CERVICAL EPITHELIUM OF THE RABBIT FOLLOWING OVARIECTOMY

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Introduction

Recent studies on egg transport in the rabbit have indicated egg expulsion through the cervix into the vagina during the preimplantation stage.[15-18,20-22] In order to elucidate the unclear phenomenon of egg explusion into the vagina, the authors had examined the morphology of the cervical epithelia during estrus, early pregnancy and pseudopregnancy, in comparison with that of the endometrium.[12-14] Consequently, it has been shown that secretory material in the cervical epithelium is reproduced anew and released into the cervical lumen prior to implantation, possibly due to an increase of estrogen output by the ovaries, although the rabbit cervix is more tolerant to hormonal events occurring after ovulation stimulus than is the uterus.[2,7] The significance of estrogens in the maintenance of the cervical epithelium has been clearly demonstrated for the rabbit.[9,10] Our previous report dealt with morphological changes in the uterine epithelium of ovariectomized rabbits and ovariectomized rabbit receiving hormonal treatments.[19] In the present study, the cervices of these same animals were investigated to determine the effect of ovarian steriods on the rabbit cervix by light (LM) and scanning electron microscopy (SEM).

Materials and Methods

The cervices used for this study were obtained from 19 female Japanese White rabbits, of which the uteri had been utilized for the previous study.[19] Ten rabbits were used after having been spayed for 3 to 18 months—two does each at intervals of 3, 6, 9 and 12 months following operation and one each at 15 and 18 months, respectively. Six more rabbits, spayed for the six postoperative intervals from 3 to 18 months, were injected subcutaneously (s.c.) with 5 μgm of estradiol benzoate (Proginon B, Schering) in

1 ml of sesame oil every 12 hours for ten days before they were killed. The remaining three does, spayed for 9, 12 and 15 months, respectively, and then given estrogen, were injected daily with 2 mg of progesterone (Upjohn) s. c. for the last three days of estradiol treatment.

The cervices obtained from each rabbit were trimmed free of extraneous tissue and the bilateral cervices were separated at the median septum. One member of each pair of cervices was assigned to LM and the other to SEM.

For LM, specimens were pinned on Sealant (Dow-Corning 780) and immediately fixed in Bouin's or Carnoy's solution. After a few hours, the specimens were cut into smaller segments and then placed separately in fresh fixative for a longer period. Paraffin sections of 5–7 μm were stained with hematoxyline and eosin (H. E.) or azan, or by the periodic acid-Shiff (PAS) method.

For SEM, cervices were cut open longitudinally and washed gently with physiological saline solution. The samples were then pinned on Sealant and immersed in 3% glutaraldehyde solution buffered with sodium phosphate for 24 hours. After fixation, the tissues were rinsed in buffer solution and cut into suitably sized blocks for mounting on the specimen stubs. These blocks were then post-fixed in 1% osmium tetroxide for an additional 1 to 1.5 hours, followed by rinsing in water, dehydration in successive grades of ethanol and transference to liquid CO_2 for critical-point drying. The dried specimens were mounted on brass stubs and sputter-coated with gold (approximately 200 Å) in an Ion Sputter (JFC-1100, JEOL, LTD.) and examined in a JEOL JSM-200 scanning electron microscope operated at 10 kV.

Results

Ovariectomized Rabbits

The diameter of each cervix had decreased considerably, to approximately 3 mm, by 9 months after spaying. Ovariectomy caused also progressive reduction in height of the cervical epithelium and in thickness of the subepithelial stroma, but did not lead to disappearance of the cervical crypts. In the cervical epithelium of rabbits spayed for 3 months, almost all non-ciliated cells had atrophied and were peg-shaped (Fig. 1). Cells containing abundant secretory material were no longer recognized. In PAS-stained sections, luminal surfaces of the epithelial cells were stained slightly (Fig. 2). SEM demonstrated the cilia-dominant surface of epithelium, because cilia hid the atrophied, non-ciliated cells. Some of non-ciliated cells, however, were occasionally found among the cilia of adjacent ciliated cells by their slightly convex apices. They were covered with densely arranged short

microvilli, containing occassional ciliary buds or shafts (Figs. 3, 4).

