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Morphological Study of the Cyclic Changes in the Endocervical Epithelium

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ABSTRACT

In order to examine the cyclic changes in the endocervical epithelium, fresh human endocervical specimens were obtained from 60 cases for observation by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The results obtained were as follows: 1) The endocervical epithelium was composed of ciliated cells and secretory cells at a ratio of 1:5—10 which did not vary by menstrual cycle, pregnancy and age. 2) Cyclic changes of ciliated cells were not observed in the menstrual cycle, but decrease in number and tip-thinning of the cilia were found in the postmenopausal phase by SEM observation. 3) Dilatation and complexity of the glandular lumen and increase in cell size reached a peak in the ovulatory phase. The findings of secretory cells in the first trimester of pregnancy were the same as those of the ovulatory phase and atrophic changes and simplified formation of the gland were observed in the postmenopausal phase. 4) SEM observation showed surface projections of various sizes and slightly dilated, round and shortened microvilli on the secretory cells in the ovulatory phase and the first trimester of pregnancy. During the postmenopausal phase, atrophic changes such as flattening of the cell surface and decrease and shortening of microvilli were observed. 5) TEM observation showed a peak in secretory granules and organelles in both number and size and exocytotic secretion during the ovulatory phase and the findings observed during the first trimester of pregnancy were similar to those during the ovulatory phase.

There are many detailed reports on the morphological cyclic changes in the human endometrium[11,14,15,19], but few reports have been made on the systemic observations of the cyclic changes in the endocervical epithelium by light microscopy, scanning electron microscopy and transmission electron microscopy despite its important functional role in producing cervical mucus. The quality and quantity of the cervical mucus change remarkably by menstrual cycle and this finding is routinely applied to clinical practice. Though the changes of cervical mucus are considered to be caused by morphological cyclic changes in the endocervical secretory cells, there is no agreement on the morphological cyclic changes[1,2,5,20,21].

In this study aimed to elucidate the endocervical cyclic changes, the endocervical morphological cyclic changes including changes in the first trimester of pregnancy and in the postmenopausal stage were discussed and compared with LM, SEM and TEM observations.

MATERIAL AND METHODS

Fresh materials were obtained from 60 operated patients (total hysterectomy), who satisfied the following four conditions. 1) Adult nonpregnant cases were not over 40 years old and had regular menstrual cycle. Pregnant cases were not over 30 years old. For cases of postmenopause, five years or more had elapsed after menopause. 2) The last menstrual period could be confirmed. 3) Inflammatory, neoplastic change and other abnormal findings were not

observed in the specimens. 4) Histological dating of the endometrium coincided with menstrual dating.

Uteruses obtained by operation were incised longitudinally, and endocervical tissues were obtained for LM, SEM and TEM from the mid portion between the inner uterine os and external os. At the same time, endometrium was obtained and stained with H-E stain for histological dating.

For LM, the sections were stained with H-E, PAS and mucicarmine stain. For SEM, the obtained tissues were fixed in 2% glutaraldehyde solution, washed in 0.1M Millonig's phosphate buffer and postfixed in 1% OsO_4 solution. Tissues were dehydrated in graded methanol solutions. Following the replacement with iso-amylacetate, these tissues were dried in the critical drying apparatus, coated with a gold sputtering apparatus and examined with SEM (JSM-T 20). For TEM observation, tissues were fixed in 2% glutaraldehyde and postfixed in 1% OsO_4 solution. These materials were dehydrated in graded methanol solutions and embedded in Epon 812. Thin sections cut on a Porter-Blum MT-1 ultramicrotome, stained with Millonig's lead solution, and coated with carbon were observed with TEM (Hitachi HS-7S).

Menstrual cycle was classified in five phases according to dating from endometrial specimens and last menstrual period as follows:

1) Menstrual phase	1st-5th day
2) Proliferative phase	6th-13th day
3) Ovulatory phase	14th-16th day
4) Secretory phase	14th-16th day
a) Early secretory phase	17th-22nd day
b) Late secretory phase	23rd-28th day
5) First trimester of pregnancy	
6) Postmenopausal stage	

RESULTS

1. Cyclic changes of ciliated cells in the endocervical epithelium.

1) Light microscopic observation (LM)

Ciliated cells were slightly larger than secretory cells and contained a central nucleus with fine granular or fine reticular chromatin. The cytoplasm was slightly clear and multiple ciliary shafts were seen on the surface border (Fig. 1). Ciliated cells were not stained with PAS and mucicarmine stain.

2) Scanning electron microscopic observation (SEM)

Many ciliary shafts showed no cyclic change in the menstrual cycle (Fig. 2), but decrease in number and deformity of the ciliary shafts were observed in the postmenopausal stage.

3) Transmission electron microscopic observation (TEM)

Ciliated cells had a centrally located oval nucleus and many ciliary shafts attached to the rootlets (Fig. 3). Mitochondria with lamellar cristal were moderately developed, but rough endoplasmic reticulum and Golgi apparatus were poorly developed, and free ribosome and glycogen aggregates were visible in the cytoplasm. In these organelles, hardly any changes could be seen due to menstrual cycle and aging.

