J. Fac. Fish. Anim. Husb.
Hiroshima Univ. (1966), 6: 357~371

Histological Observations on the Quail Oviduct; On the Secretions in the Mucous Epithelium of the Uterus

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(Text-fig. 1; Plates 1-4)

In the previous paper[1] (1965), the present authors mainly reported that the ciliated apical cells in the epithelium of the quail uterus contained the pigment granules belonging to porphyrin, which might be released from the cells to form the cuticular pigment of the quail egg, and that, in addition to this, the same cells contained the PAS-positive large granules.

In the uterus of the domestic fowl, the mucous surface epithelium is composed of the apical ciliated cells and the basal mucous cells (RICHARDSON[2], 1935; JOHNSTON *et al.*[3], 1963). Functions, however, of these two types of cells have not been clearly interpreted as far as relating to the egg formation. Thus, it is necessary to clear that each of these two types of the epithelial cells produces what kinds of secretion materials concerning the egg components.

For this purpose, the present authors observed the secretion materials of the mucous epithelial cells of the quail uterus, with reference to their histochemical properties and their features alternated with the egg formation stages.

MATERIALS AND METHODS

The tissues of the uterus were obtained from 36 cases of the Japanese quail (*Coturnix coturnix japonica*), of which oviducts belonging to various stages of the egg formation. Stages were divided 1 to 6, as follows: Stage 1; a soft egg covered with albumen was possessed in the magnum, Stage 2; an egg covered with shell-membrane in the isthmus, Stage 3; an egg covered with shell-membrane in the uterus, Stage 4; an egg covered with incompletely or completely formed shell in the uterus, Stage 5; an egg covered with colored cuticule in the uterus and Stage 6; no egg in the oviduct after laying.

Specimens were fixed with the following fixatives; CARNOY's, BOUIN's, 10% neutral formol, alcohol-formol, trichloroacetic acid-formol, alcohol, aceton and ZENKER-formol. Then they were embedded in paraffin and sectioned at 5 to 7 μ in thickness.

The used staining methods and procedure were as follows:

1. Hematoxylin-eosin
2. HEIDENHAIN's azan
3. Chrome alum hematoxylin (CH) (GOMORI[4], 1941)

4. Alcian blue (AB) (MOWRY[5], 1956)
5. Periodic acid-SCHIFF (PAS) (MCMANUS[6], 1948)
6. Alcian blue-periodic acid SCHIFF (AB-PAS) (MOWRY and WINKLER[7], 1956)
7. Periodic acid-phenylhydrazine-SCHIFF (PAPS) (SPICER[8], 1961)
8. Aldehyde fuchsin (AF) (HALMI[9], 1951)
9. Aldehyde fuchsin-Alcian blue (AF-AB) and Alcian blue-aldehyde fuchsin (AB-AF) (SPICER and MEYER[10], 1960)
10. Metachromasia with buffered azure A (SPICER and DUVENCI[11], 1964)
11. Mercury-bromphenol blue (HgBPB) (BONHAG[12], 1955)
12. Ninhydrin-SCHIFF (NS) (YASUMA and ICHIKAWA[13], 1953)
13. Performic acid-SCHIFF (PFAS) (PEARSE[14], 1951)
14. Performic acid-Alcian blue (PFAAB) (ADAMS and SLOPER[15], 1956)
15. Dimethylaminobenzaldehyde-nitrite (DMAB) (ADAMS[16], 1957)
16. Aceton Sudan Black (BERENBAUM[17], 1954)
17. Salivary digestion (ARCADI[18], 1952)

In addition to these paraffin sections, the specimens were cut into 1 mm^3 pieces, fixed in 1% osmium tetroxide (MILLONIG[19], 1961), dehydrated, embedded in Epon 812 (LUFT[20], 1961), sectioned on an ultramicrotome and stained with toluidine blue.

RESULTS

The mucosal epithelium of the uterus in the quail, as well as in the domestic fowl, is composed of two types of cells; the apical ciliated and the basal mucous (Fig. 1). The apical cells contain the pigment granules and the PAS-positive large granules (Fig. 20), and the basal cells weakly PAS-positive mucin (Fig. 2). The pigment granules in the apical cells are unstained, as mentioned previously (TAMURA *et al.*[1], 1965), with ordinary staining methods including polysaccharide-stains. The mucin granules in the basal cells are so fine that their granular structure is only clearly found in the ultrathin sections (Fig. 1).

