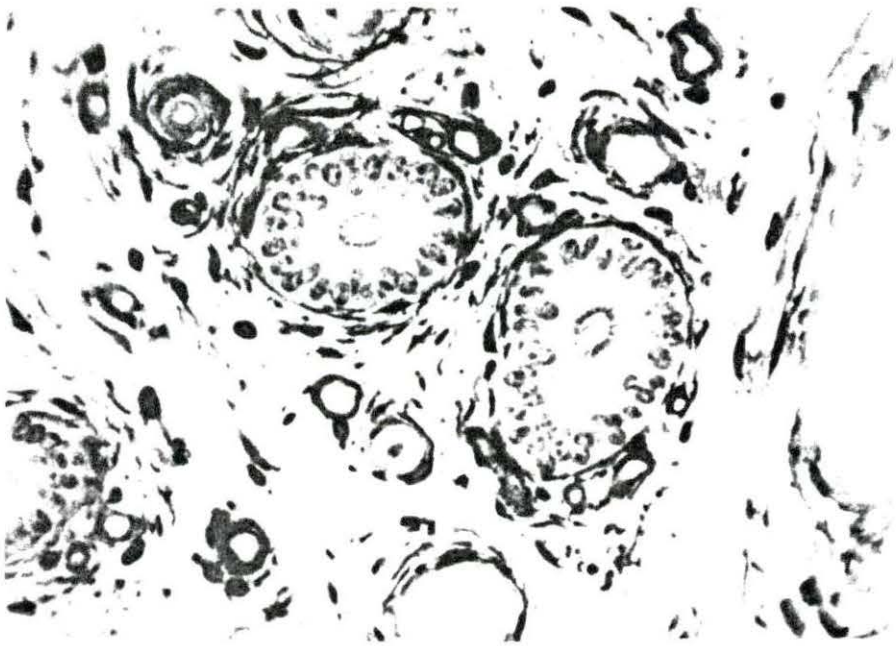
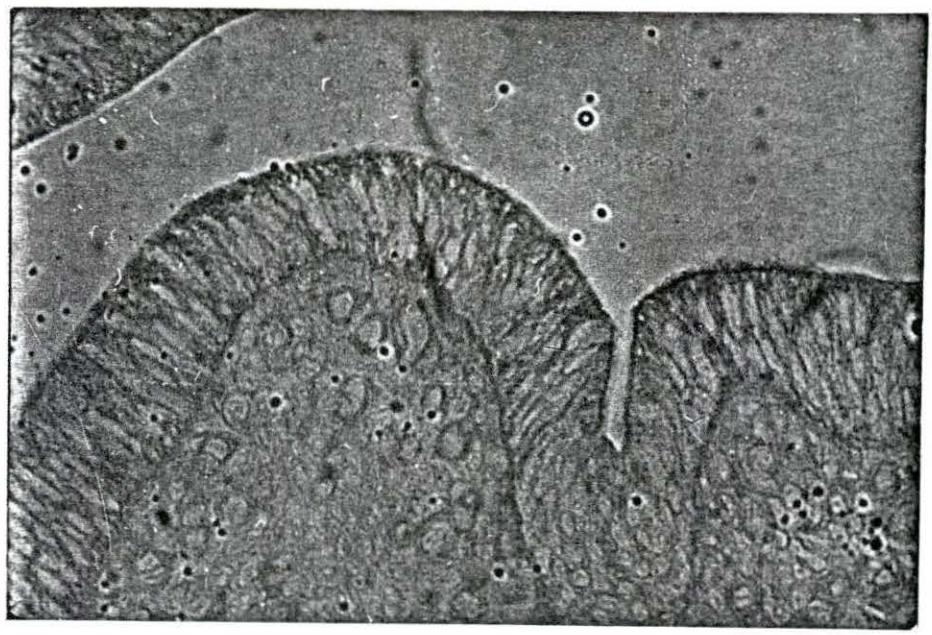


Fig. 25. Uterine surface epithelium 18 days post estrus. Cow 32, 7 years old. P.A.S. positive reaction. 420x magnification.

Fig. 26. Uterine surface epithelium 2 days post estrus. Cow 29, 6 years old. P.A.S. positive reaction. 420x magnification.



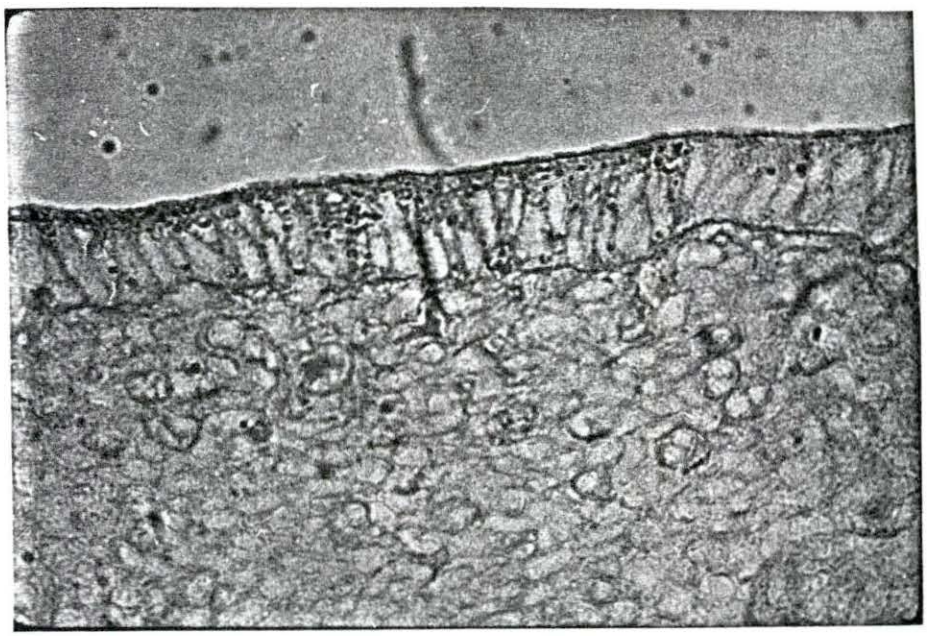
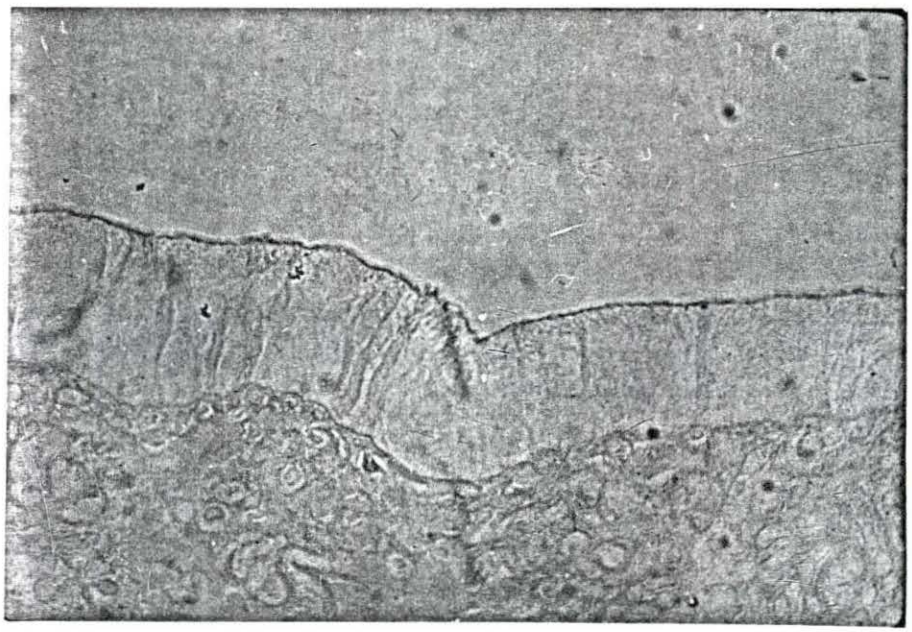


Fig. 27. Uterine surface epithelium 5 days post estrus. Cow 35, 6 years old. P.A.S. positive granules disappeared from cells after diastase incubation and only a line of the distal part of the cells appears positive. 420x magnification.

Fig. 28. Uterine glandular epithelium at estrus. Cow 34, 6 years old. P.A.S. positive reaction in glands. 420x magnification.



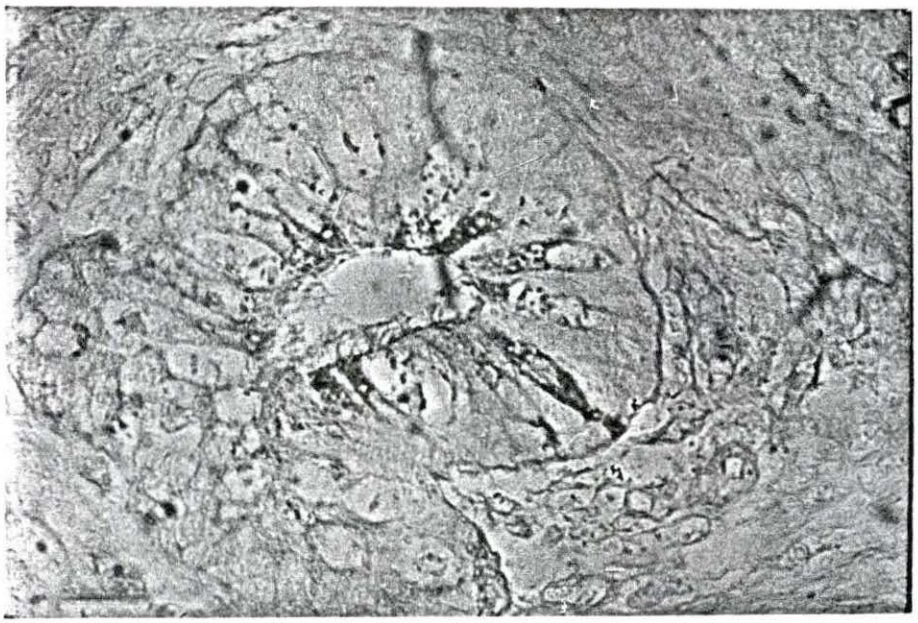
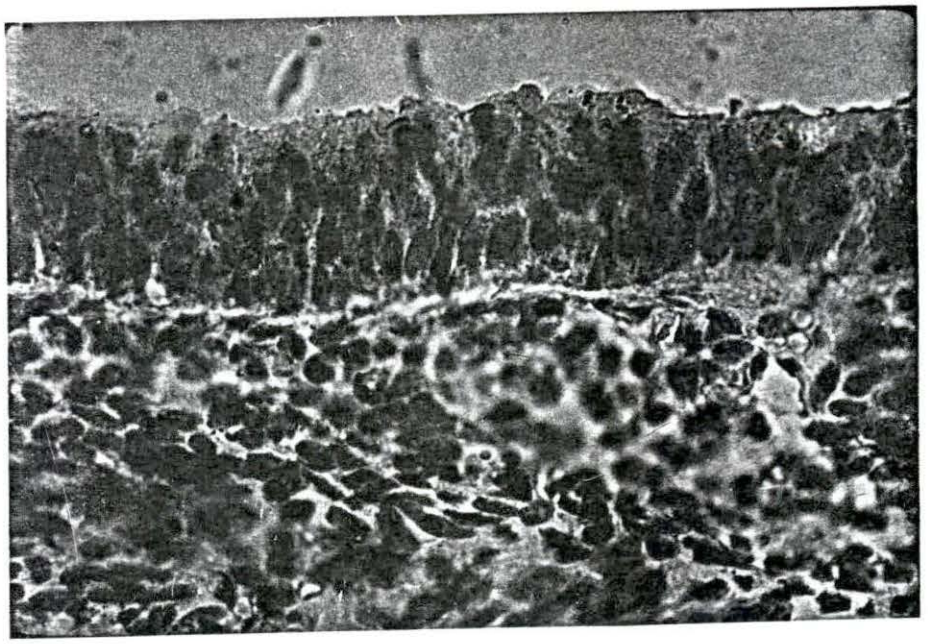


Fig. 29. Uterine surface epithelium 18 days post estrus. Cow 32, 7 years old. Oil red O staining positive material in cells. 420x magnification.

Fig. 30. Uterine surface epithelium 16 days post estrus. Cow 38, 8 years old. Oil red O staining. Positive material in cells. 420x magnification.



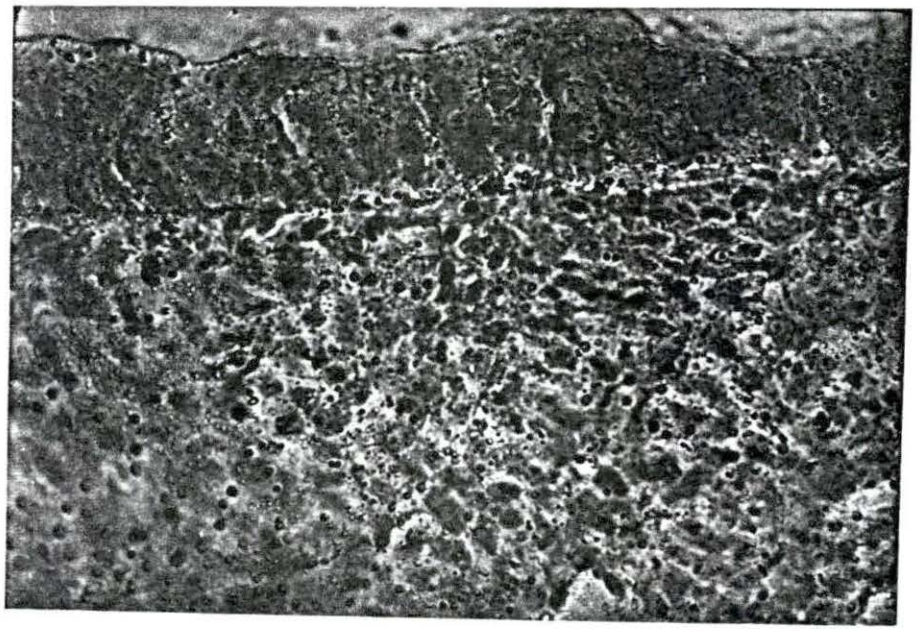


Fig. 31. Cervical epithelial cells during estrus. Cow 90, 4 years old. Stained with Hematoxylin-Eosin. 420x magnification.

Fig. 32. Cervical epithelial cells five days post estrus. Cow 12, 3 years old. Stained with Hematoxylin-Eosin. 420x magnification.

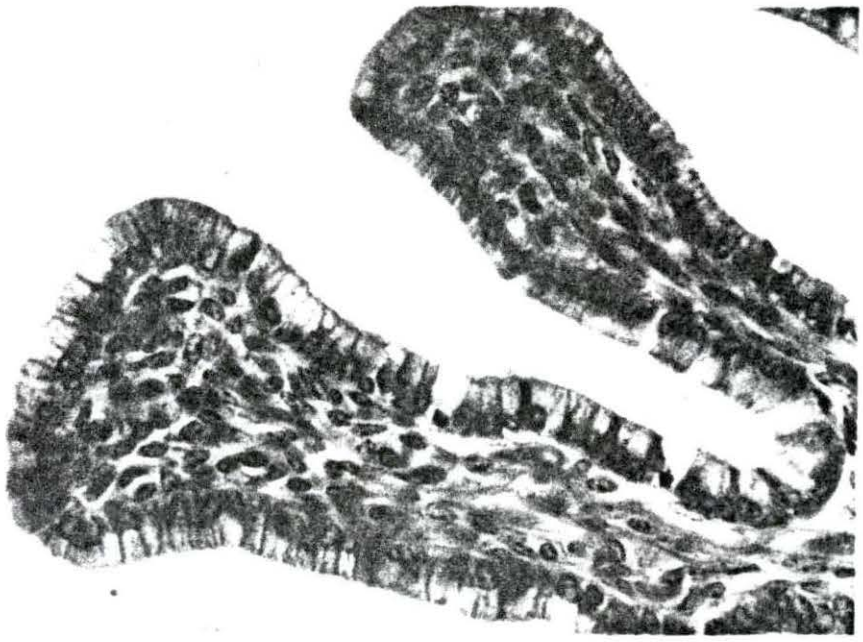


Fig. 33. Cervical epithelium 7 days post estrus. Cow 28, 6 years old. Stained with Hematoxylin and Eosin. 420x magnification.

Fig. 34. Cervical epithelium 10 days post estrus. Cow 94, 4 years old. Stained with Hematoxylin and Eosin 420x magnification.