4) The ratio of ciliated cells to secretory cells.

The ratio of ciliated cells to secretory cells was calculated by SEM in the surface epithelium and by LM in the glandular epithelium. The ratio of ciliated cells to secretory cells was calculated to be 1:5-10, and no evident variation could be observed during the menstrual cycle, pregnancy and postmenopausal stage. No marked change in cell size could be noted.

2. Cyclic changes of secretory cells in the endocervical epithelium.

1) Menstrual phase

By LM study, the secretory cells in the endocervical epithelium were arranged in a regular monolayer and individual cell was observed as tall columnar cell (Fig. 4). Cytoplasm was poorly stained with PAS and mucicarmine stain and secreted material could hardly be observed in the gland.

SEM revealed that the cell surface was either relatively round or doom in shape and covered with numerous microvilli (Fig. 5). No evidence of secretion could be seen.

By TEM study, few secretory granules were observed in the cytoplasm, and the secretory cells had poorly developed small mitochondria and Golgi apparatus, moderately developed rough endoplasmic reticulum and moderate number of free ribosome (Fig. 6).

2) Proliferative phase

During the proliferative phase, LM study showed that the surface and glandular epithelia were orderly arranged as a monolayer, and in-

dividual cells were taller than in the menstrual phase (Fig. 7). Cytoplasm was well stained with PAS and mucicarmine stain.

SEM findings did not show any marked change in individual cells except a few cytoplasmic projections considered to be secretions were seen in some of the secretory cells (Fig. 8).

On the other hand, by TEM observation, secretory cells had numerous secretory granules having various density (Fig. 9). Exocytotic secretion was observed at the end of the proliferative phase. Well developed mitochondria, rough endoplasmic reticulum and Golgi apparatus were also seen.

3) Ovulatory phase

In this phase, by LM observation, glandular lumens were dilated and tortuous, and the cell size was larger than in the previous phase (Fig. 10). Cytoplasm and intraluminal substance were well stained with PAS and mucicarmine stain, and projections were observed on the cell surface.

SEM showed that the size of individual cells was larger than in the proliferative phase and many cytoplasmic projections considered to be secretions were observed (Fig. 11).

By TEM observation, many secretory granules containing low electron dense material were found in the cytoplasm. These were mutually fused and exocytotic secretion was apparent on the surface of secretory cells (Fig. 12). As for other organelles compressed with secretory granules, their development reached a peak. During this phase, fibrous structures (arrow) were observed among secretory granules in the cytoplasm.

4) Secretory phase

a. In the early secretory phase, LM observation showed that the cell size of secretory cells was slightly smaller and the structure of the glands became relatively simple (Fig. 13). Mucinous substance in the cytoplasm and lumen was decreased and poorly stained with PAS and mucicarmine stain.

By SEM observation, cytoplasmic projections considered to be secretion on the cell surface were decreased and the cell surface was low doom in shape and covered with numerous microvilli (Fig. 14).

By TEM observation, secretory granules containing relatively high electron dense material were remarkably decreased in the cytoplasm. As for other organelles, mitochondria, rough endoplasmic reticulum and Golgi apparatus could be well observed because of the decrease in secretory granules (Fig. 15).

b. Late secretory phase

In this phase, LM observation revealed that glands became straight and simple in structure, and individual cells became smaller and flat (Fig. 16).

By SEM observation, secretory cells were low doom in shape as in the proliferative and early secretory phases, but cytoplasmic projections were hardly found and microvilli were slightly elongated (Fig. 17).

By TEM observation, hardly any secretory granules were observed in the cytoplasm and the development of mitochondria, rough endoplasmic reticulum and Golgi apparatus was poor (Fig. 18).

5) First trimester of pregnancy

LM observation showed that endocervical secretory cells in the first trimester of pregnancy were regularly arranged in a monolayer in both surface and glandular epithelia, but glandular lumen was tortuous and cells became large and tall (Fig. 19 and 20). Cytoplasm and intraluminal substance were well stained with PAS and mucicarmine stain.

By SEM observation, the shape of secretory cells covered with numerous microvilli were round and doom in shape. Cytoplasmic projections were also observed (Fig. 21 and 22).

TEM showed that the cytoplasm was filled with secretory glanules containing low electron dense substance which were fused with each other (Fig. 23), and exocytotic secretion (arrow) was observed in many cells in the cell surface (Fig. 24). The other organelles were not well visible due to compression of numerous secretory granules. These findings were similar to those in the ovulatory phase.

6) Postmenopausal stage

LM observation showed that the individual cells became small and cuboidal in shape (Fig. 25 and 26) during the postmonopausal stage. The reaction of PAS and mucicarmine stain in the cytoplasm was decreased, and intraluminal substances were rarely observed.

SEM revealed that the cell surface was remarkably flattened and microvilli were short-

ened (Fig. 27), and loss and tip-thin transformation of ciliary shaft were observed (Fig. 28).