With AB stain, the mucin was stained, but not the PAS-positive granules in the apical cells. With AB-PAS procedure, the PAS-positive granules were stained pink to red of PAS-reactivity, inversely, the mucin stained blue for AB-stainability (Fig. 3). In consequence, three types of granules were distinguished in the epithelial cells, the pigment granules and the PAS-positive granules in the apical ciliated cells, and the mucin or basophilic granules in the basal mucous cells. With CH stain for mucin, only the mucin was stained (Fig. 4). The PAS-reactivity of the PAS-positive granules as well as the mucin was uneffected respectively with diastase digestion.

With azure A buffered at pH 5.0, 3.5, 2.0, 1.0 and 0.5, only at pH 5.0, β-metachromasia was induced to the mucin (Fig. 5). With AF stain, a positive coloration was found in the mucin (Fig. 6). Moreover, it was stained with the first dye in the respective procedure of AF-AB and AB-AF. With PAPS procedure, the PAS-reactivity of the PAS-positive granules disappeared, but the reactivity of the mucin was somewhat retained. In addition to these, the PAS-positive granules disappeared

in the cells with acidic fixatives, as CARNOY's and BOUINS (Fig. 2).

Then, the proteins and amino-groups contained in these secretion materials were examined. With HgBPB method for proteins, these three types of granules were stained blue or green (Figs. 7, 8), as well as with NS method for amino-radical stained pink (Fig. 9). With PFAAB procedure for cystine or cystine-compound, the mucin granules were stained bluish-green (Fig. 10). With DMAB method for tryptophan, the PAS-positive granules were stained light green or blue, and the pigment granules somewhat paler (Fig. 11). With Sudan for bound lipids, the PAS-positive granules were clearly stained black, and the pigment granules and the mucin somewhat weakly stained.

In addition to these histochemical characters of the secretion materials, the relationships between their changes and the egg formation stages were examined. These results are illustrated in the Text-Figure 1.

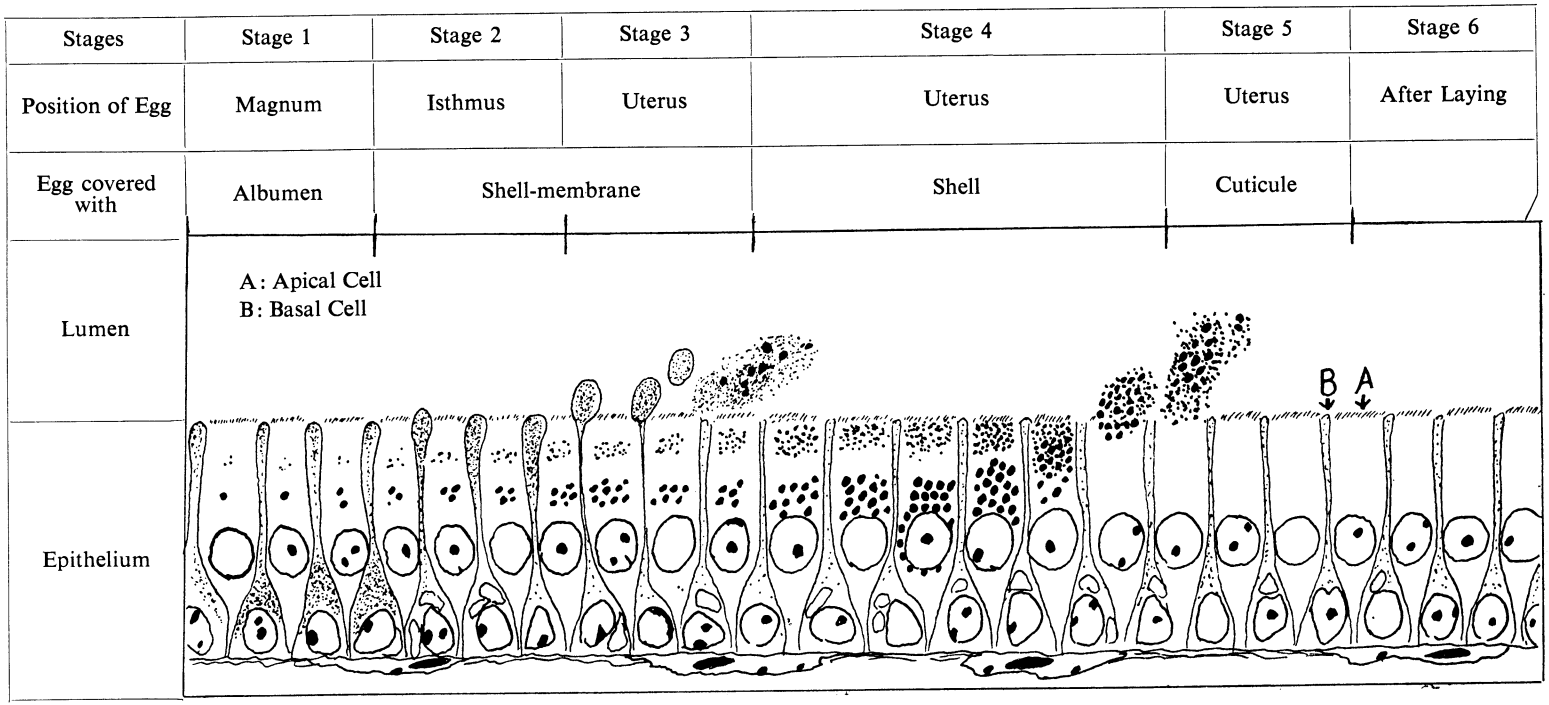
On the apical cells, the changes in quantity of the pigment and the PAS-positive granules were seen in parallel relation in each stage. In the Stage 1, both of them were scarcely found in the cells, then as the stages progressed (Figs, 14, 17, 20), they increased up to Stage 4. In Stage 4, in many cases, the cells were filled with their granules and presented a typical pattern of the cells (Fig. 20); the pigment granules accumulated in the apical cytoplasm, and the PAS-positive granules were swarmed near the nucleus. In some cases belonging to the stage, both granules were mingled and collected in the apical cytoplasm of the cells (Fig. 22), and also, agglutinated structures consisted of the PAS-positive and the pigment granules were found in the uterine lumen. In Stages 5 and 6, both granules in the cells were scarcely or not observed.