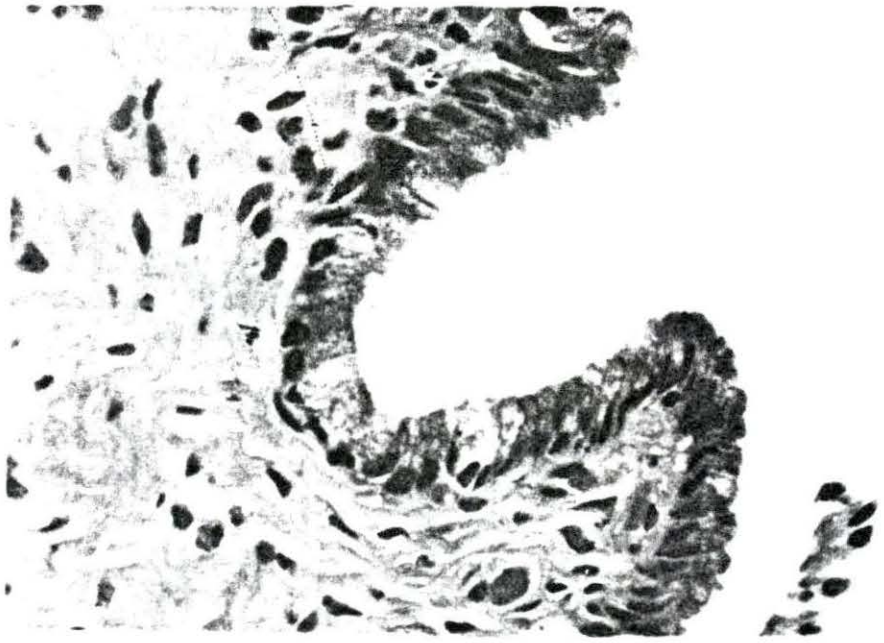
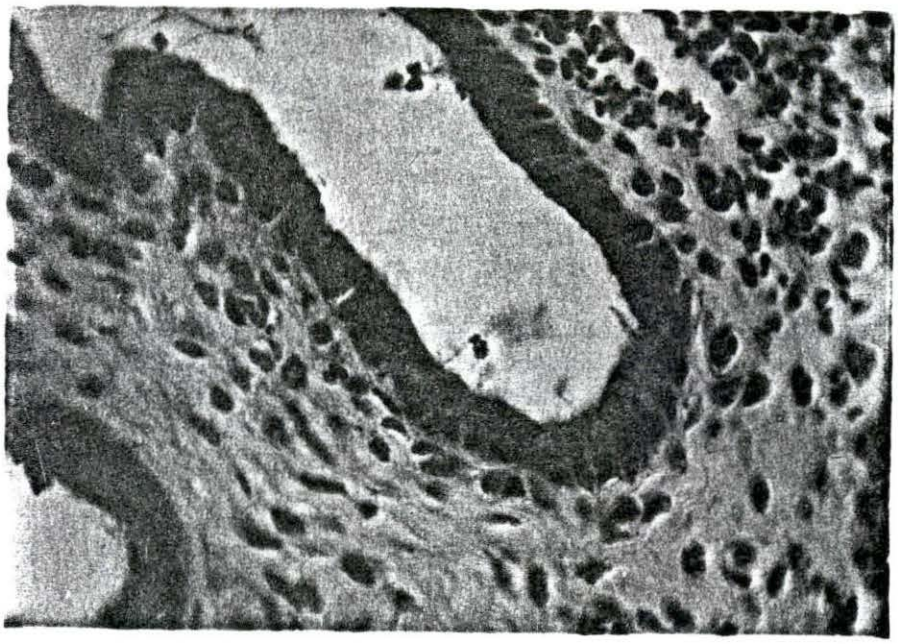


Fig. 35. Cervical epithelium 14 days post estrus. Cow 35, 6 years old. Stained with Hematoxylin and Eosin. 420x magnification.

Fig. 36. Cervical epithelium 17 days post estrus. Cow 33, 8 years old. Stained with Hematoxylin and Eosin. 420x magnification. Note dark ciliated cell in the epithelium.



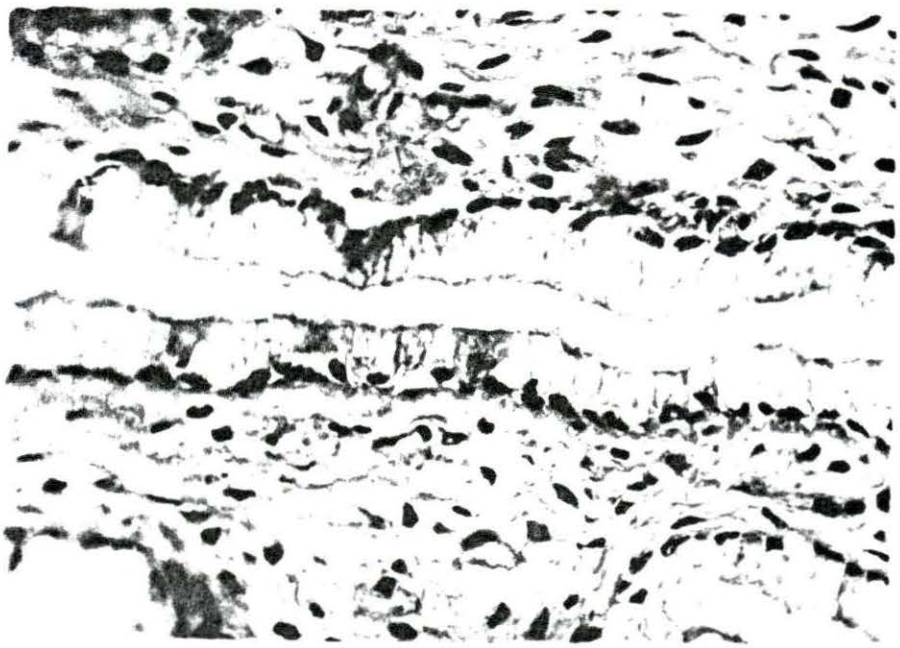


Fig. 37. Cervical epithelium 7days post estrus. Cow 28, 6 years old. Alkaline phosphatase preparation. 420x magnification. Note negative staining of the epithelium and the slight positive response of the connective tissue.

Fig. 38. Cervical epithelium 18 days post estrus. Cow 9, 3 years old. Alkaline phosphatase 420x magnification. Positive reaction in the distal part of cells and in connective tissue.

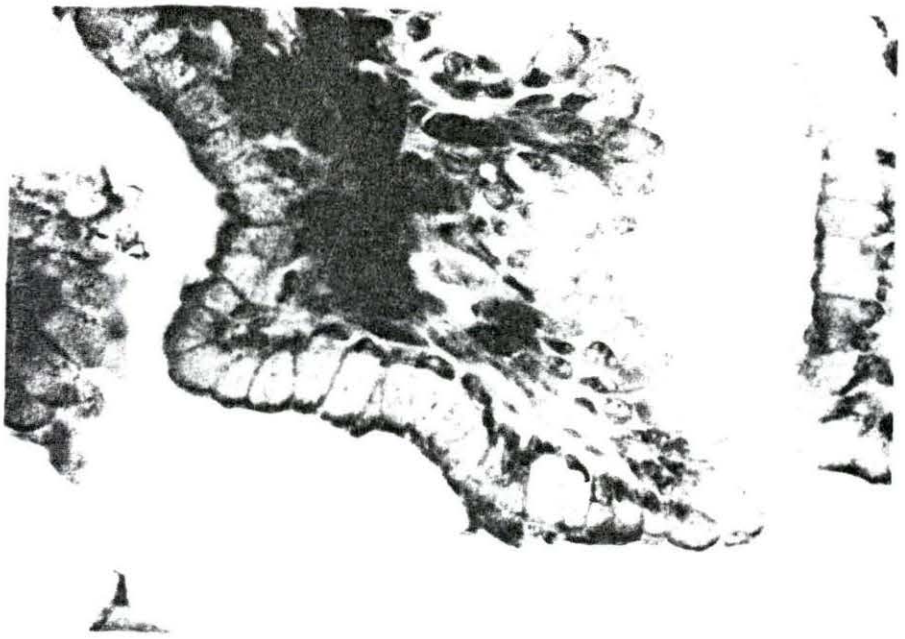
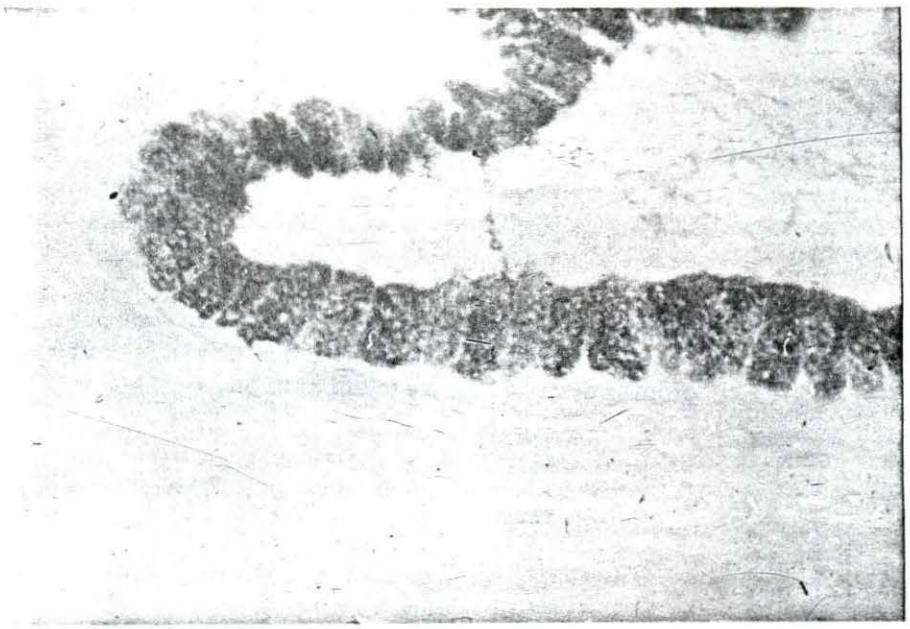


Fig. 39. Cervical epithelium during estrus. Cow 90, 4 years old. P.A.S. positive material in cells 420x magnification.

Fig. 40. Cervical epithelium day 14 post estrus. Cow 35, 6 years old. Less P.A.S. positive material in cells than during estrus.



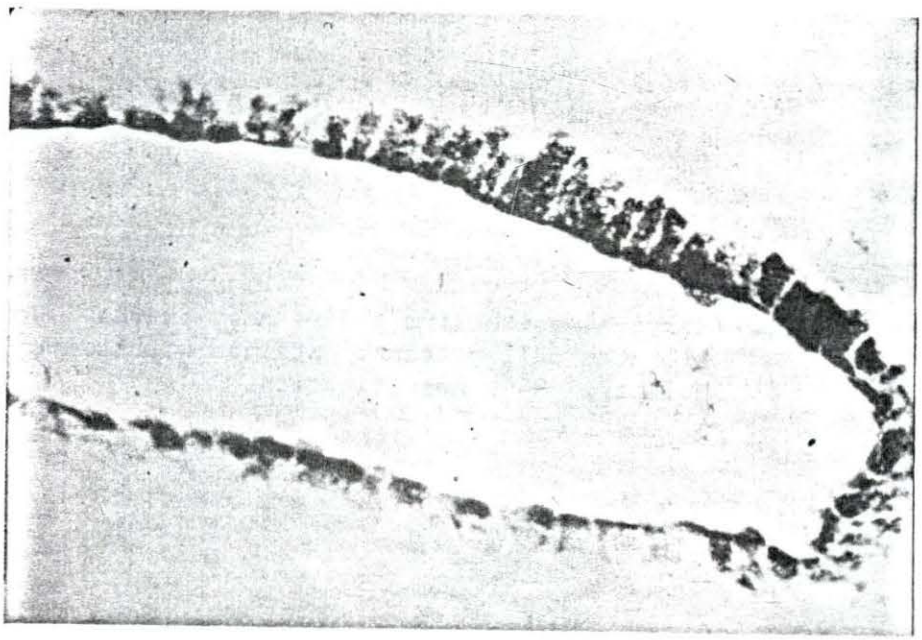
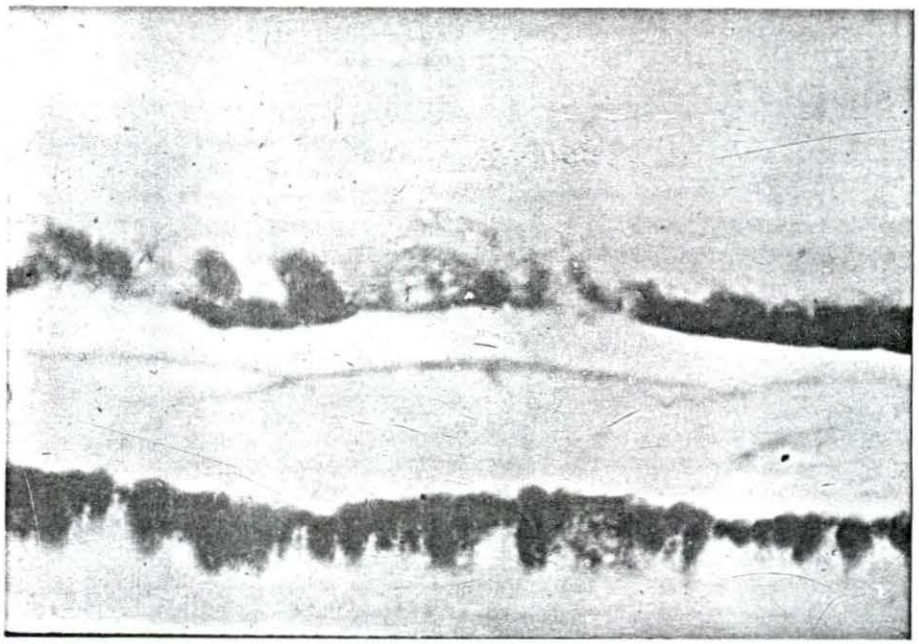


Fig. 41. Cervical epithelium 18 days post estrus. Cow 9, 3 years old. P.A.S. positive material in cells starts to increase in quantity. 420x magnification.

Fig. 42. Cervical epithelium 5 days post estrus. Cow 12, 3 years old. Ciliated cell present. Stained with tri-chrome method of Mallory. 960x magnification.



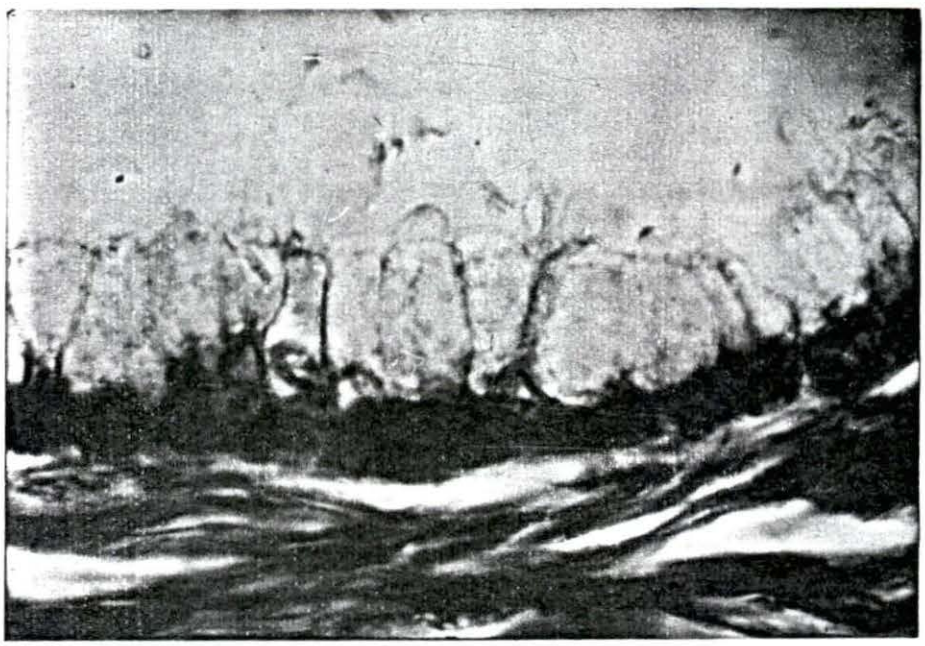


Fig. 43. Uterine surface epithelium at day 7. Cow 28, 6 years old. Magnification 10,000x. Pseudostratified columnar epithelium resting on basement membrane (Bm). Nucleus (N), Microvilli (Mv), and Lumen (L).