Six and 9 months following ovariectomy, surfaces of the non-ciliated cells had become flat, with microvilli decreased in length and nub-like in shape (Fig. 5). Some of the ciliated cells displayed fewer cilia per cell on their surfaces (Fig. 5).

In rabbits spayed 12 and 15 months previously, the ciliated type of epithelial cell had disappeared in limited areas (Fig. 6), and cells possessing decreased number of cilia per cell were frequently noted (Fig. 7). Some of these cells displayed crooked, slender cilia on their free surfaces (Fig. 8). Microvilli on the surfaces of non-ciliated cells had disappeared partially (Fig. 7).

Cervical epithelium 18 months after ovariectomy consisted generally of cuboidal cells, and in some areas the cell arrangement was disordered (Fig. 9). SEM revealed that the non-ciliated type of epithelial cell was dominant and the cell surfaces had become polygonal in appearance (Fig. 10). Although most of non-ciliated cells were densely covered with short microvilli (Figs. 11, 12), some cells were devoid of microvilli and exhibited wavy surfaces (Fig. 13). It was found occasionally that cell surfaces were ruptured, exposing their internal structure (Figs. 11–13).

Hormonal Treatment in Ovariectomized Rabbits

Exogenous estrogen administration caused a remarkable swelling of the cervix (to 6～10 mm in diameter), regardless of the duration following ovariectomy. In rabbits spayed more than 12 months previously, especially after 18 months, estrogen treatment resulted in dramatic alterations in appearance of the cervical mucosa as compared to that from spayed animals. LM showed that mucosal folds had become broader due to edematous changes in the subepithelial stroma, and the epithelial cells appeared to be hyperplastic (Fig. 14). Abundant secretory material was found in both epithelial cells and cervical lumen (Fig. 15). In SEM, many convex apices of non-ciliated cells with elongated microvilli, and with occasional cytoplasmic projection, were seen among the well-developed cilia (Figs. 16, 17). Most of ciliated cells were fully ciliated, but some cells had normally long cilia only at their peripheries, exposing a bare microvillous surface in the central portion with or without some short cilia (Fig. 18).

Progesterone administration following estrogen priming led to a decrease in height of the epithelial cells and in density of secretory material within the cells (Fig. 19). More abundant secretory material was found in the cervical and cryptal lumina than in the cells. In SEM, apical surfaces of the non-ciliated cells appeared to be less elevated and their microvilli had

become shorter than those seen in the rabbits treated with estrogen alone. Cytoplasmic projections or buds were frequently observed on the microvillous surfaces. In one of three animals examined, ciliated cells had considerably decreased in number, but their surface morphology did not appear to have been altered (Fig. 20).

Cytoplasmic Cell Inclusions

Cytoplasmic cell inclusions were never observed in the cervical epithelium of spayed rabbits, regardless of the duration following ovariectomy. However, these inclusions appeared frequently in the cervical epithelium of ovariectomized rabbits after estrogen administration. The incidence of such inclusions became extremely low after additional progesterone administration.

Discussion

The present study showed that ovariectomy causes a cessation of secretory activity of the cervical epithelium. This confirms the findings of ODOR[9] and RICHES *et al.*[10], who reported that following castration secretory granules disappear and the cell apices of secretory cells become flat. Our investigation also demonstrated that a partial loss of cilia advances as the duration of ovariectomy becomes longer, and consequently a widespread deciliation occurs (18 months after spaying). Those observations disagree partially with the one previous study[10], in which a patchy deciliation resulted only 15-18 months following ovariectomy. In the present study, degenerative features of the epithelial cells were obvious in the rabbit spayed for 18 months.