On the other hand, TEM revealed that secretory granules containing high electron dense material filled the cytoplasm, but secretion from the cell surface could not be observed. Development of organelles was not visible due to compression in with numerous secretory granules (Fig. 29).

DISCUSSION

Detailed reports on the cyclic changes in the human endometrium by light and electron microscopy have been published[11,14,15,19]. As for human endocervix originating embryologically from müllerian duct similar to the endometrium, epithelia are not ablated during menstruation. A remarkable variation in the quality and quantity of cervical mucus secretion is observed with a peak seen at ovulation. These findings are different from the endometrium. The views on the endocervical morphological changes can be classified into the following three: 1) Cyclic changes can hardly be observed histologically[2,5]. 2) Evaluation on the presence of cyclic changes is impossible[21]. 3) The cyclic changes are present though not remarkable[1,20].

Generally, cyclic changes of the cervical mucus showing a peak at ovulation are applied in clinical practice for estimating ovarian function and ovulation phase. Morphological cyclic changes can thus be predicted.

The first report of SEM observation of the uterine cervix was made by Frencyzy et al[6] in 1973 regarding the findings of the transformation zone. Thereafter, Ludwig has classified the menstrual cycle into three phases (postmenstrual stage, midcycle stage and secretory stage), and reported numerous secretory findings during the midcycle stage and increase in secretory cell volume during the premenstrual stage through evaluation of each stage. Chretien and Guillon[4] reported that though ciliated cells did not show any remarkable changes, apocrine-like projections were observed in secretory cells during the ovulatory phase. The results of the present study did not reveal any apparent cyclic changes in the ciliated cells, but cyclic changes in secretory cells with a peak at ovulation were observed on the cell surface such as swelling of cell surface, cytoplasmic projection and bulgy changes of microvilli. However, it could not be confirmed whether the secretion type was apocrine type or exocytotic type by SEM findings.

With regard to cyclic changes observed by TEM, Hashimoto[10] reported remarkable changes of organelles, secretory granules, mitochondria, Golgi apparatus and rough endoplasmic reticulum in secretory cells. In 1963, Komori[12] observed that ciliated cells did not show cyclic changes, but secretory cells showed remarkable changes in organelles. As for cyclic changes of secretory granules, Komori[12] and Hamada[9] reported cyclic changes which peaked during ovulation. According to the present study, secretory granules showed cyclic changes of increasing number, volume and electron density which peaked during the ovulatory phase. In earlier studies on the ultrastructure of the human endocervix, two types of secretions have been discussed, the apocrine type[9,10,12] and exocytotic type[16,18]. In the present investigation, the secretion type was all the exocytotic type in the ovulatory phase and first trimester of pregnancy and the apocrine type secretion was not observed. The significance and role of fibrous structure observed during the ovulatory phase and first trimester of pregnancy are unknown, but Philipp[17] has suggested a relation with secretory granules. In this study, many fibrous structures were seen during the ovulatory phase and pregnant stage and their presence between multiple secretory granules suggests a relation with the mechanism of discharge of secretory granules.

With regard to LM findings during pregnancy, Fluhmann[8] reported in his morphological observation that there were complexities of dendriform structures of the gland and increase of the number and size of glandular epithelial cells. In the present investigation, findings similar to those in the ovulatory phase were seen such as increase of cell volume, dendriform structures of gland, and cytoplasm and intraluminal substance which stained well with PAS and mucicarmine stain.

There are only a very few reports on SEM observation during pregnancy. It was only Ludwig[13] who observed swelling of cells shortening of microvilli and ruptured holes of various size. In this study, only cytoplasmic projections of various sizes were observed. As

for TEM findings during pregnancy, Komori[12] and Chapman[3] observed that the cytoplasm of secretory cells was filled with numerous secretory granules. Granules containing the low electron dense material and exocytotic secretion were also observed in the present study.

Bradburn[2] who investigated by light microscopy, 15 cases of postmenopausal endocervix observed such findings as simplified shape and diminished number of glands and flattening and reduction of cells. Similar findings were made in the present study. Staining of PAS and mucicarmine stain of secretory cells were reduced and no secreted material was seen in the lumen of the gland. Further, Ludwig[13] in this SEM observation reported the change with sharp boundary of cells, pavement-like flattened cells and shortening of microvilli. In the present study, microvilli of secretory cells were shortened and atrophic changes were apparent. These findings are similar to those of postmenopausal uterine endometrium reported, by Ferenczy and Richart[7]. Ciliary shafts of ciliated cells were decreased in number and tips of ciliary shafts were deformed to tip-thin shape. These findings suggest loss of function. In TEM observation of the posmenopausal stage, Komori[12] also noted numerous secretory granules in cytoplasm. In the present study, secretory cells were filled with electron dense granules, but secretory findings could not be observed from the cell surface.