On the changes in the mucin of the basal cells, the characteristic figures were found both in Stages 2 and 3. In the former stage (Fig. 13), most of the basal cells contained a large quantity of the mucin, showing the most developed figure of the cells. Moreover, in the latter stage (Figs. 15, 16), numerous cells filled the mucin and projected into the lumen showing large apocrine projections, some of which were released into the lumen. The released structures were PAS-positive and consisted of fine granular materials and globular bodies (Fig. 18). In them, no pigment granules were found. In other stages, the basal cells were slender showing undeveloped figures.

DISCUSSION

The mucosal epithelium of the uterus in the quail is consisted of the apical ciliated cells and the basal mucous cells, as in case of the domestic fowl described by RICHARDSON[2] (1935) and JOHNSTON *et al.*[3] (1963). These two types of cells are characterized by their morphological features and secretion materials in them. The apical cells contain the pigment granules and the PAS-positive granules. And the basal cells do the basophilically stained mucin. The pigment granules are porphyrin (TAMURA *et al.*[1], 1965) and average in size and unstained with ordinary stain-

Text-Figure 1. Changes of Secretions in Uterine Mucous Epithelium.



ing methods including polysaccharide-stains. The PAS-positive granules are larger in diameter, and are unstained with polysaccharide-stains with the exception of the PAS method. The PAS-reactivity of it is blocked by phenylhydrazine, so that they may contain neutral mucopolysaccharide (SPICER[8], 1961) in their structures. The mucin is composed of very fine granules. They are weakly PAS-positive, AB-positive with both methods of AB, and AB-PAS, and weakly metachromatic with azure A at pH 5.0. On the basis of the conceptions of mucopolysaccharides (MOWRY[5], 1956, MOWRY and WINKLER[7], 1956; MOWRY and MORAND[21], 1957; SPICER and MEYER[10], 1960; SPICER[8], 1961), the mucin is thought to contain some acidic mucopolysaccharide. Moreover, according to the results of AF, AF-AB and AB-AF (SPICER[8], 1961), it is regarded to contain surphyl radical in the acid mucopolysaccharide polymer, namely, sulfated mucopolysaccharide.

In the domestic fowl, also, the granules similar to one in the quail in the apical cells were described by RICHARDSON[2] (1935) and JOHNSTON *et al.*[3], (1963). The former described them as basophilic granules and the latter as basophilic and PAS-negative. Therefore, the characters of the granules in the apical cells are presented in marked contrast between these two kinds of the fowl.