The endometria of the same rabbits used in the present study were previously examined by LM and SEM[19], and disappearance of cilia was noted in the endometrium 12-18 months after ovariectomy. RUMERY and EDDY[11] reported that the oviductal epithelium in the rabbit undergoes a widespread loss of cilia following long-term castration (15-18 months). Therefore, ciliated cells in the cervical epithelium seem not to be as sensitive to estrogen depletion as those in the oviductal and uterine epithelium.

Cells possessing fewer cilia per cell appeared increasingly with lapse of time after ovariectomy. Such a change in ciliated cells may indicate one made of deciliation, in which individual cilia were falling off of the cell surface. Despite the patchy deciliation which occurred in the cervices of spayed animals, stubby, short cilia, which do resemble the immature or developing cilia demonstrated in SEM by other investigators[1,3,5,10], were frequently observed. This indicates that ciliation is induced also in the cervix under

estrogen deficiency. A similar tendency was found in the uterine epithelium of rabbits spayed for a relatively short period (3-9 months).[19]

Since estrogen treatment as administered in this study resulted in hyperplasia of the cervical mucosa, as compared to the morphology of the estrous cervix[12,13], the dose of estrogen seems to be above the normal physiological level. However, it is evident that estrogen brought about a dramatic restoration in the cervical epithelium. The mucosal surface was thickly ciliated again and the non-ciliated cells had prominent convex apices among the cilia. These observations agree well with the findings of ODOR[9] and RICHES *et al.*[10] A similar regeneration is observed in the oviductal[11] and uterine epithelium.[19] On the other hand, treatment with progesterone following estrogen priming caused release of the secretory material from the epithelial cells and occasional deciliation.

These results strongly indicate that estrogen is important in the maintenance of ciliated cells and secretory cells in the cervical epithelium of the rabbit, and that estrogen stimulates production of secretory granules in the cells and progesterone facilitates their release, supporting the general view on the activity of the mammalian cervix.[4] However, the cervix is less sensitive to ovarian steroids than is the uterus, when compared with previous findgins.[19]

Our observations concerning the movement of cytoplasmic cell inclusions makes clear that their production is dependent on estrogen and their discharge into the cervical lumen is under the influence of progesterone. This explains well their movement in the cervical epithelium of the rabbit during luteal phases.[6,8,13,14]

Summary

The cervical epithelia of ovariectomized rabbits, and ovariectomized rabbits receiving estrogen with or without progesterone, were investigated with light and scanning electron microscopes.

Following ovariectomy, non-ciliated secretory cells were atrophied and their secretory activities were almost ceased. In rabbits spayed 12 to 15 months previously, a partial loss of cilia from the cervical epithelium was evident, and in animals spayed for 18 months cilia had disappeared from almost all epithelial cells.

Estrogen given to these animals caused a dramatic restoration of the cervical epithelium, whereas progesterone following estrogen priming resulted in release of secretory material from secretory cells and in occasional deciliation.

It is concluded that estrogen plays an important role in the maintenance of ciliated cells and secretory cells in the cervical epithelium, but that secretory material is released from the cells under the influence of progesterone.

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PLATE 1

EXPLANATION OF FIGURES

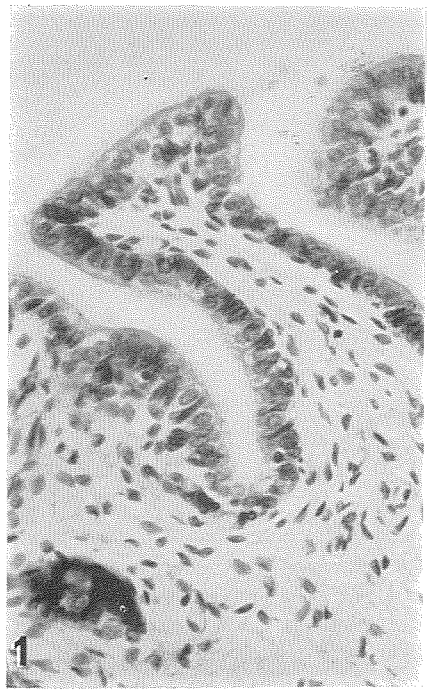
Fig. 1. LM appearance of cervical mucosa 3 months following ovariectomy, showing atrophic peg-shaped, non-ciliated cells. Ciliated cells seem to exhibit no changes. H.E. ×320.