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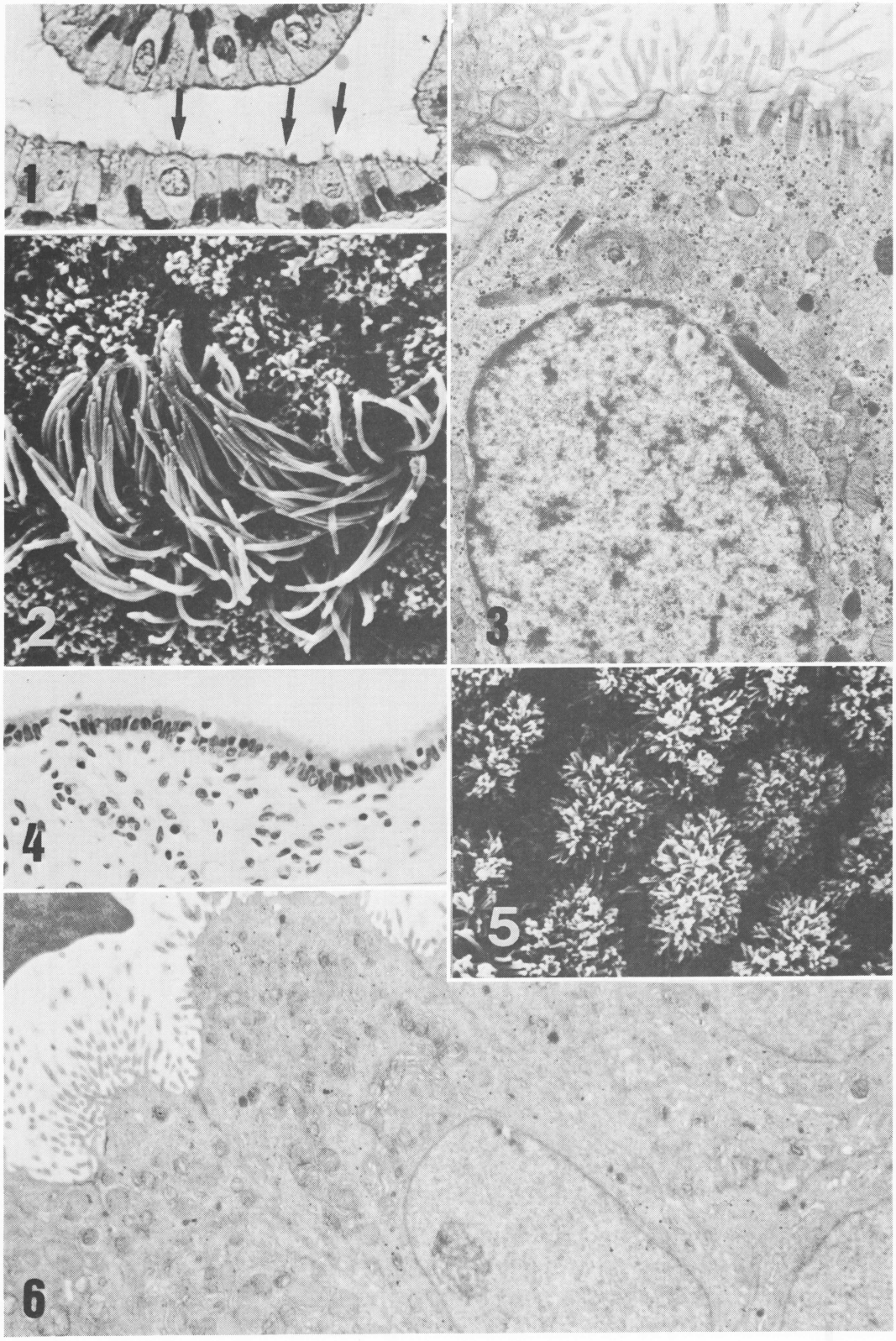
EXPLANATION OF FIGURES