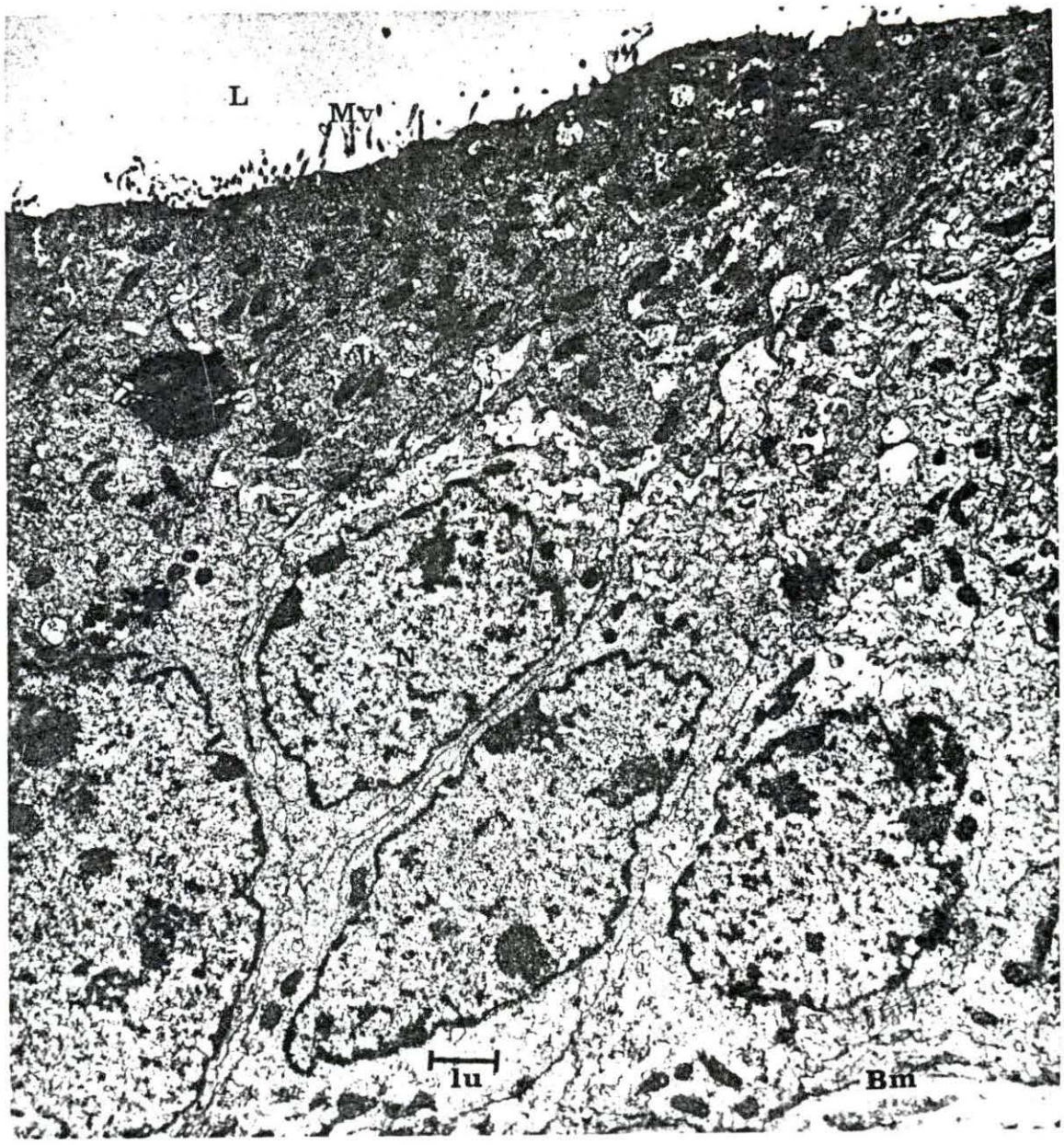
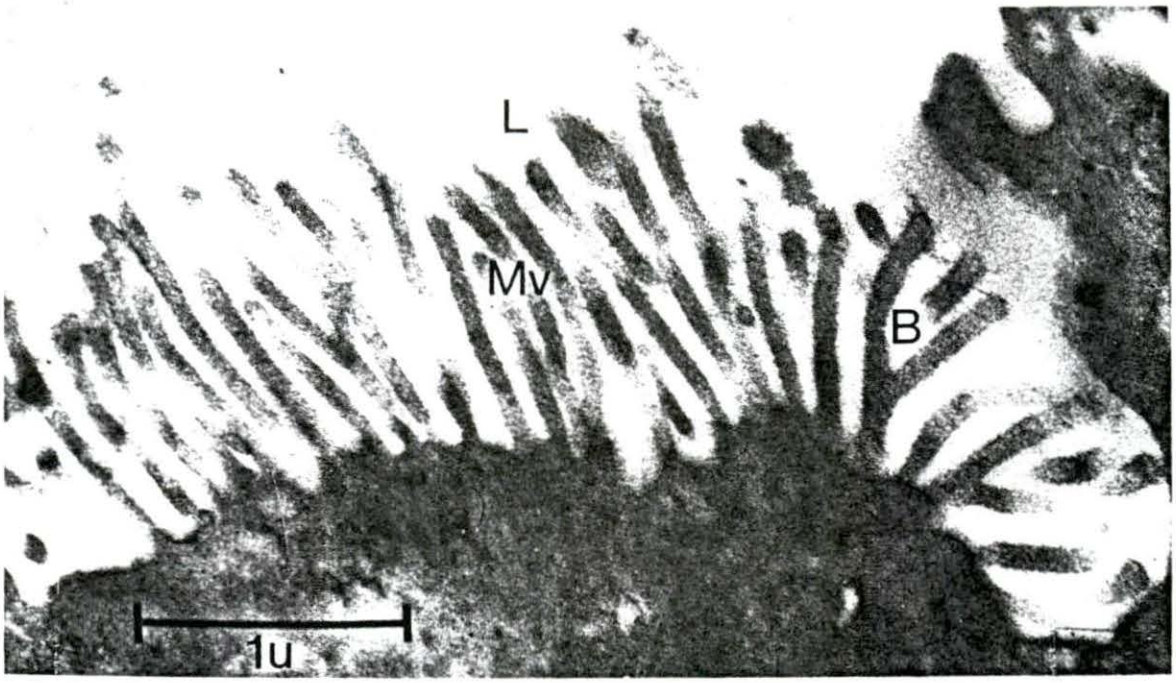

L
Mv
N
lu
Bm

Fig. 44. Uterine surface epithelium at estrus. Cow 34, 6 years old. Magnification 30,000x. Small numbers of long microvilli (Mv) present. A vacuole (V) of unknown origin can be observed in the cell. Mitochondria (Mi), cell membrane (Cm), desmosome (D).


Mv
V
Mi
Cm
D
1u

Fig. 45. Uterine surface epithelium at day one. Cow 93, 4 years old. Magnification 37,000x. Note the branching (B) of the microvilli (Mv). Lumen (L).

Fig. 46. Uterine surface epithelium at day 16. Cow 33, 8 years old. Magnification 37,000x. Microvilli (Mv), mitochondria (Mi).


L
Mv
B
1u

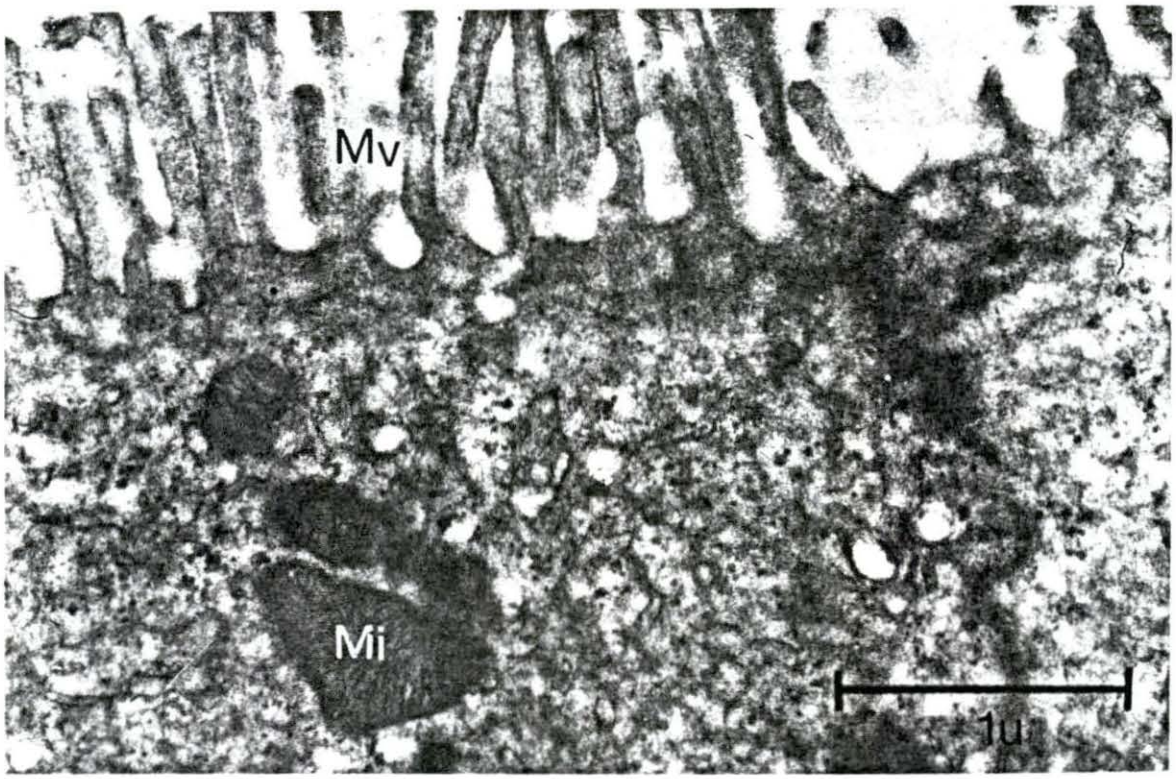

Mv
Mi
1u

Fig. 47. Uterine surface epithelium at day 13. Cow 30, 2 years old. Magnification 11,000x. Note the lack of microvilli. This is considered to be an artifact. Terminal bars (Tb), cell membrane (Cm), nucleus (N), mitochondria (Mi), lumen (L).

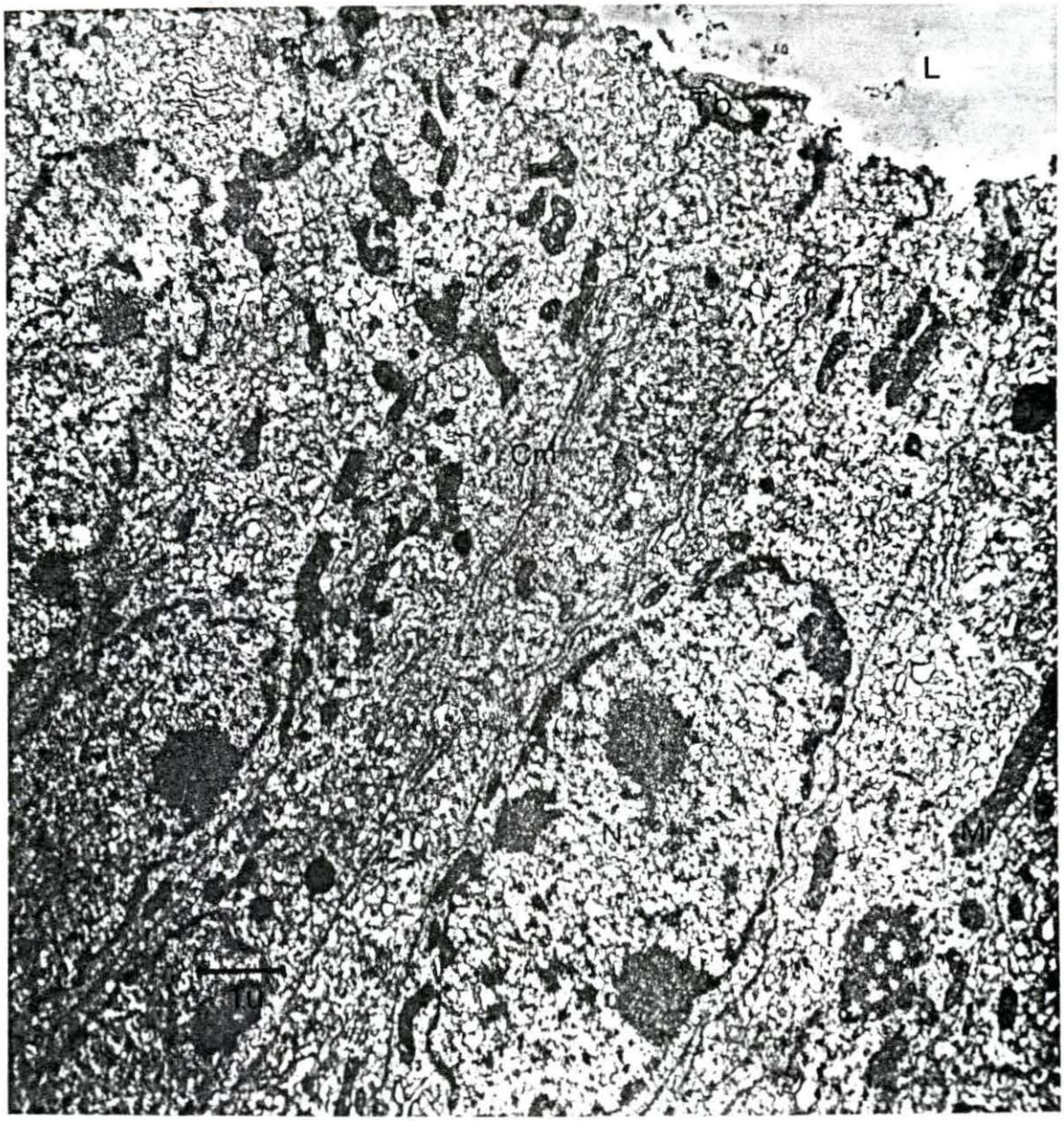

L
Cm
N
M
1U

Fig. 48. Uterine surface epithelium at day 13. Cow 30, 2 years old. Magnification 40,000x. Note the presence of ciliated cells (Ci) in the surface epithelium. Lumen (L), mitochondria (Mi), microvilli (Mv).


L
Mv
C
Mi

Fig. 49. Uterine surface epithelium at day 5. Cow 35, 6 years old. Magnification 15,000x. Note the presence of terminal bars (Tb) that extend across the cell. Cell membrane (Cm) interdigitation of adjacent cell membranes may also be observed. Lumen (L), mitochondria (Mi), nucleus (N), secretion (S), vacuole (V).

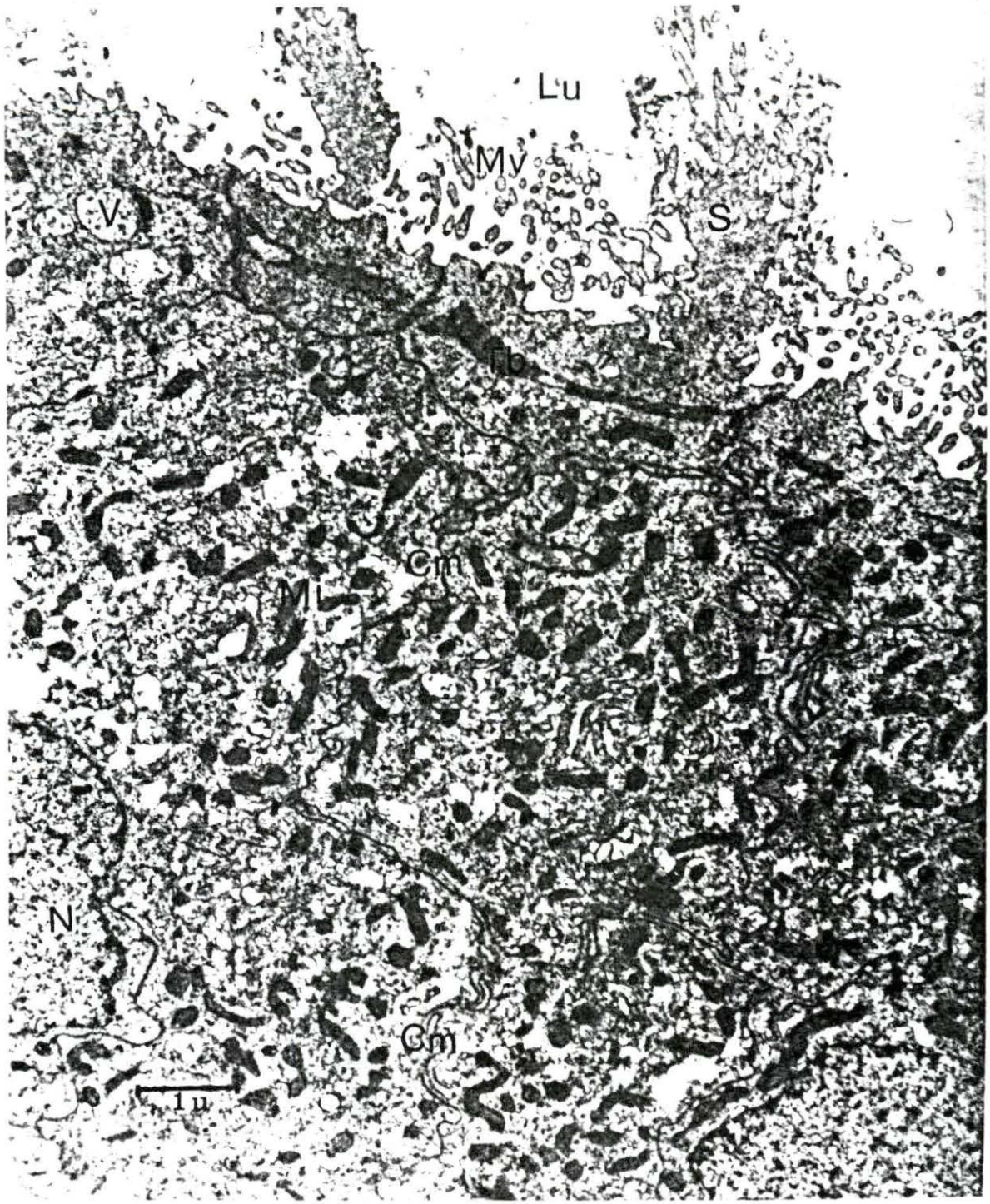

Lu
My
V
S
Tb
Cm
Mi
N
Cm
1 u

Fig. 50. Uterine surface epithelium at day five. Cow 35, 6 years old. Magnification 17,000x. Cell membrane (Cm), lumen (L), microvilli (Mv), and secretory mass (S) separating from the cell body.


L
MV
S
Tb
Cm
1u

Fig. 51. Uterine surface epithelium at day 10. Cow 31, 6 years old. Magnification 28,000x. Note two types of mitochondria (Mi) present. Cell membrane (Cm).