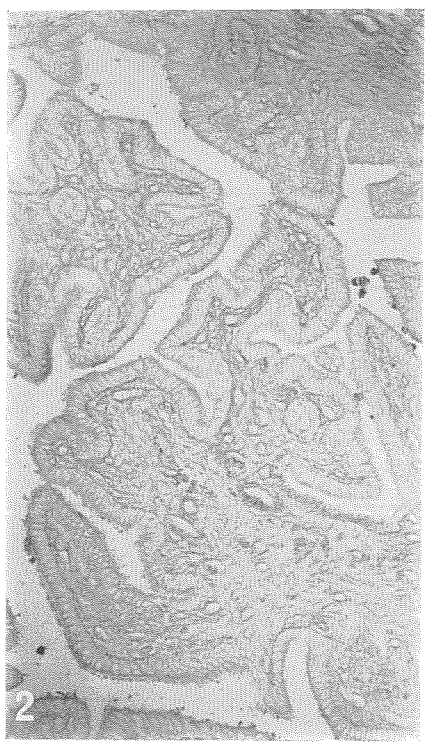
Fig. 2. PAS-stained section of cervical mucosa from a rabbit spayed for 3 months, showing weakly positive material at the apical part of the epithelial cells. ×160.

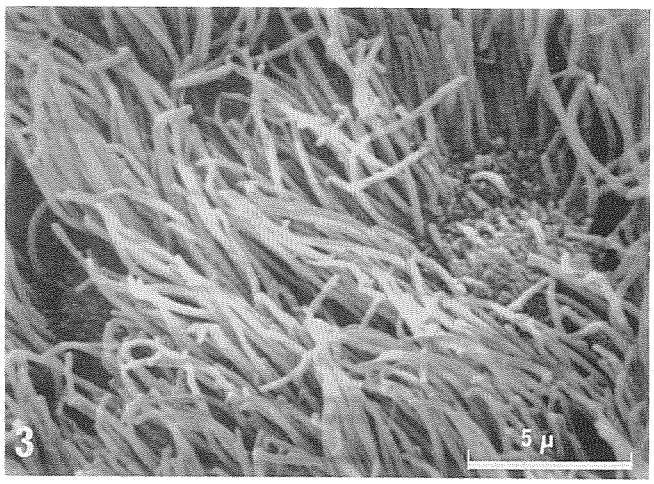
Fig. 3. SEM features of cervical epithelium 3 months after spaying. The majority of the cells are ciliated. A cell containing several ciliary buds is located on the right side of this figure.

Fig. 4. SEM features of cervical epithelium 3 months after spaying. Note developing cilia on the right side of this figure.

Fig. 5. SEM features of cervical epithelium 9 months following ovariectomy, showing very small microvilli on the surfaces of the non-ciliated cells. Note decreased number of cilia per cell.