Fig. 1. Ciliated cells with ciliary shafts (arrow) are observed among the secretory cells. (HE stain × 400)
Fig. 2. Ciliated cells have many long cilia on the cell surface. (SEM × 3500)
Fig. 3. Ciliated cells have many ciliary shafts attached to the rootlet. (TEM × 8200)
Fig. 4. Menstrual phase: Epithelium is composed of single columnar cells with basal nuclei. (HE stain × 200)
Fig. 5. Menstrual phase: Secretory cells are round or oval in shape and covered with numerous microvilli. Finding of secretion is not observed. (SEM × 2940)
Fig. 6. Secretory cells during menstrual phase: Poorly developed rough endoplasmic reticulum, Golgi apparatus and small mitochondria are observed, but no secretory granule is found in the cytoplasm (TEM × 4800)
Fig. 7. Proliferative phase: The glandular lumen becomes dilated and tortuous, and the vacuole is apparent in the cytoplasm. (HE stain × 200)
Fig. 8 Proliferative phase: The SEM findings of this phase are similar to those of the menstrual phase. (SEM × 2450)
Fig. 9. Secretory cells during proliferative phase: Secretory granules appear in the cytoplasm. The development of mitochondria, rough endoplasmic reticulum and Golgi apparatus can be seen. (TEM × 4400)