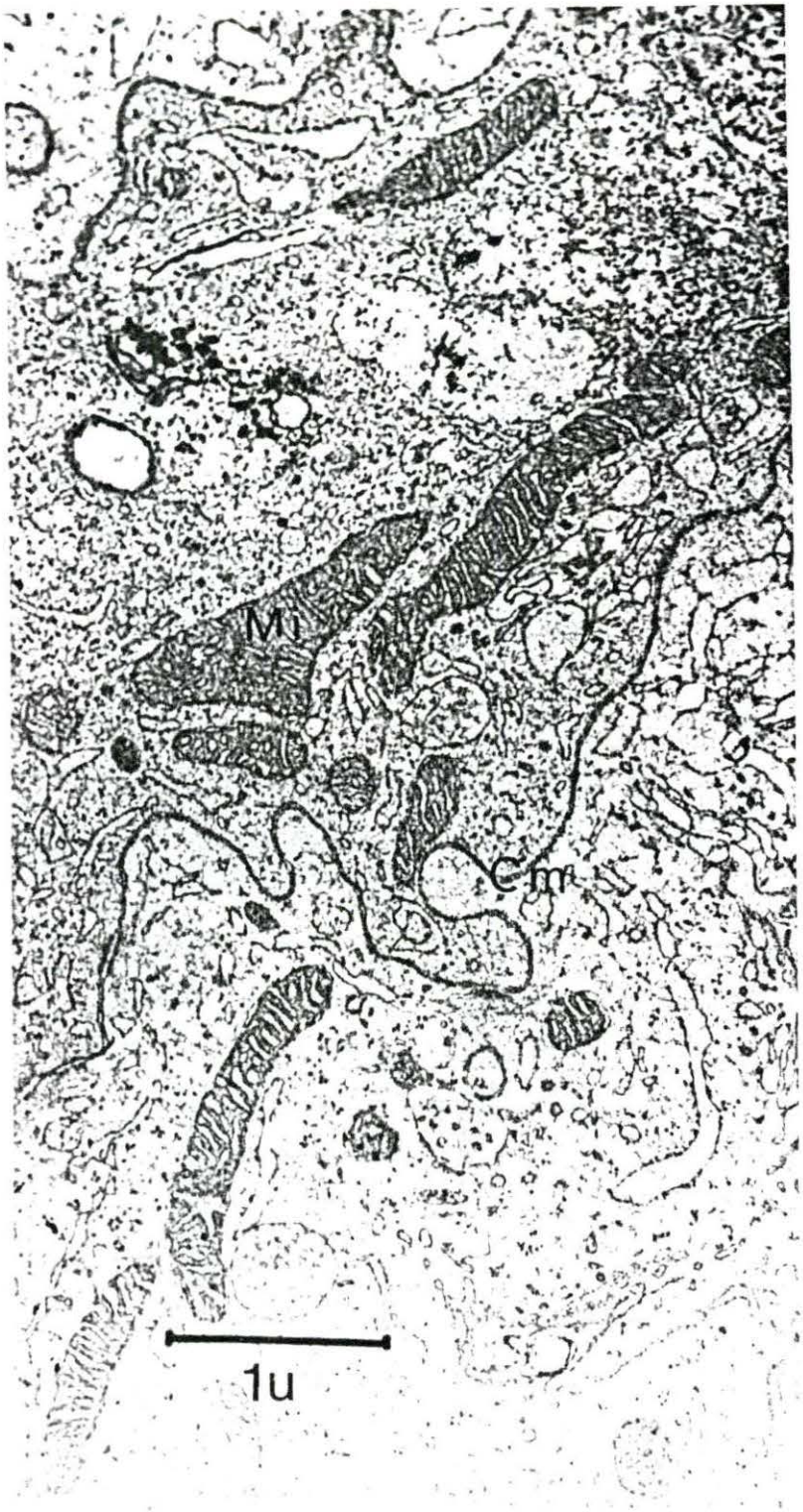

M
em
1u

Fig. 52. Uterine surface epithelium at day 13. Cow 30, 2 years old. Magnification 11,000x. Note the presence of lipid granules (Li). The cells are resting on a basement membrane (Bm) that is separated from the connective tissue, (Ct). Nucleus (N).

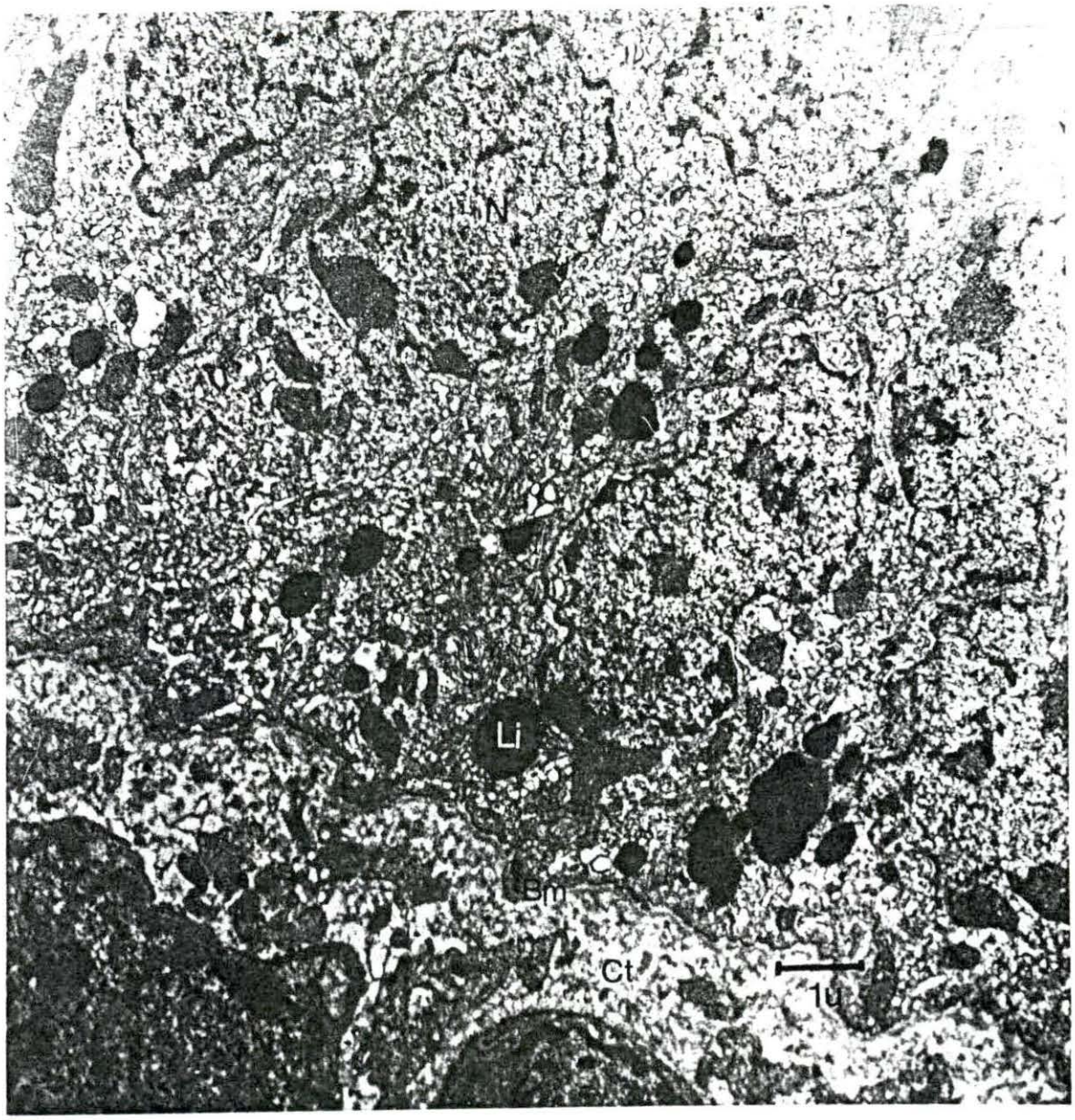

N
Li
Lsm
Ct
1u

Fig. 53. Uterine glandular epithelium at estrus. Cow 33, 8 years old. Magnification 10,000x. Cilia (Ci), cell membrane (Cm), endoplasmic reticulum (E), Golgi apparatus (G), mitochondria (Mi), Apocrine secretion process (S).

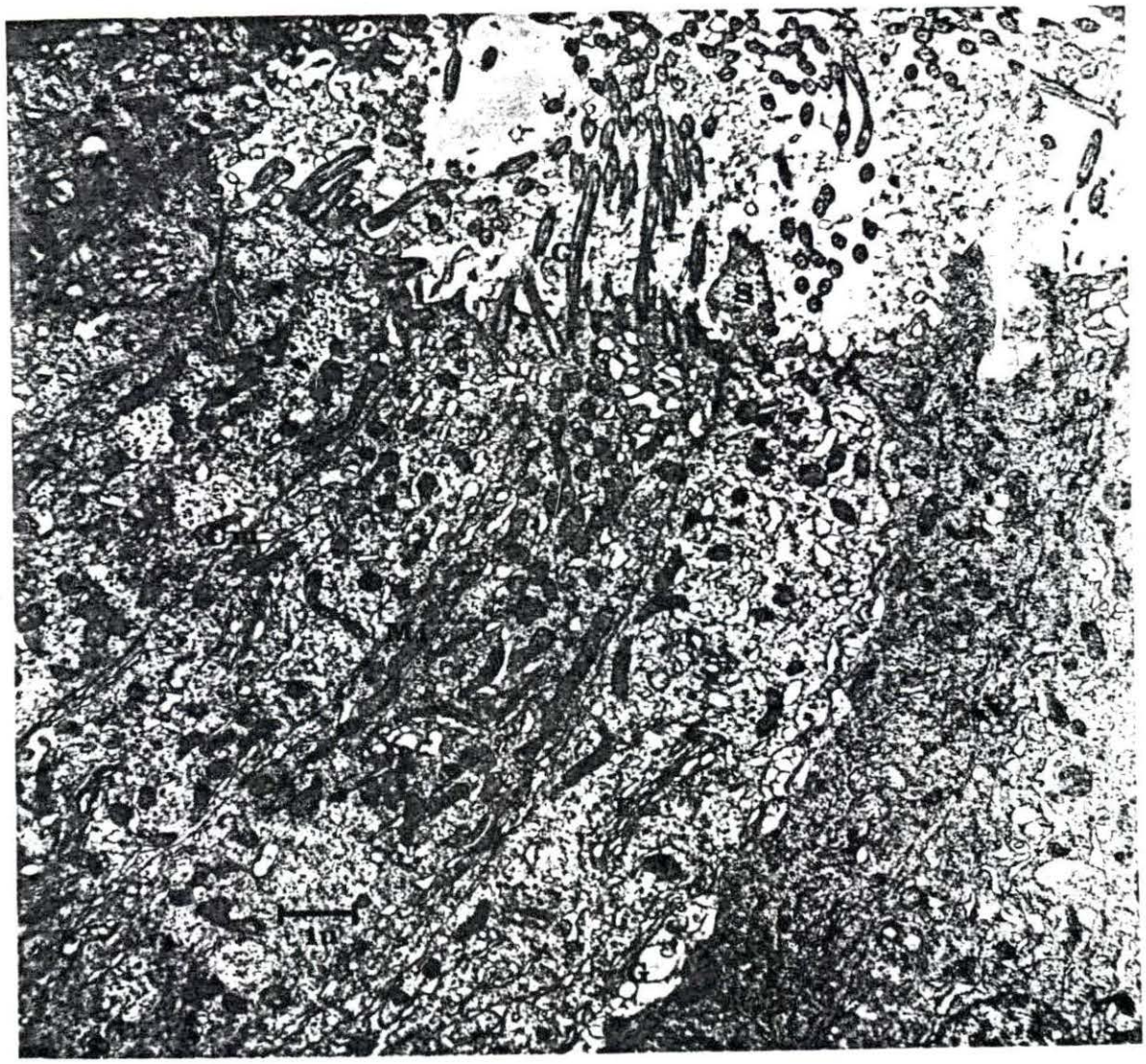

1μ

Fig. 54. Uterine glandular epithelium at day 16 of the cycle. Cow 33, 8 years old. Magnification 15,000x. Note the secretory material (S) in the lumen (L). Cilia (Ci), desmosome (D), terminal bars (Tb).

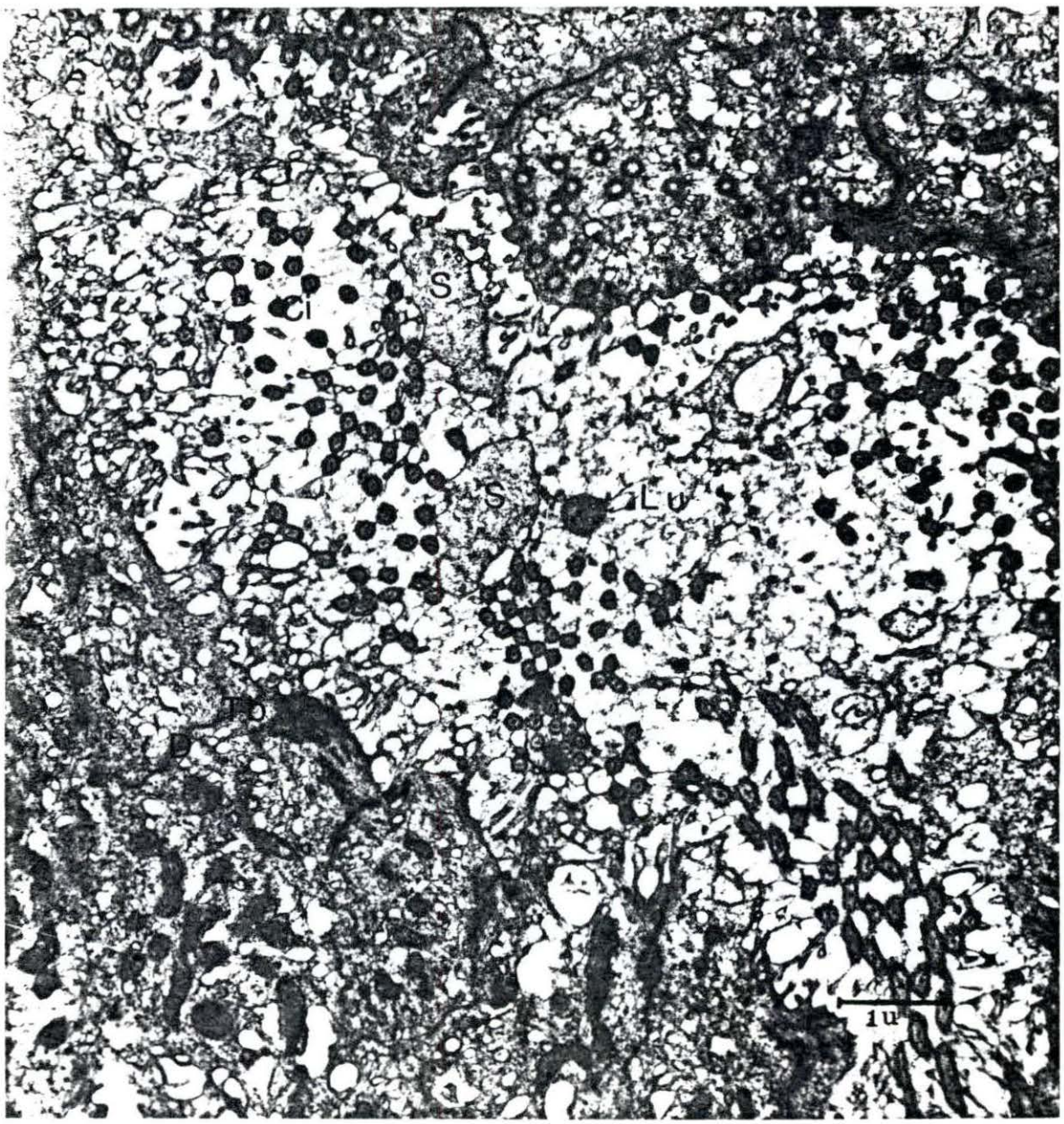
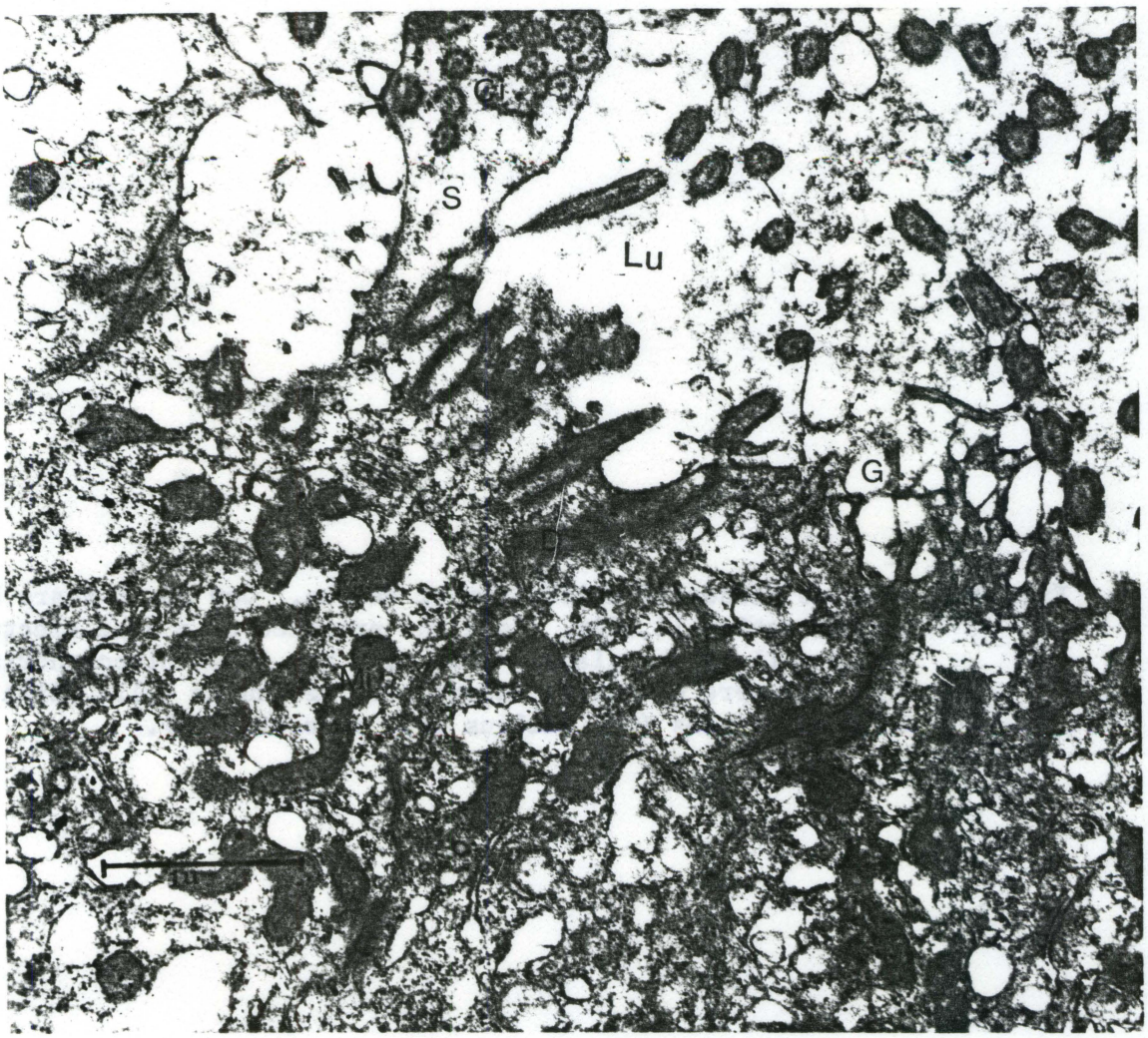

Ci
S
S
Lu
1u

Fig. 55. Uterine glandular epithelium at day 16 of the cycle. Cow 33, 8 years old. Note the breaking off of secretory material (S) into the lumen (Lu) and the presence of Golgi apparatus (G) in the secretion. Cross section of cilia (Ci) can be observed in the secretory mass. Desmosomes (D) and mitochondria (Mi).