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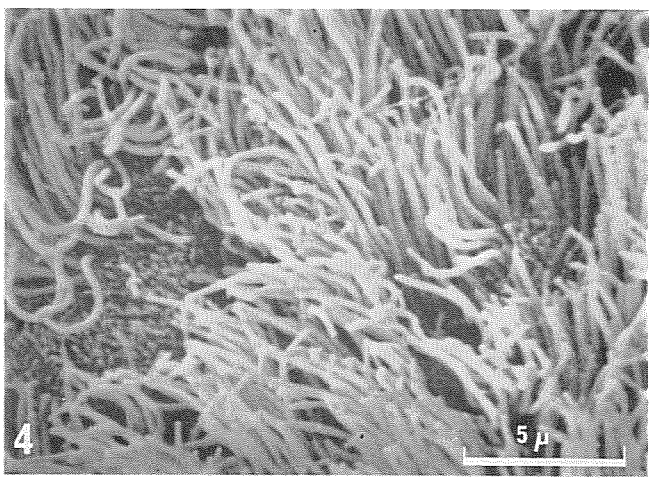
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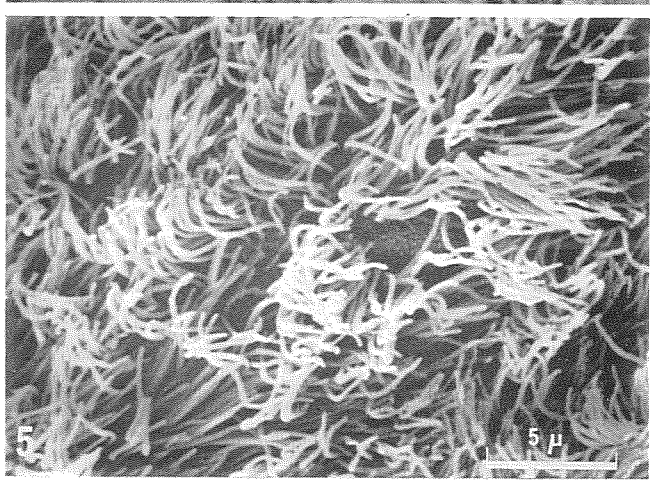
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PLATE 2

EXPLANATION OF FIGURES

Fig. 6. SEM features of cervical epithelium of rabbit spayed for 15 months, showing a patchy loss of cilia.

Fig. 7. Higher magnification of an area in Fig. 6. Some cells display fewer cilia per cell on their surfaces.

Fig. 8. Higher magnification of another area in Fig. 6, showing crooked, slender cilia.

Fig. 9. LM features of cervical mucosa of rabbit spayed for 18 months. The epithelial cells have become atrophic and the cell arrangement is partially in disorder.

Figs. 10–13. SEM features of cervical epithelium of rabbit spayed for 18 months, showing various regressive features. Ciliated cells have almost disappeared from the epithelium.

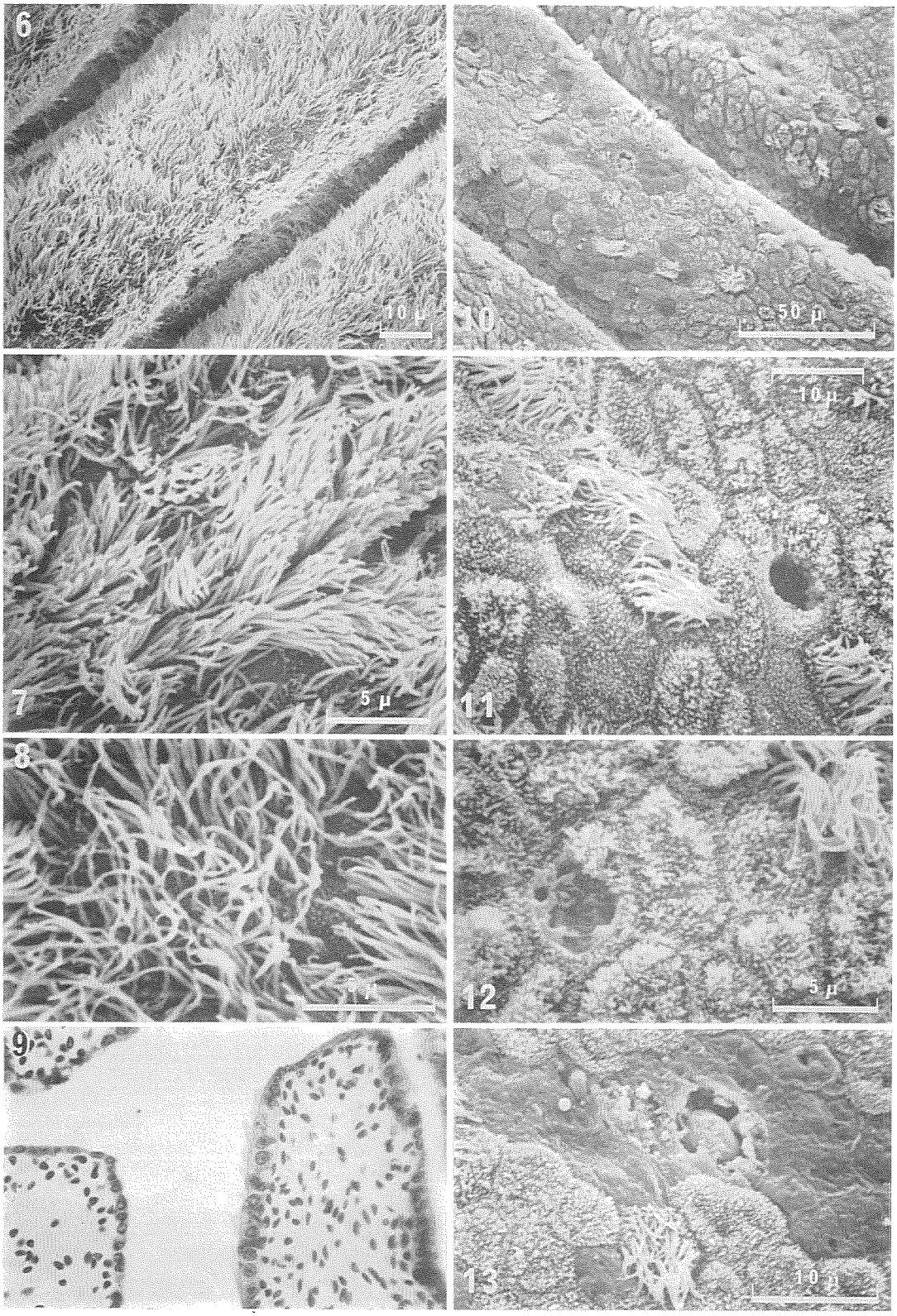
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PLATE 3