Fig. 10. Ovulatory phase: Formation of vacuole in the cytoplasm is observed and cell surface shows irregular pattern. (HE stain × 400)
Fig. 11. Ovulatory phase: Secretory cell has on the cell surface protrusions of cytoplasms of various size and shapes. (SEM × 5250)
Fig. 12. Secretory cells during ovulatory phase: Cytoplasm is filled with secretory granules containing low electron dense material and fibrous structures are observed among them (arrow). Exocytotic type secretions are seen on the cell surface. (TEM × 4100)
Fig. 13. Early secretory phase: The structure of glands becomes slightly simple. The size of cells becomes small and secreted material is scanty in the glandular lumen. (HE stain × 200)
Fig. 14. Early secretory phase: The protrusions of cytoplasm are hardly observed. (SEM × 1400)
Fig.15. Secretory cells during early secretory phase: Secretory granules in the cytoplasm are remarkably decreased in number, so organelles are well observed. (TEM × 4800)
Fig. 16. Late secretory phase: The structure of glands is more straight and simple and the secretory material cannot be observed in the lumen. (HE ×200)
Fig. 17. Secretory cells during the late secretory phase: Formation of cytoplasmic protrusion is not seen. The microvilli are slightly elongated. (SEM × 1680)
Fig. 18. Secretory cells during late secretory phase: Secretory granules are not observed in the cytoplasm. (TEM × 5800)
Fig. 19. First trimester of pregnancy: The size of each cell is increased. The formation of vaculoes is remarkable and the nuclei are located in the lower part of the cells. (HE stain × 200)
Fig. 20. First trimester of pregnancy: The structure of the gland is tortuous and cell size becomes large and tall with clear cytoplasm. (HE stain × 200)
Fig. 21—22. First trimester of pregnancy: The cytoplasmic protrusions of various size are observed on the surface of secretory cells. (Fig. 21 SEM × 4900, Fig. 22 SEM × 3010)
Fig. 23. Secretory cells during the first trimester of pregnancy: The cytoplasm is filled with secretory granules containing low electron dense material which are fused each other. (TEM × 4500)
Fig. 24. Secretory cells during the first trimester of pregnancy: Exocytotic type secretions are observed from the cell surface. (TEM × 9200)
Fig. 25. Postmenopausal stage: The gland of simple structure is formed by small cells. (HE stain × 200)
Fig. 26. Postmenopausal stage: The structure of glands is small and the secretion is not observed in the glandular lumen. (HE stain × 200)