S
Lu
G

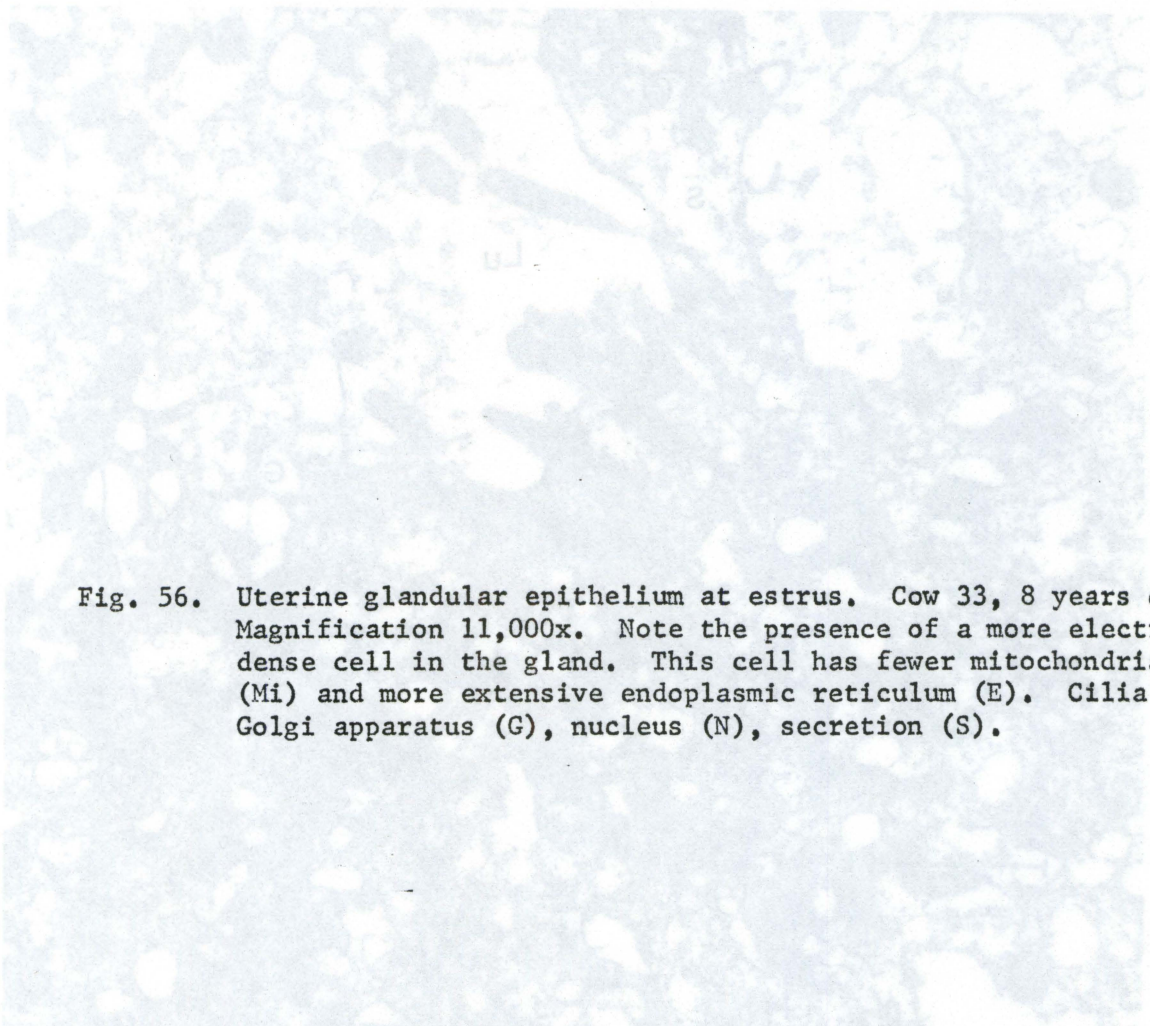


Fig. 56. Uterine glandular epithelium at estrus. Cow 33, 8 years old. Magnification 11,000x. Note the presence of a more electron dense cell in the gland. This cell has fewer mitochondria (Mi) and more extensive endoplasmic reticulum (E). Cilia (Ci) Golgi apparatus (G), nucleus (N), secretion (S).

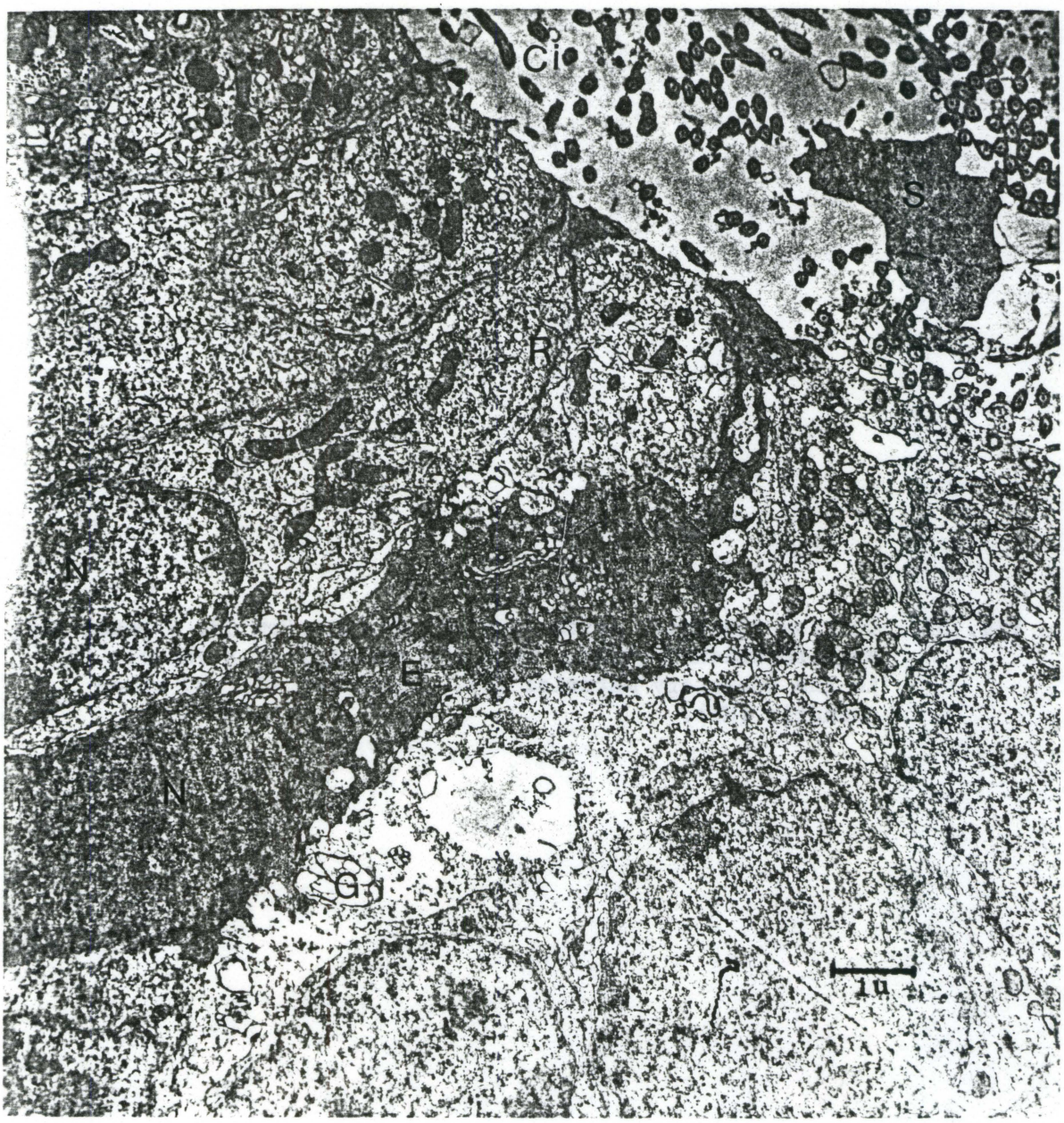

Ci
S
R
N
E
N
G
1u

Fig. 57. Uterine glandular epithelium at day 18. Cow 33, 8 years old. Magnification 10,000x. Note the presence of a vacuole (V) in the secretory material (S). Desmosome (D) and mitochondria (Mi).

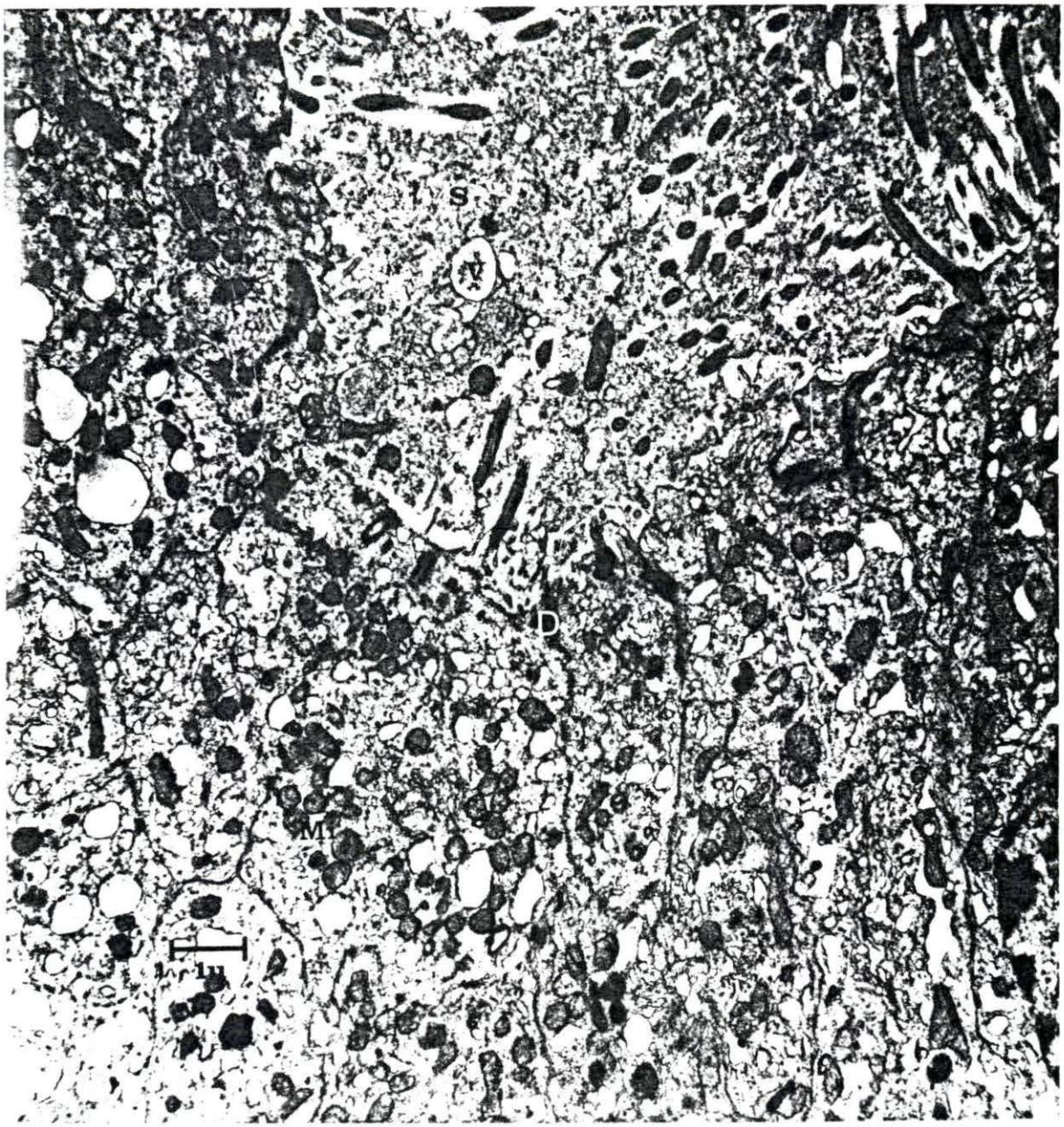

S
N
D
M
I
A

Fig. 58. Uterine glandular epithelium at day 18. Cow 33, 8 years old. Magnification 10,000x. The lumen of the gland is filled with secretion (S). Terminal bars (Tb) are present. Golgi apparatus (G), nucleus (N), cilia (Ci).

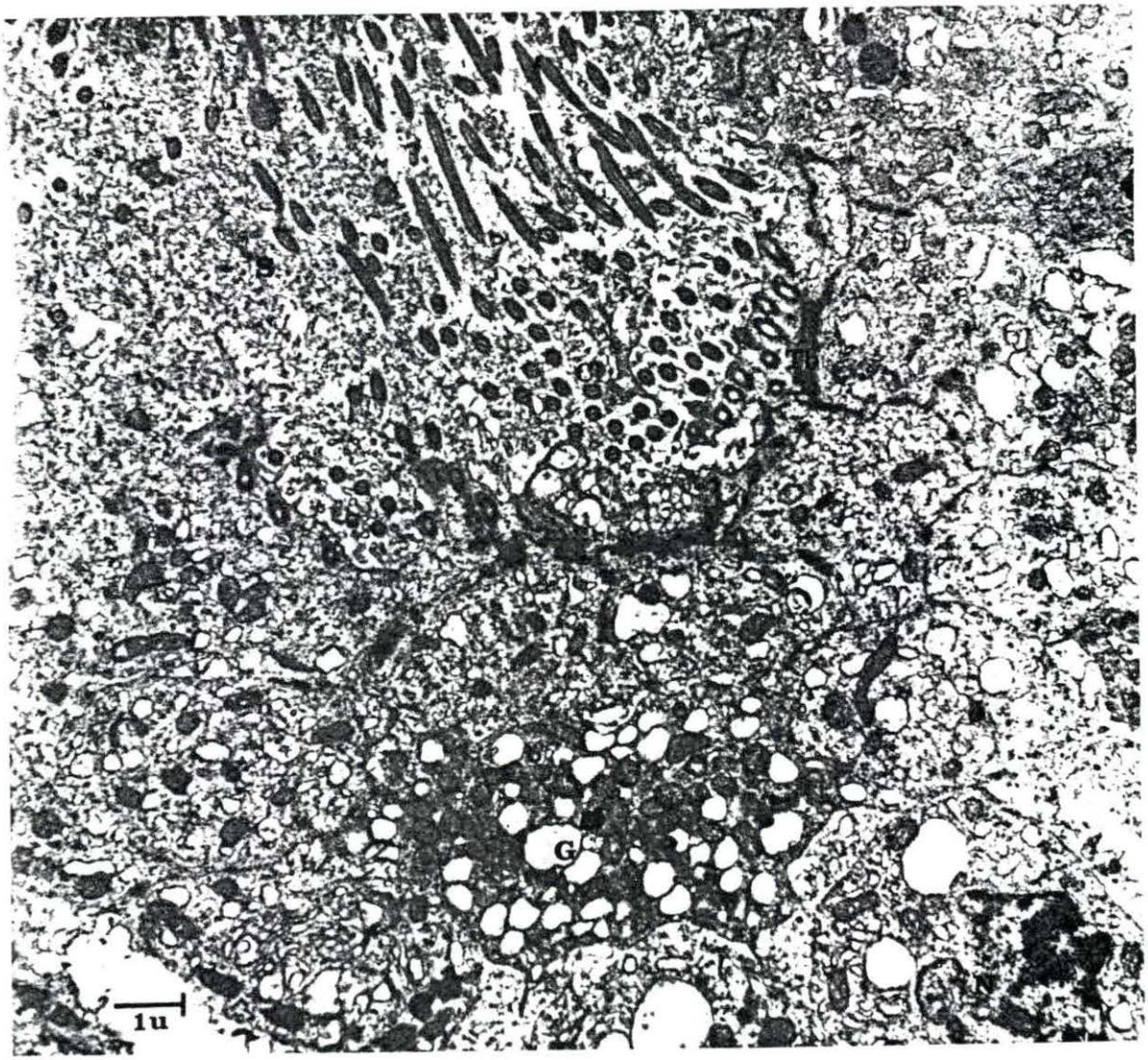

Tb
G
1u

Fig. 59. Uterine surface epithelium at day 2. Cow 29, 6 years old. Magnification 10,000x. Cell membrane (CM), lumen (L), mitochondria (Mi), microvilli (Mv), nucleus (N).