On the basal cells in the uterus of the domestic fowl, BRADLEY[22] (1928) and RICHARDSON[2] (1935) described respectively that the mucin was scarcely unstained with mucin stains. Recently, in the domestic fowl, CHAKRAVARTI and SADHU[23] (1961) mentioned that the mucin was acid mucopolysaccharide, inversely, FUJII *et al.*[24] (1965) that they were neutral mucopolysaccharide. JOHNSTON *et al.*[3] (1963) distinguished the basal cells into two types; those containing sulfated and acid mucopolysaccharide in the anterior uterus, and others containing neutral mucopolysaccharide in the posterior uterus. In the quail, however, the basal cells are same in their morphology and in the staining characters, throughout the uterus, and they are corresponded with the posterior uterine type of JOHNSTON *et al.*[3] Thus, these results on the characters of the basal cells are different according to the different authors.

With both procedures of HgBPB and NS, these three types of granules were stained, so that the pigment granules are presented as a pigment-protein, the PAS-positive granules as neutral mucopolysaccharide-protein complex, and the mucin granules as acid mucopolysaccharide-protein complex.

Each procedure of PFAAB (ADAMS and SLOPER[15], 1956) and DMAB (ADAMS[16], 1957) has been respectively estimated as the most specific and valuable method for cystine or cystine-compounds, and for tryptophan (PEARSE[25], 1961). Therefore, the results in these methods may introduce the interpretation that the PAS-positive granules, perhaps also the pigment granules, contain tryptophan, and the mucin granules cystine or cystine-compounds.

In addition to these characters, the PAS-positive granules disappeared in acidic fixatives. Also, the pigment granules were dissolved in acidic solutions (TAMURA *et al.*[1], 1965). Thus, some relationships between the PAS-positive granules and the pigment granules are suggested.

It is quite interesting that, in the apical cells of the quail, the PAS-positive

granules and the pigment granules showed a series of parallel changes. And these two types of granules were released at the same time into the uterine lumen, and, in the shell formation stage, the most developed figures of the apical cells were found. These findings are regarded as the following: The pigment and the PAS-positive granules in the apical cells increase and are stored in the stage of formation of the shell, and then, as soon as the shell is completely formed, these granules are released into the lumen to form the cuticule of the egg surface.

On the function of the granules of the apical cells, RICHARDSON[2] (1935) suggested the formation of the organic matrix of the shell, as JOHNSTON *et al.*[3] did (1963).

The present authors rather suggest that the mucin of the basal cells is related with the shell matrix, and, inversely, the apical cells in the quail are connected with the cuticule formation, because of the fact that they are changed with the pigment granules.

In the quail, the basal cells, relating to secretion, indicated the most developed and active figures in Stages 2 and 3, in which an egg covered with the shell-membrane was possessed in the isthmus and uterus. On the other hand, RICHARDSON[2] (1935) and JOHNSTON *et al.*[3] (1963) described respectively that the basal cells in the domestic fowl showed the maximum activity after the shell completed, and mentioned that the basal cells were related with the cuticule formation.

SIMKISS and TYLER[26] (1957) observed histochemically the shell, especially the organic matrix of it. They concluded that the organic matrix of the shell was protein-acid mucopolysaccharide complex. In this study, the mucin of the basal cells of the quail uterus contain acid mucopolysaccharide and protein, so the results are much similar to the results of SIMKISS and TYLER[26].

Therefore, the present authors suggest that the mucin of the basal cells of the quail uterus is released as soon as the egg reaches to the uterus, and is related to the formation of organic matrix of the shell. And those conceptions that the apical cells relate to the cuticule colored with the pigment and the basal cells to the shell matrix in the quail, are just contrary to the suggestions by RICHARDSON[2] (1935), and partially JOHNSTON *et al.*[3] (1963), that the apical cells relate to the shell matrix and the basal cells to the cuticule in the domestic fowl.

SUMMARY

In this investigation, the observations were made on the secretion materials in the surface epithelium of the quail uterus, with reference to their histochemical properties and their quantitative changes related to the egg formation. These results are summarized as follows:

1. The mucous surface epithelium in the uterus of the quail consisted of the apical ciliated cells and the basal mucous cells as described in the domestic fowl by RICHARDSON[2] (1935) and JOHNSTON *et al.*[3] (1963).
2. Three types of secretion materials were found in the epithelium; the pigment

granules (porphyrin) and the PAS-positive granules in the apical cells and the basophilic mucin granules in the basal cells.