Fig. 27. Postmenopausal stage: The microvilli in the secretory cells are decreased in number and length. (SEM × 6300)
Fig. 28. Postmenopausal stage: The number of cilia is decreased, and tip-thin deformities are remarkable. (SEM × 5180)
Fig. 29. Secretory cells during postmenopausal stage: The cytoplasm is filled with secretory granules containing high electron dense material, but secretory finding cannot be observed (TEM ×7900)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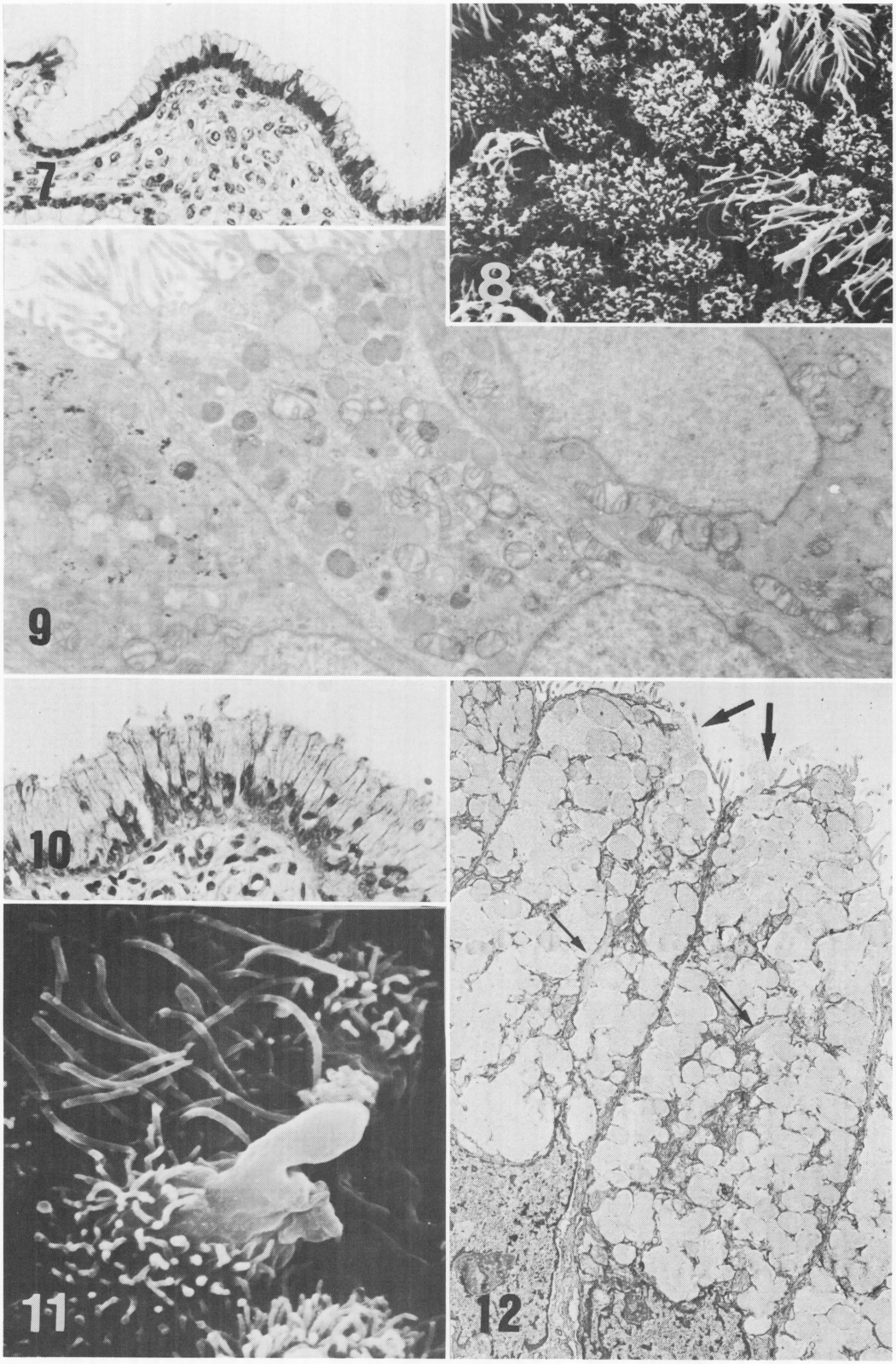
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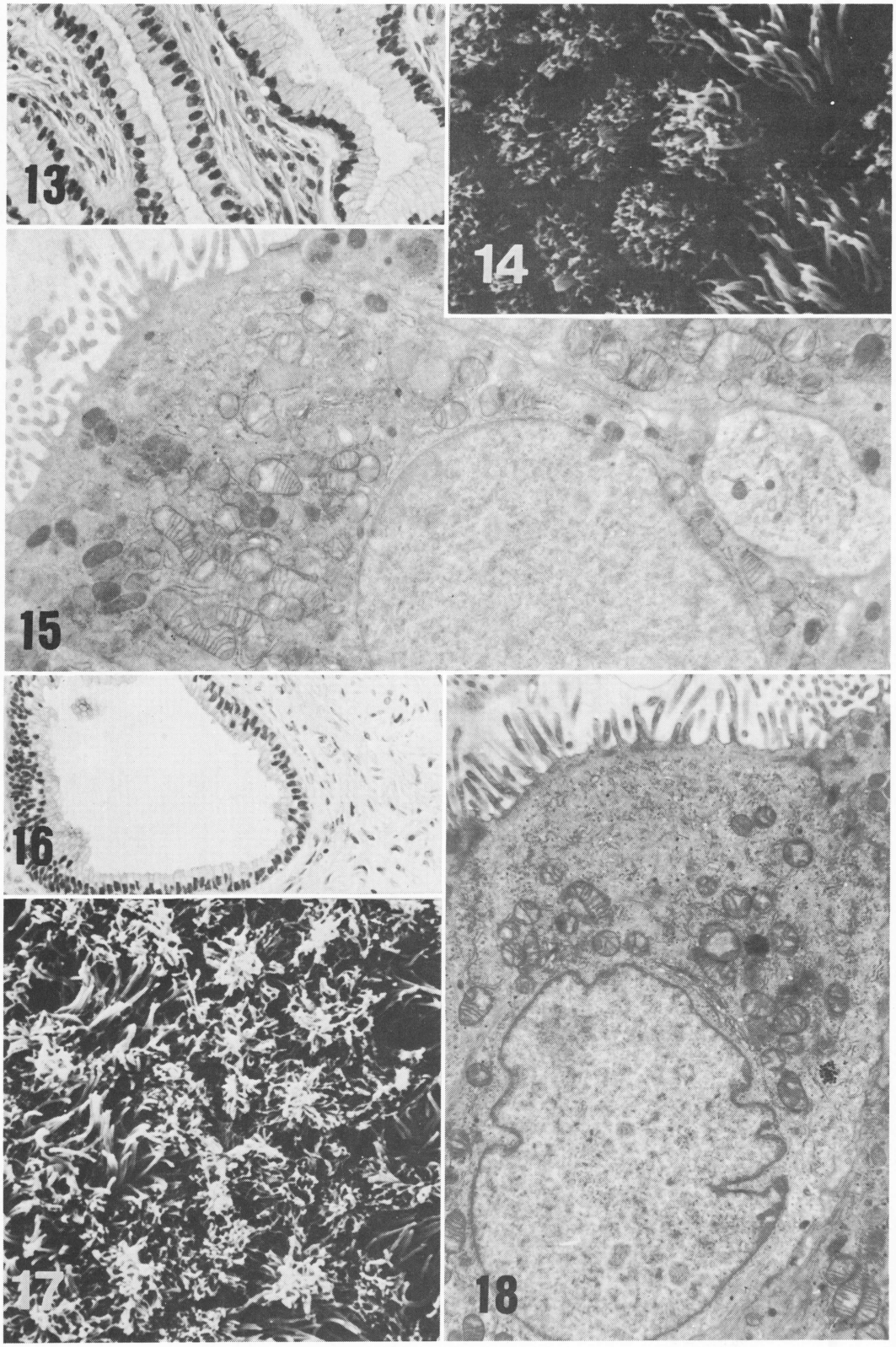
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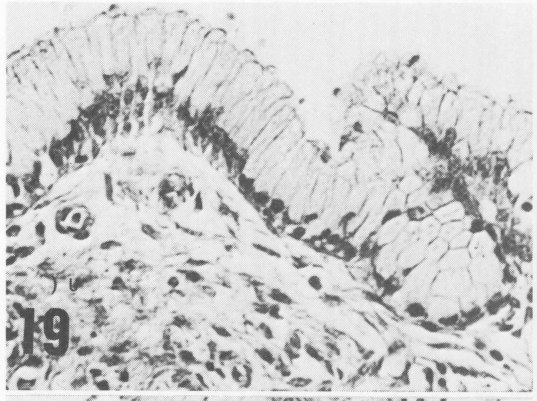
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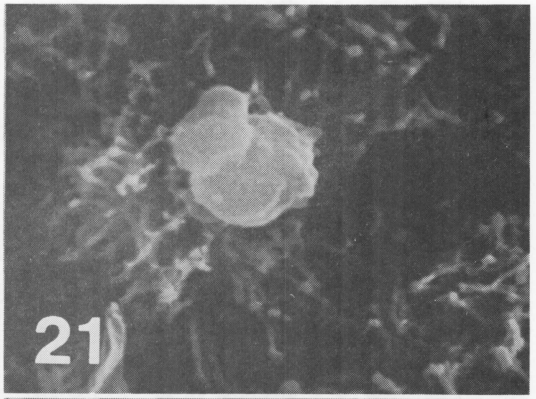
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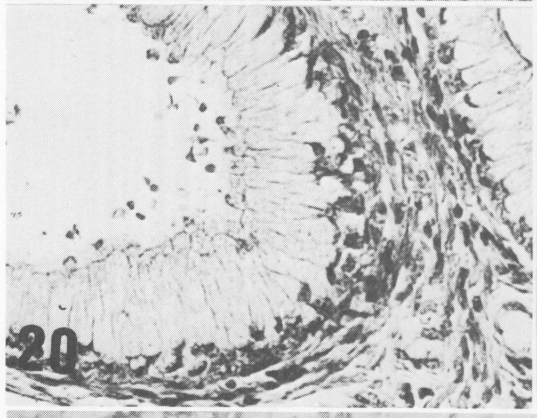
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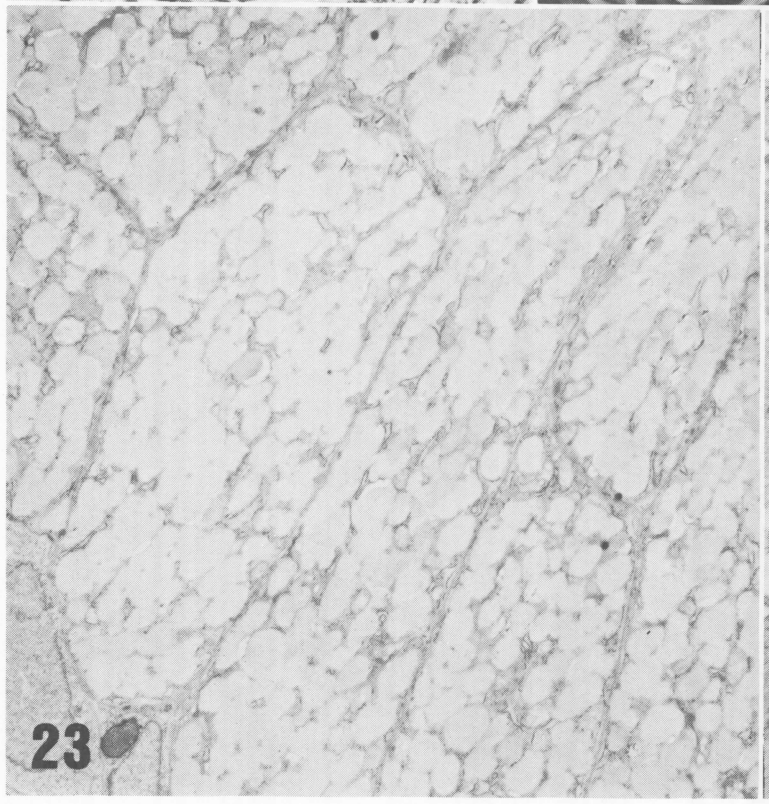
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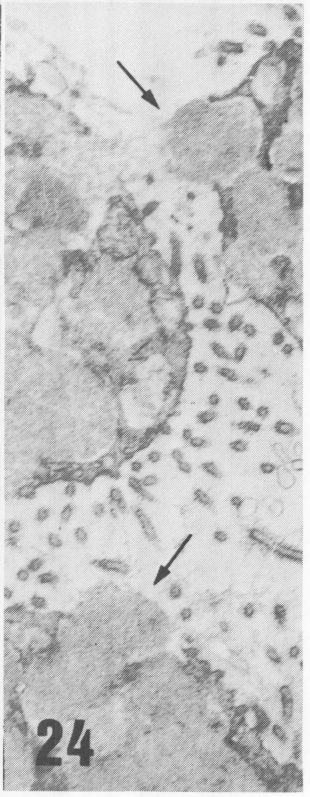
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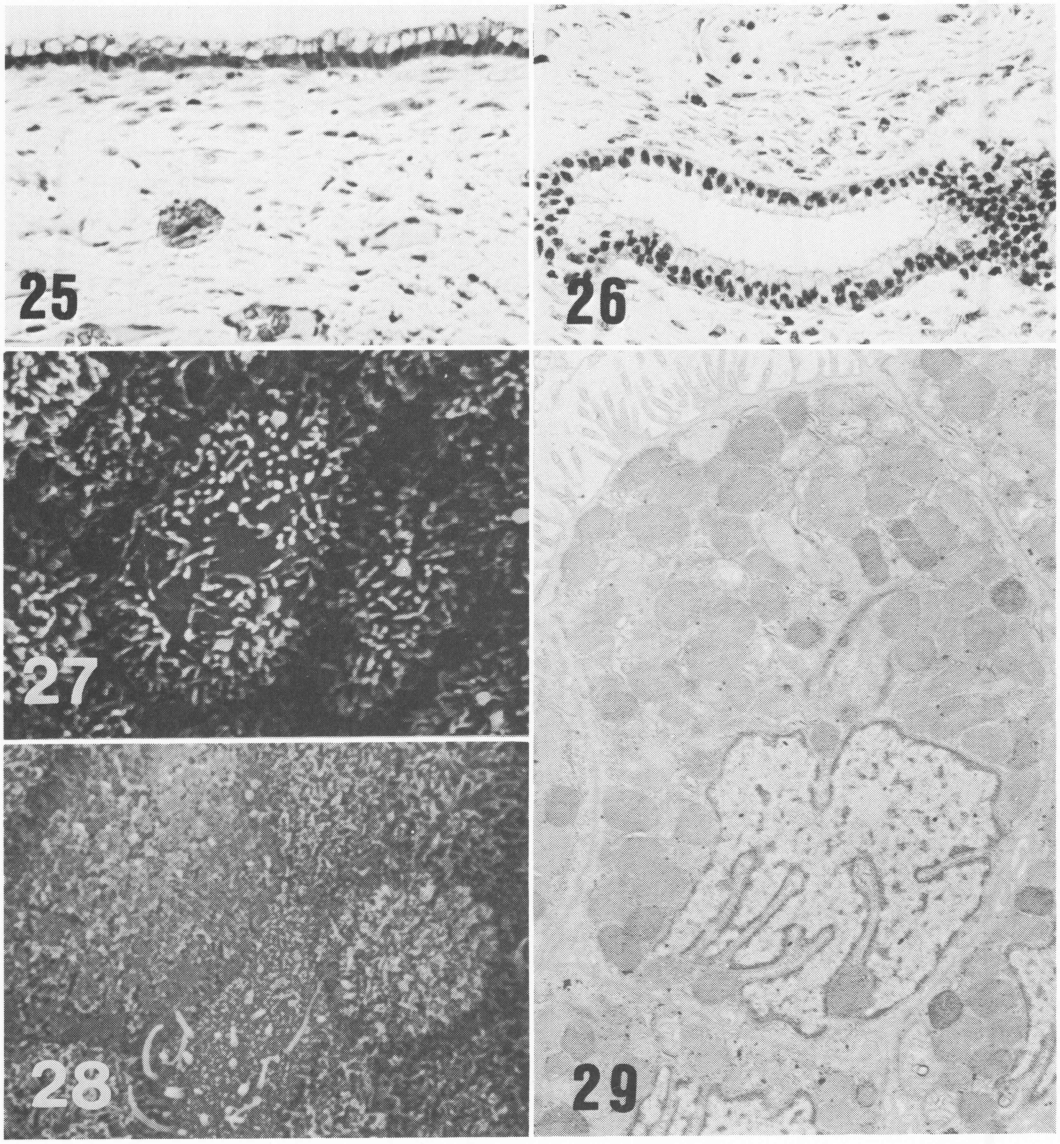
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