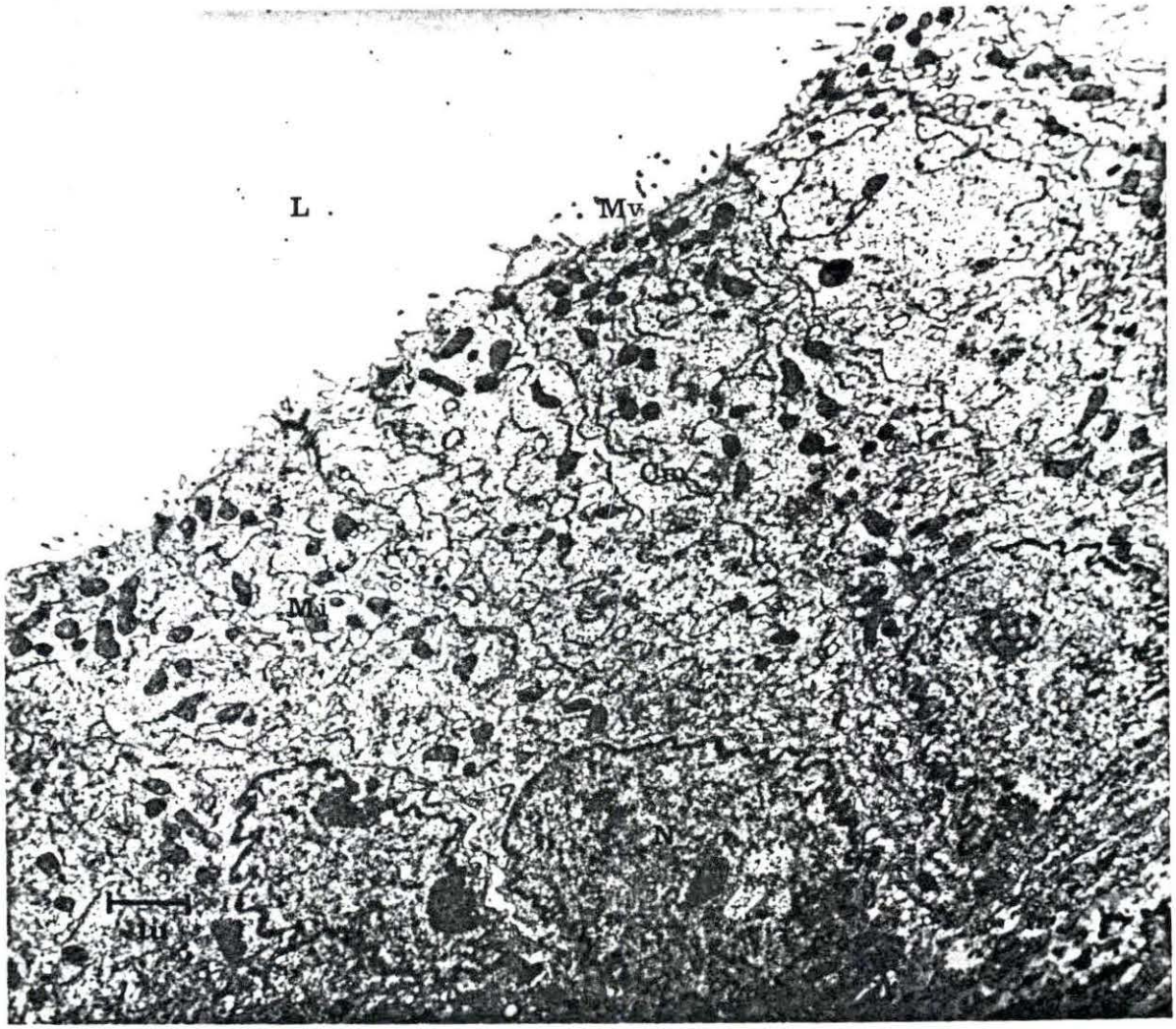

L
Mv
Mi
N
1μ

Fig. 60. Uterine surface epithelium at day 7. Cow 28, 6 years old. Magnification 10,000x. Note the intercellular channels (C) extending toward the lumen (L). Basement membrane (Bm), cell membrane (Cm), microvilli (Mv).

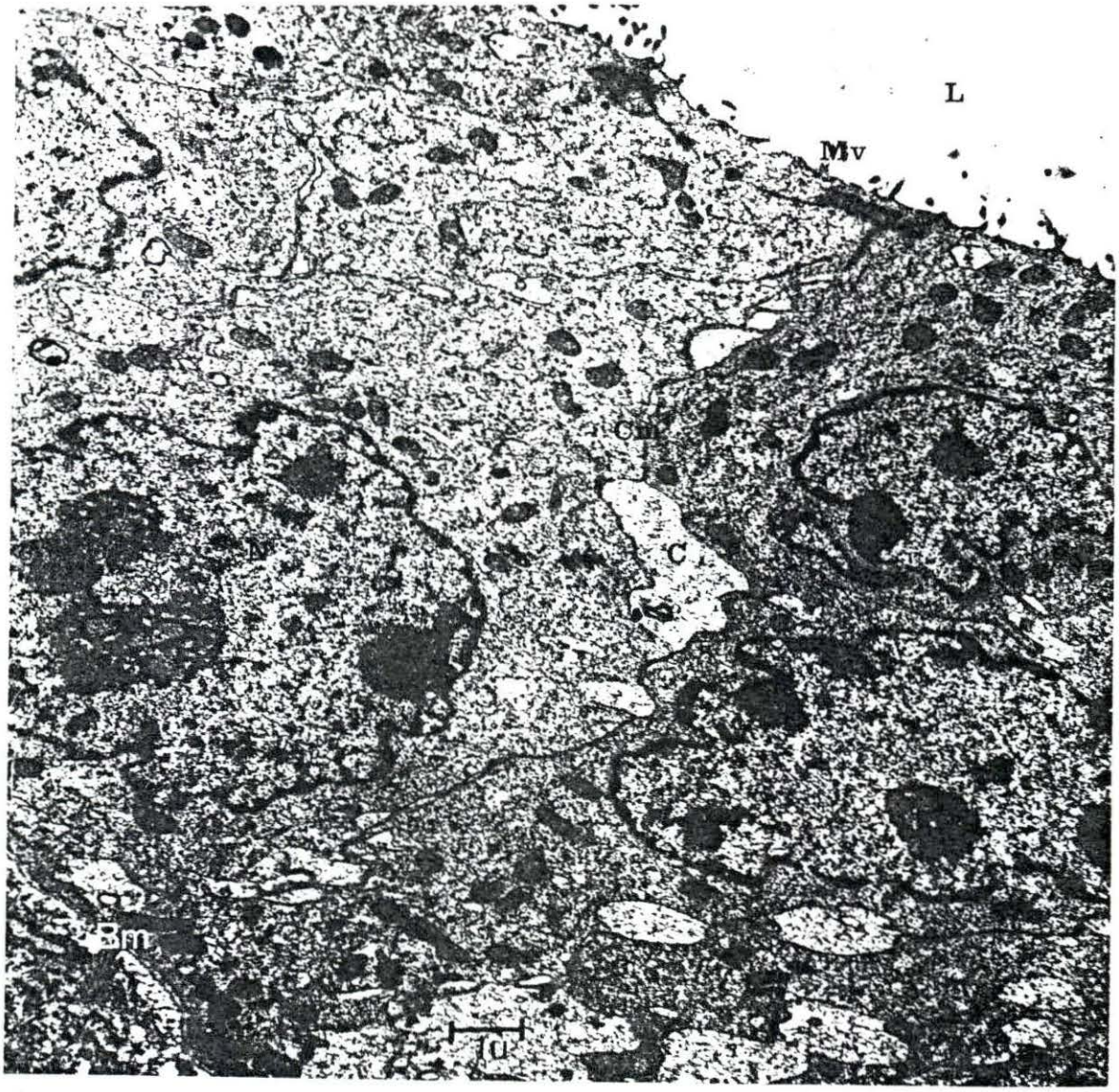

L
Mv
Cm
C
Bm
1u

Fig. 61. Uterine surface epithelium at day 18. Cow 32, 7 years old. Magnification 30,000x. Cell membrane (Cm), lumen (L), mitochondria (Mi), microvilli (Mv).

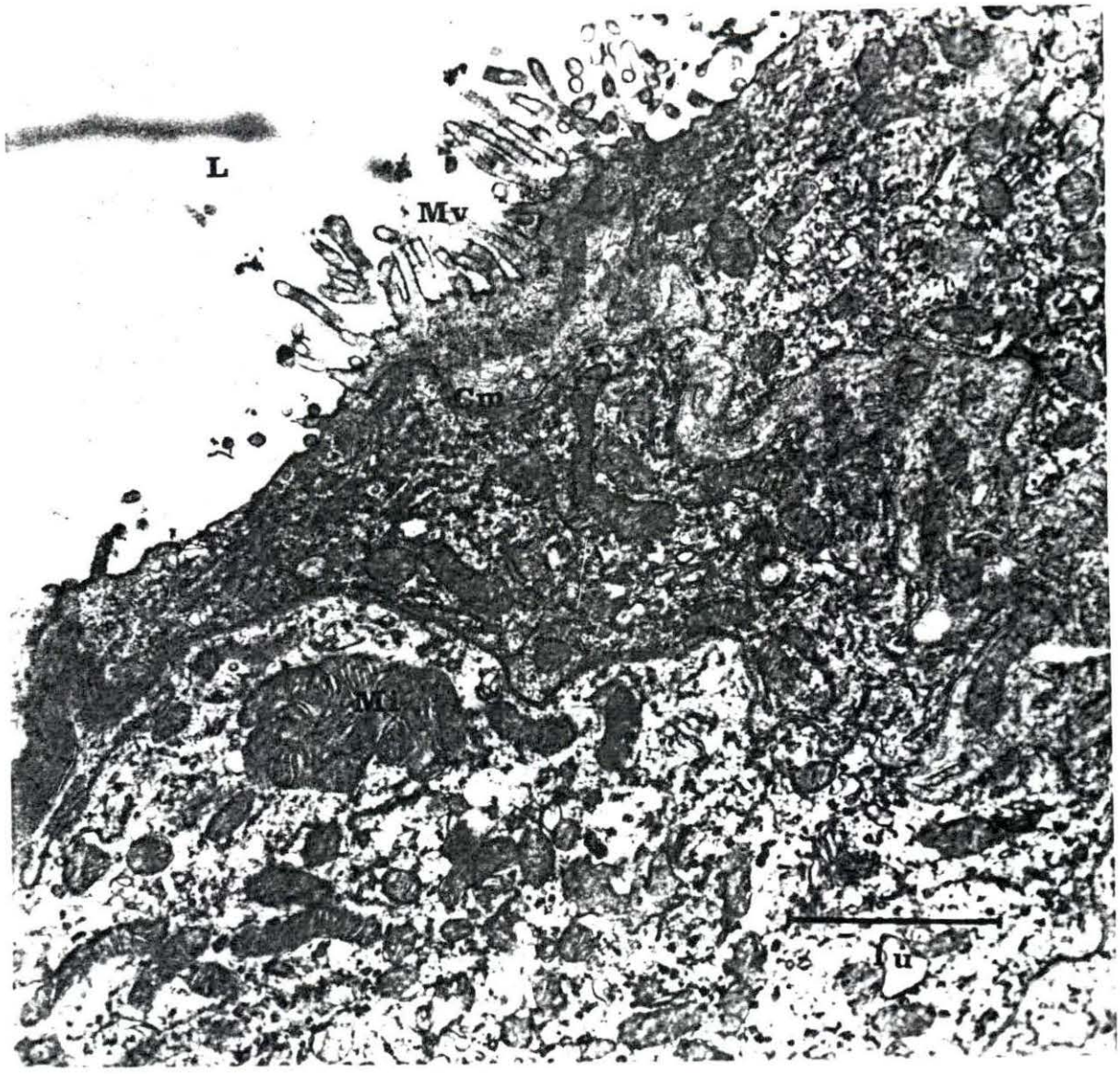

L
Mv
Gm
Mi
u

Fig. 62. Uterine surface epithelium at day 20. Cow 34, 6 years old. Microvilli (Mv) are seen projecting into the lumen (L). Mitochondria (Mi) are present. Magnification 20,000x. Vacuole (V), cell membrane (Cm).


L
Mv

Fig. 63. (Left) Uterine surface epithelium at day 7. Cow 28, 6 years old. Magnification 8,000x. A clear cell can be observed next to the basement membrane (Bm). Channels (C) are seen between the cells.

Fig. 64. (Right) Uterine surface epithelium at day 7. Cow 28, 6 years old. Magnification 13,000x. A leucocyte (Le) is present next to the basement membrane (Bm). Channels (C) are seen between cells.

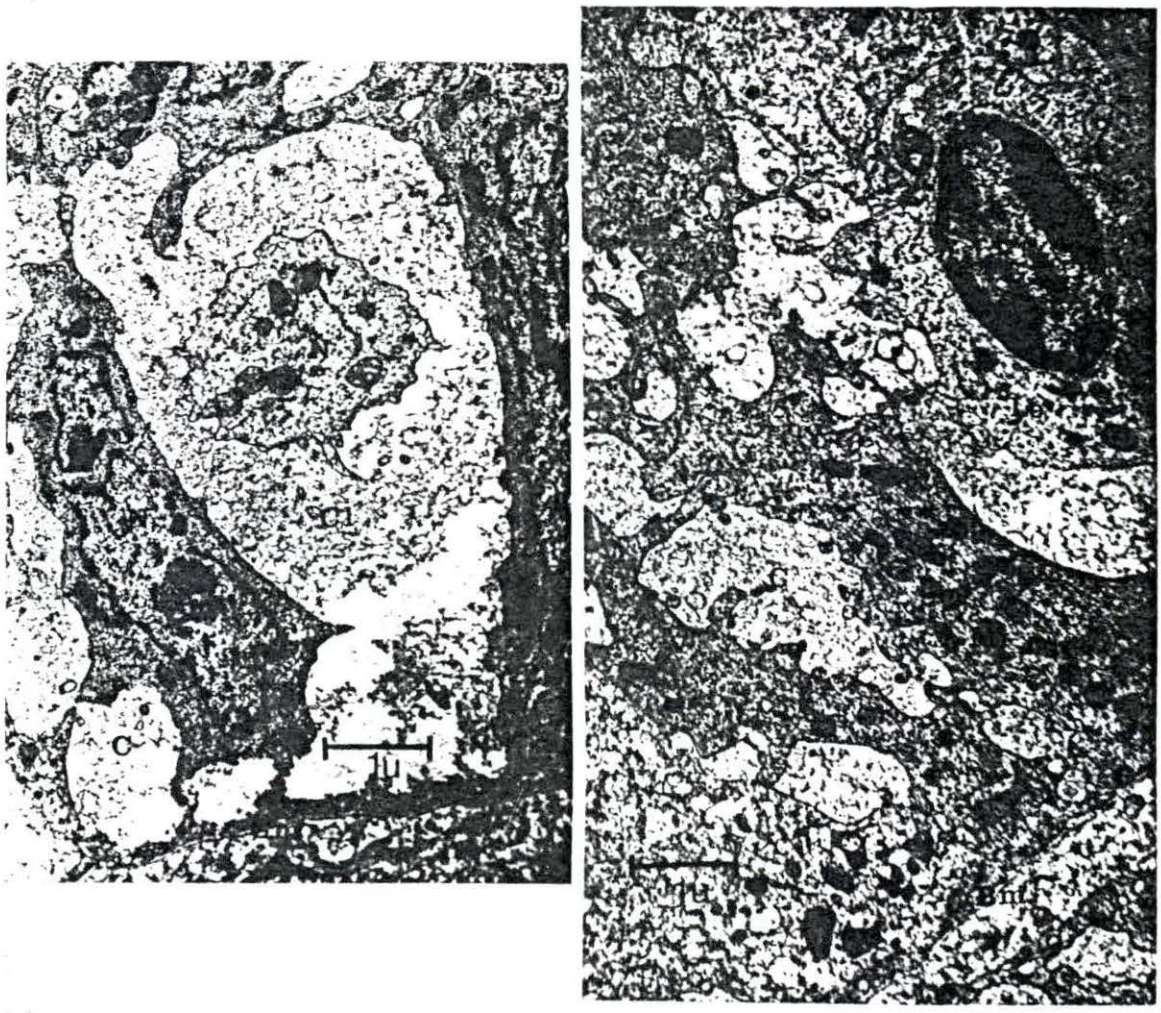

C
1μ
Bm
1μ

Fig. 65. Uterine glandular epithelium at day 5. Cow 35, 6 years old. Magnification 23,000x. Cilia (Ci), and cell membrane (Cm).


M
Cm
1u

Fig. 66. Cervical epithelium at day 7. Cow 38, 6 years old. Magnification 14,000x. A ciliated cell is between two secretory cells. Cilia (Ci), cell membrane (Cm), desmosome (D), mucous granules (M), mitochondria (Mi), nucleus (N), vacuole (V).