3. Histochemically, the pigment granules were presented as pigment protein, the PAS-positive granules as neutral mucopolysaccharide-protein complex and the mucin granules as acid mucopolysaccharide-protein complex.
4. The PAS-positive granules and the pigment granules showed parallel appearances in their quantitative changes in the cells; they were maximum in the stage of the shell formation, then released into the uterine lumen to form their mingled structures, which were considered as the cuticular material.
5. The mucin granules in the basal cells were maximum in the stage of shell-membrane formation, and then released into the lumen, prior to the pigment and the PAS-positive granules. They were considered as related to the formation of the organic matrix of the shell.

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うずら卵管の組織学的研究；子宮部の粘膜上皮の分泌物について

田村達堂・藤井俊策

前報 (1965) では，うずら卵管子宮部の粘膜上皮の繊毛細胞 (apical cell) の色素 (porphyrin) について報じたが，子宮部粘膜上皮と卵形成との関係を明らかにするために，その粘膜上皮を構成している繊毛細胞 (apical cell) と粘液細胞 (basal cell) のそれぞれの分泌物について，組織化学的に観察し，またこれらの分泌像が卵の形成の各時期でどのように変化するかについて検索した．

該上皮の分泌物は，apical cell における色素顆粒 (porphyrin) および PAS 陽性顆粒，basal cell における塩基性粘液顆粒である．色素顆粒は蛋白反応にのみ陽性で，色素蛋白であること，PAS 陽性顆粒は中性粘液多糖類—蛋白複合体であること，粘液顆粒は酸性粘液多糖類—蛋白複合体であることがそれぞれ示された．

一方各時期における分泌像の変化については，basal cell は，峡部に卵殻膜で包まれた軟卵がある時期に多量の粘液を充し，ついでその卵が子宮部に至ると，卵殻形成以前に粘液を放出する．apical cell の両顆粒は常に相平行した関係を示し，卵が子宮部にあって卵殻が形成されている間に顆粒は著しく増加し，卵殻完成に伴なって放出される．

これらのことから，basal cell の粘液顆粒は卵殻の有機基質の形成に，apical cell の色素顆粒および PAS 陽性顆粒はクチクラ形成に関係するものと考えられる．

EXPLANATION OF FIGURES

Plate 1

Fig. 1. The mucous epithelium of the uterus is consisted of apical ciliated cells and basal mucous cells. The mucin granules are clearly presented. Ultrathinsection embedded in Epon 812. Toluidine blue stain. ×1,650.

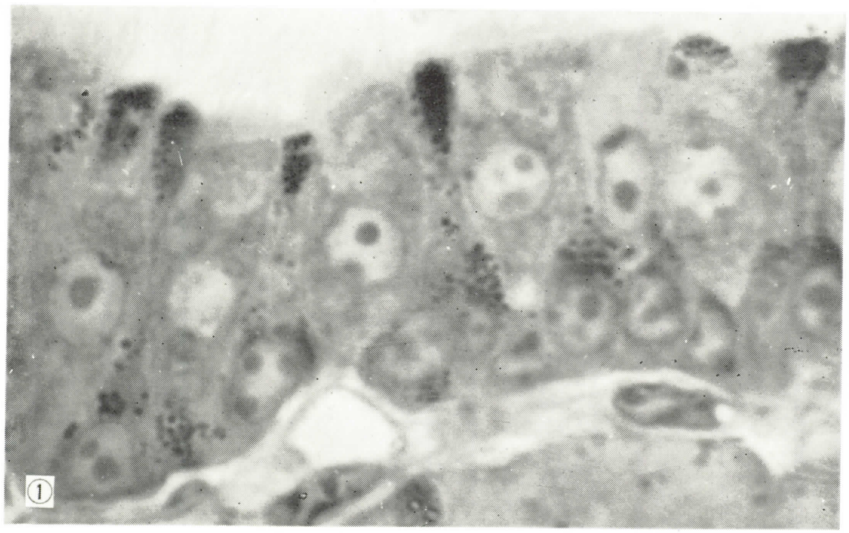
Fig. 2. The mucin in the basal cells is stained. PAS method. ×1,400.

Fig. 3. The apical cells contain PAS-positive granules and the mucin granules are stained with Alcian blue. AB-PAS procedure. ×1,000.

Fig. 4. The mucin is stained. CH stain. ×1,000.

Fig. 5. The mucin presents weak β-metachromasia. Azure A at pH 5.0. ×1,000.