EXPLANATION OF FIGURES

Fig. 14. LM features of the cervical mucosa, 18 months after spaying and following estrogen treatment. A dramatic restoration is evident in comparison with Fig. 9. H.E. ×320.

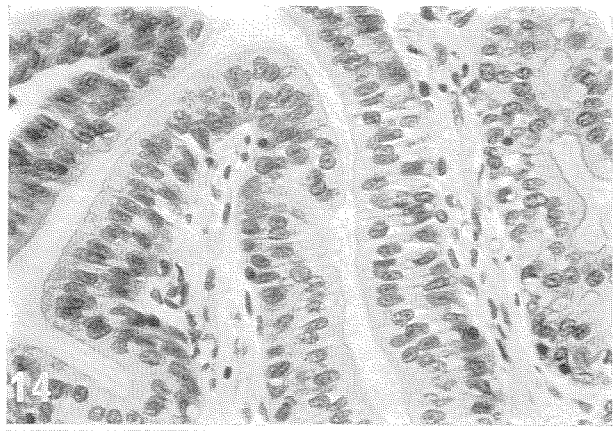
Fig. 15. PAS-stained section of cervical mucosa obtained from the same rabbit as in Fig. 14. ×160.

Fig. 16. SEM features of cervical epithelium from the same rabbit as in Fig. 14, showing numerous ciliated cells and dome-like apical surfaces of non-ciliated cells.

Figs. 17 & 18. Higher magnification of the cervical epithelium of the same rabbit as in Fig. 16.

Fig. 19. PAS-stained section of cervical mucosa, 15 months after spaying and following estrogen and progesterone treatment. Positive material is seen only at the apical parts of the cells. Abundant secretory material is located in the cervical crypts. ×160.