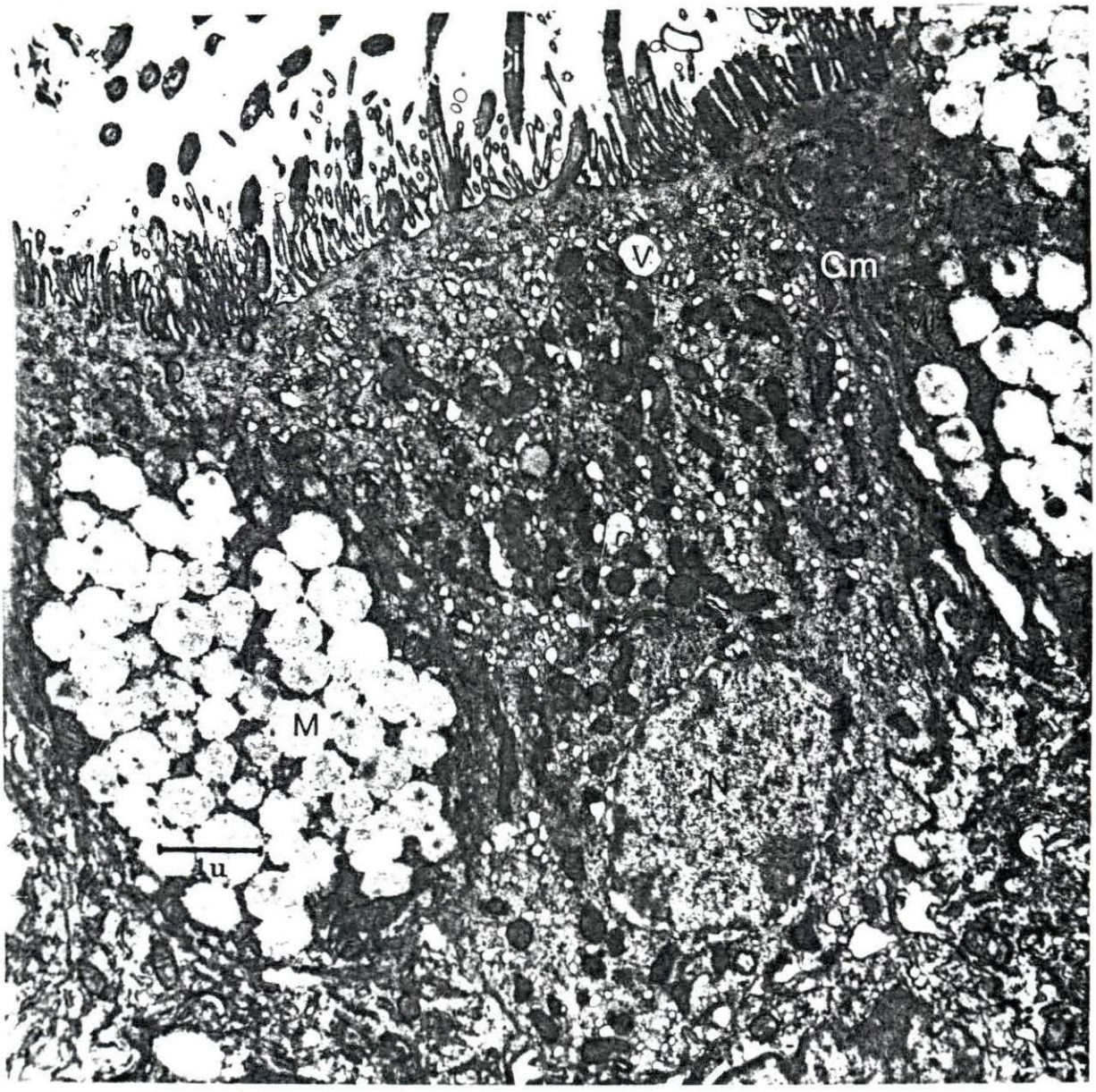

V
Cm
M
N
1u

Fig. 67. Cervical epithelium at day 7. Cow 28, 6 years old. Magnification 20,000x. Cilia (Ci) are observed in cross section. Desmosome (D), mucous (M), mitochondria (Mi), microvilli (Mv), vacuole (V).

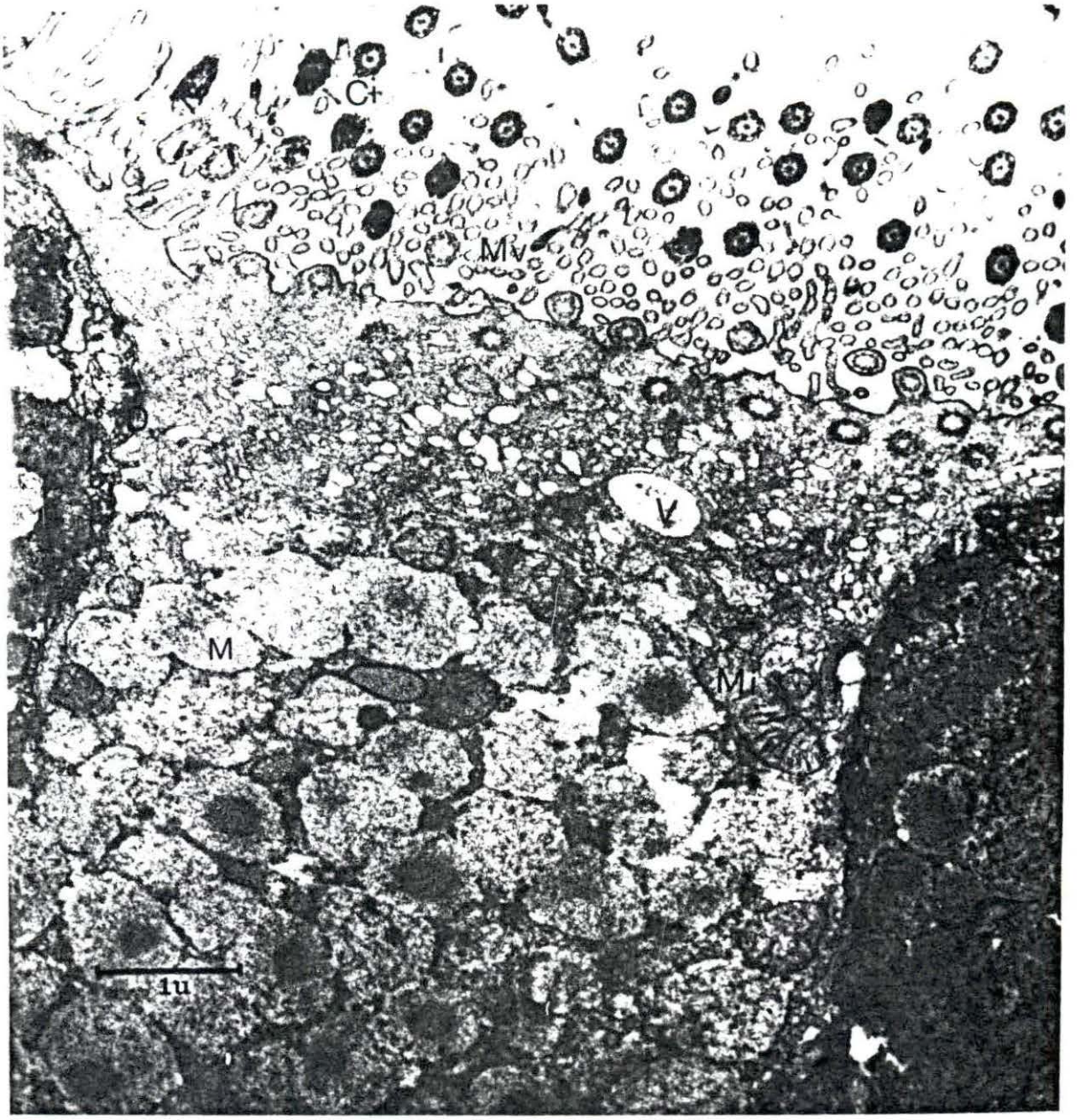

Ct
Mv
V
M
Mi
1u

Fig. 68. Cervical epithelium at estrus. Cow 31, 6 years old. Magnification 13,000x. Secretory cells full of mucous granules (M). Microville (Mv), nucleus (N).

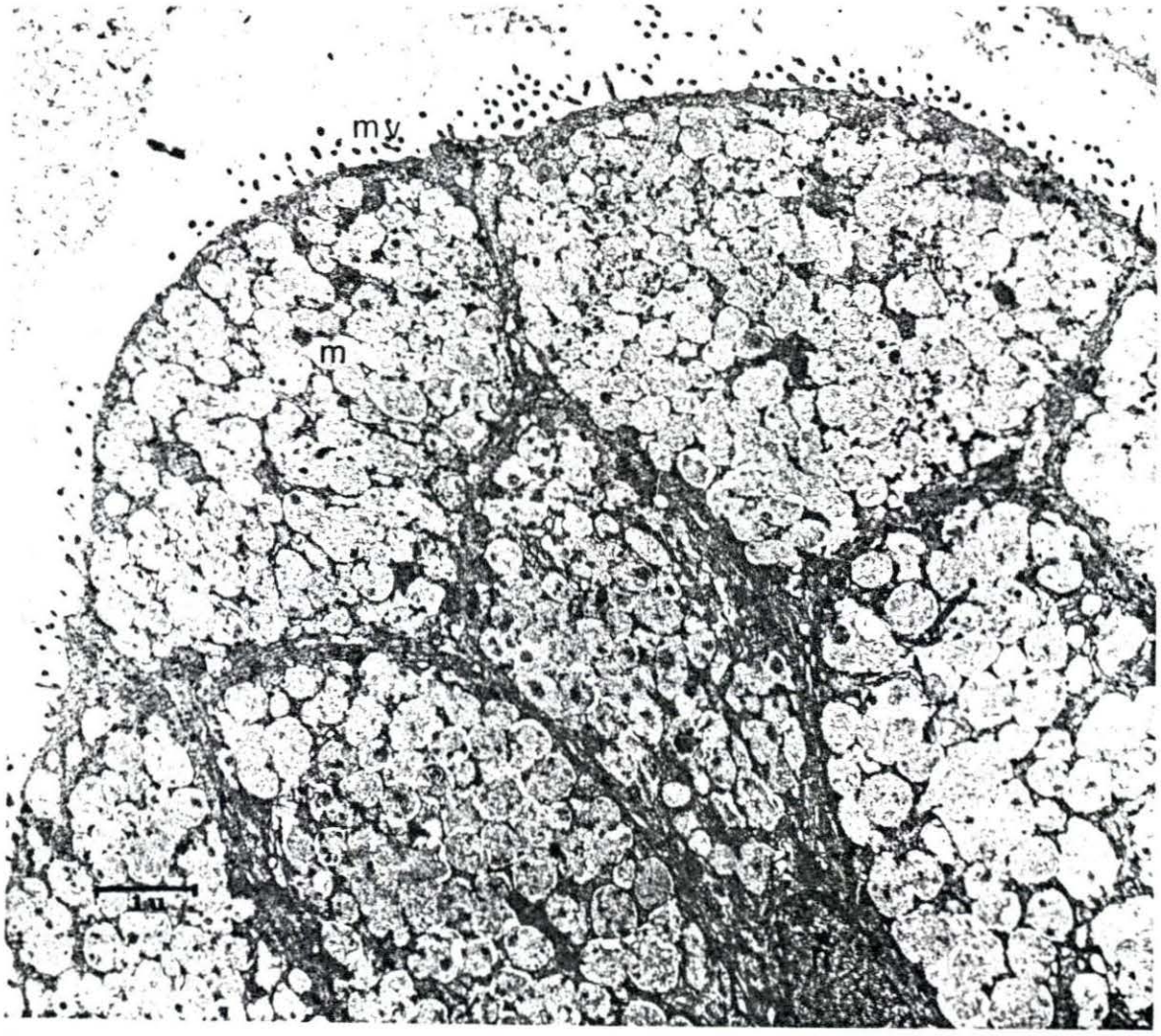
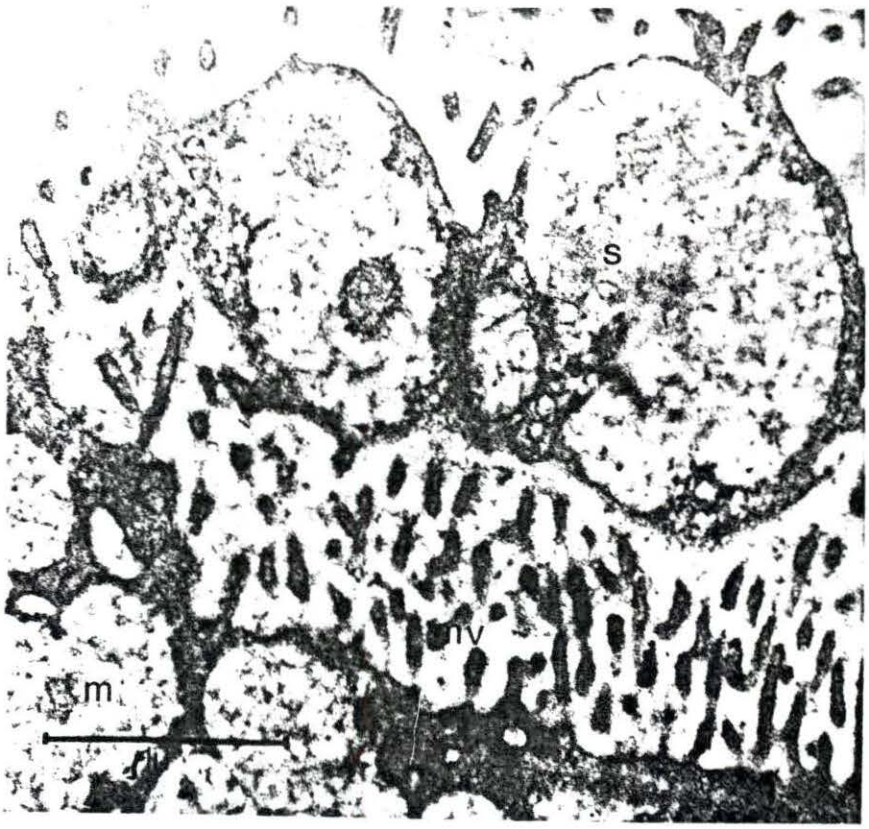

my
m
1μ

Fig. 69. Cervical epithelium at estrus. Cow 93, 4 years old. Magnification 13,000x. Some of the secretory granules (M) have an electron dense center. The nuclei (N) are small and pushed toward the basement membrane.


M
N
1μ
N

Fig. 70. Cervical epithelium at estrus. Cow 93, 4 years old. Magnification 31,000x. Membrane bound secretory granules (S) are present in the lumen. Microvilli (Mv), mucus (M).

Fig. 71. Cervical epithelium at day 10. Cow 31, 6 years old. Secretion process in one cell (arrow). Endoplasmic reticulum (E), mucus (M), mitochondria (Mi), microvilli (Mv), nucleus (N).


s
mv
m

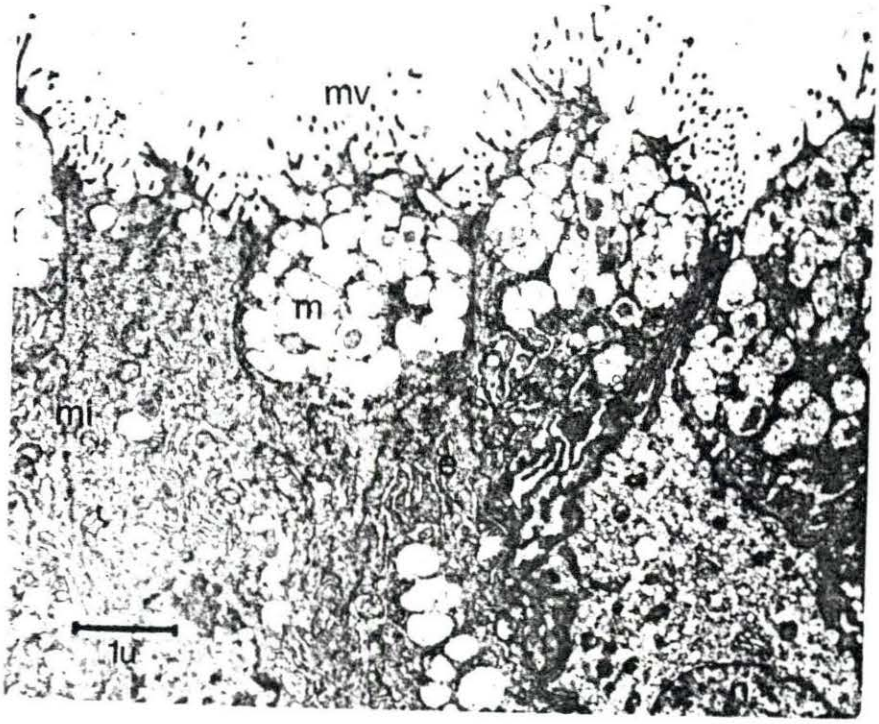

mv
m
ml
e
1u

Fig. 72. Cervical epithelium at 7 days post estrus. Cow 28, 6 years old. Magnification 15,000x. Mucous granules (M) and cilia (Ci) are noted. Breaks in the plasma membrane allow mucous granules to enter the lumen.

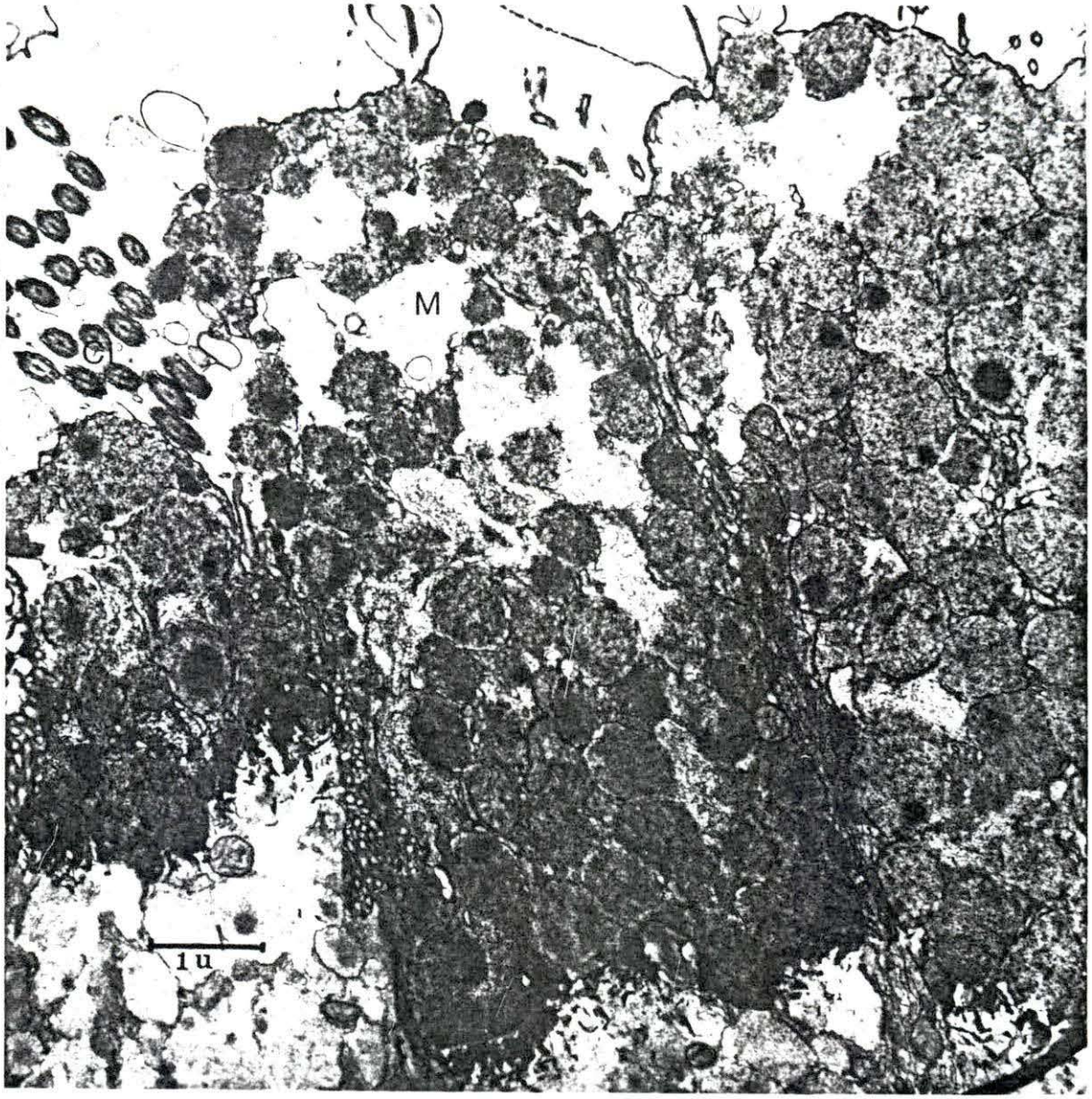

M
1u

Fig. 73. Cervical epithelium at day 3 of the cycle. Cow 90, 4 years old. Magnification 12,000x. Mucous cell is nearly empty. Some mucous (M) is present, however, the cell has an abundance of endoplasmic reticulum (E) and Golgi apparatus (G). Ciliated cells have cilia (Ci), a small number of mitochondria and Golgi.

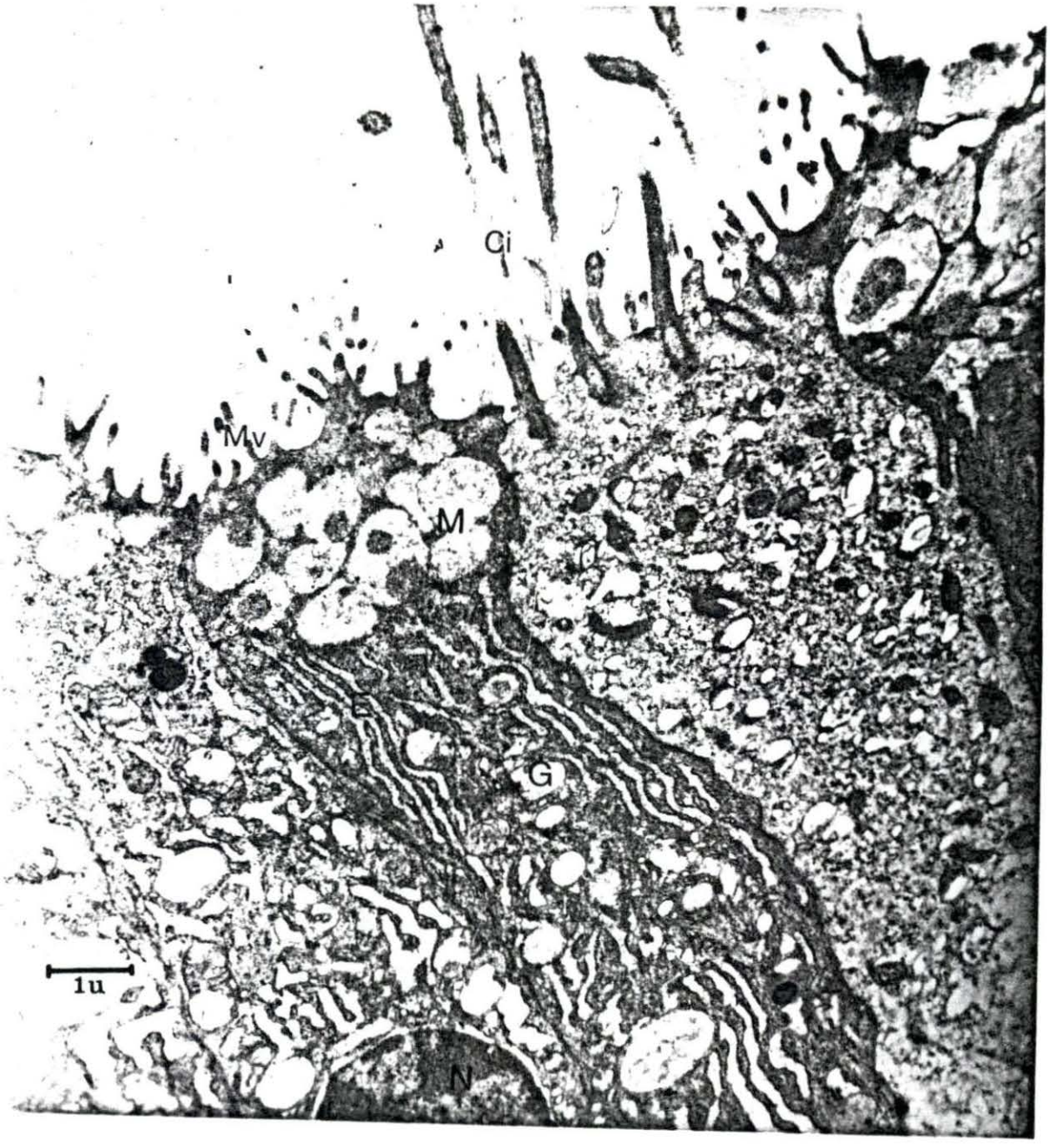

Ci
Mv
M
G
1u
N

Fig. 74. Cervical epithelium at day 7. Cow 28, 6 years old. Secretory mucous granules (M) are present and mitochondria (Mi) are scattered throughout the cells and near the basement membrane (Bm). Magnification 13,000x.

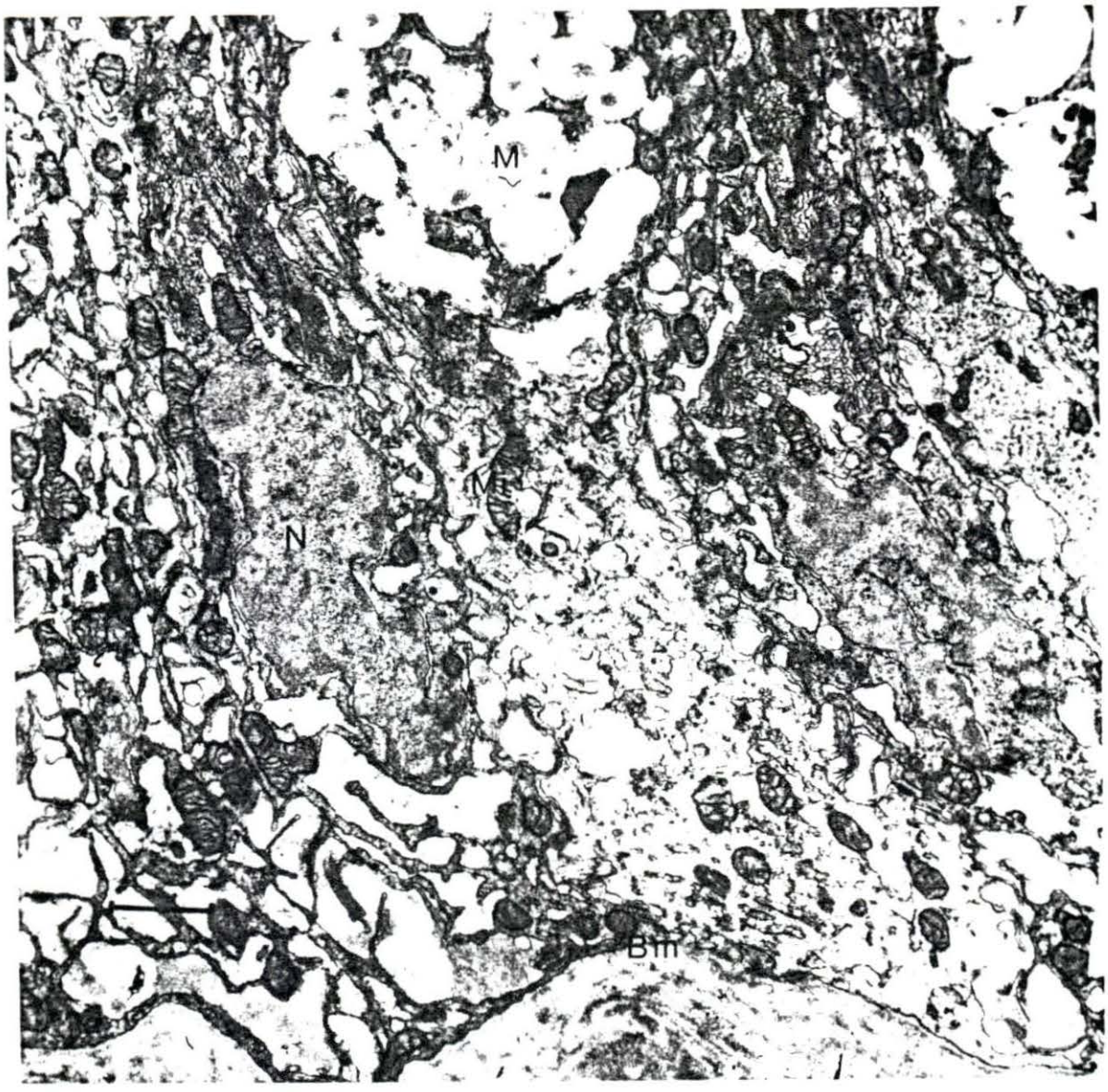

M
N
Bm

Fig. 75. Cervical epithelium at day 7. Cow 28, 6 years old. Magnification 7,000x. Secretion of electron dense granules is noted (arrow). Intercellular channels are observed between cells. Basement membrane (Bm), Golgi (G), mucous (M), microvilli (Mv), nucleus (N).

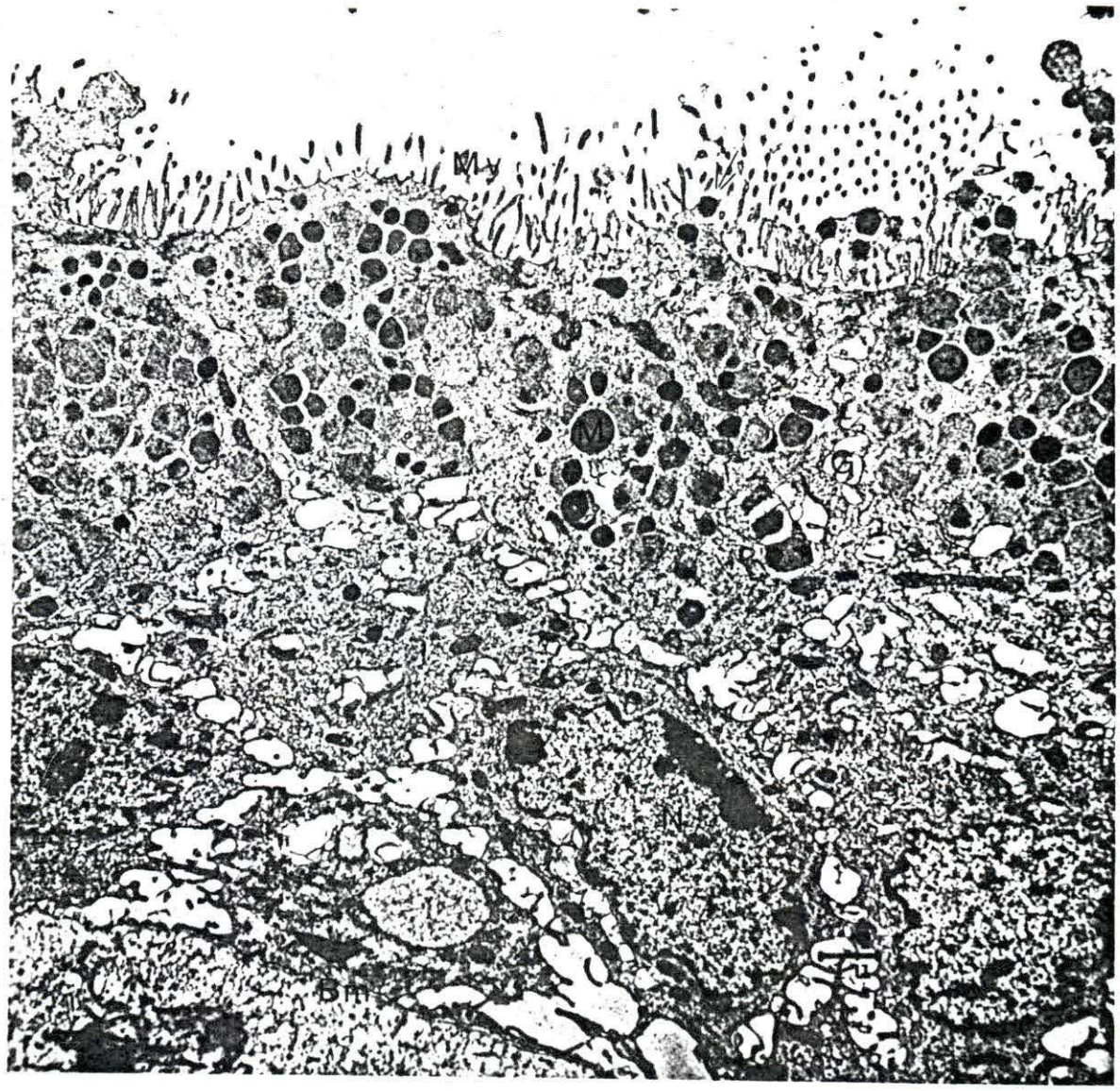
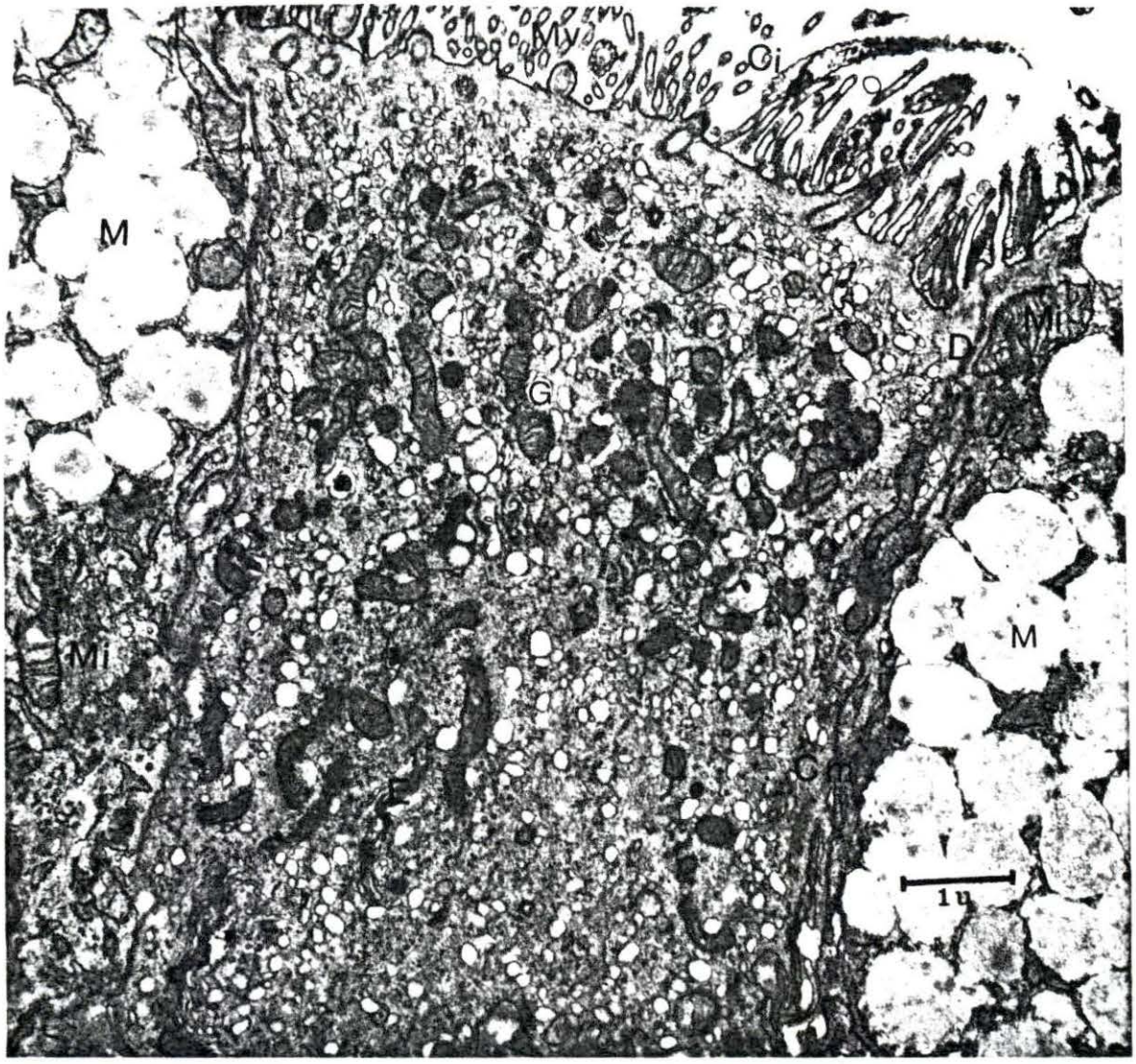

Bm

Fig. 76. Cervical epithelium at estrus. Cow 35, 6 years old. Magnification 15,000x. Cilia (Ci), cell membrane (Cm), desmosome (D), endoplasmic reticulum (E), Golgi (G), mucous (M) mitochondria (Mi), Microvilli (Mv).


Mv
Ci
M
Mi
D
G
Mi
M
Cm
1u