Fig. 20. SEM features of cervical epithelium of the same rabbit as in Fig. 19, showing a widespread deciliation.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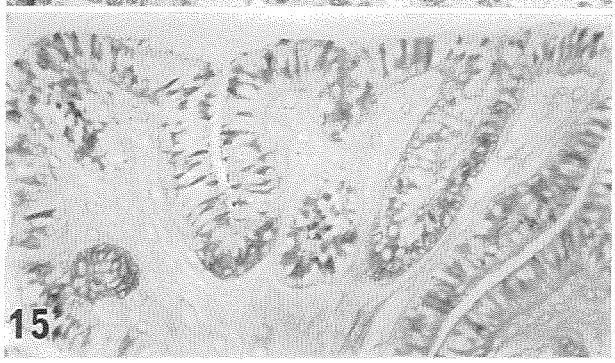
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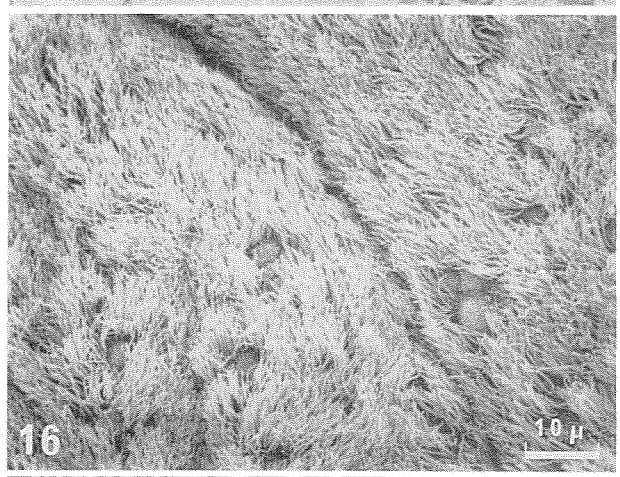
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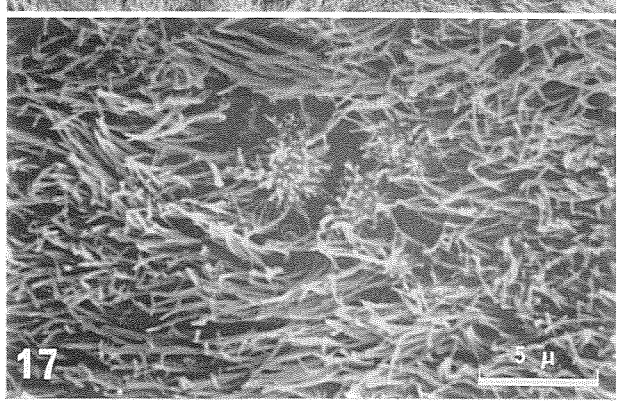
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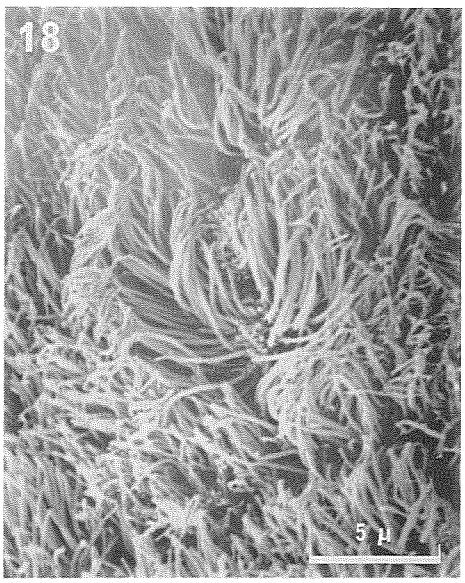
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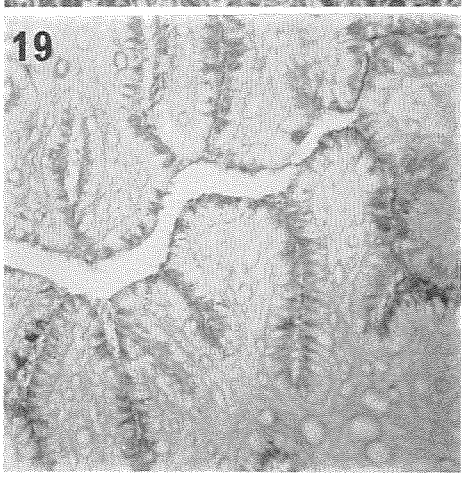
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