Fig. 6. The mucin is stained. AF stain. ×1,000.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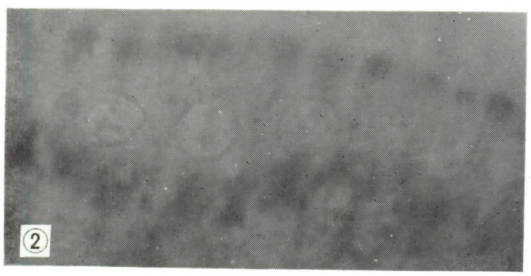
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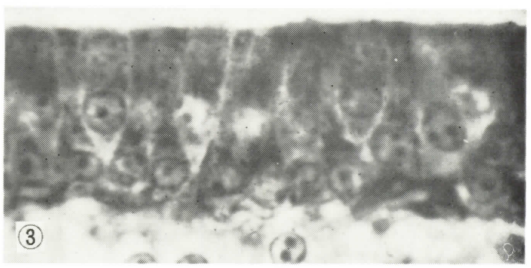
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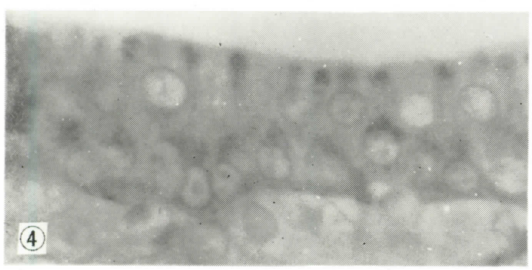
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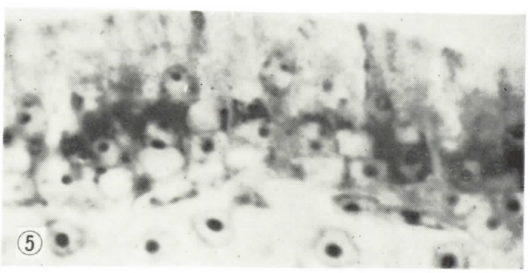
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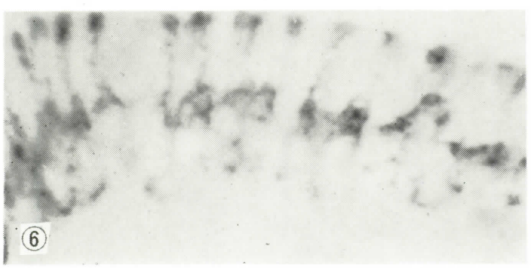
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Plate 2

Fig. 7. The pigment granules and the PAS-positive granules in the apical region (↑) of the apical cells are stained clearly. HgBPB method for protein. ×1,000.

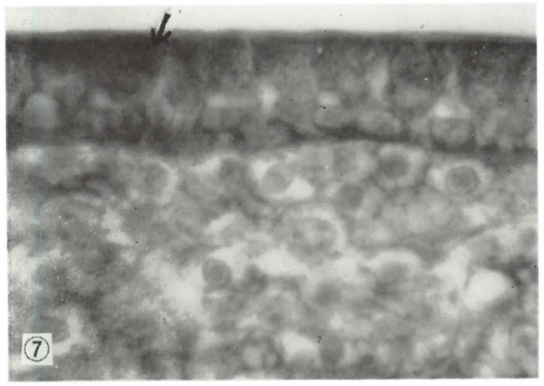
Fig. 8. The mucin of the basal cells is stained. HgBPB method. ×1.000.

Fig. 9. The granules in both types of cells are stained. NS method for amino-radical. ×1,000.

Fig. 10. The mucin is sharply stained. PFAAB procedure for cystine or cystine-compounds. ×1,000.

Fig. 11. In the apical cells, the PAS-positive granules are stained in bluish-green color and the pigment granules in somewhat paler color. DMAB method for tryptophan. ×1,000.

Fig. 12. The granules in both types of cells are stained. Aceton Sudan Black [stain] for bound-lipids. ×1,000.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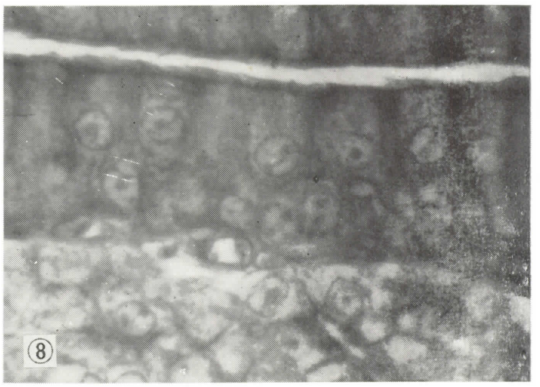
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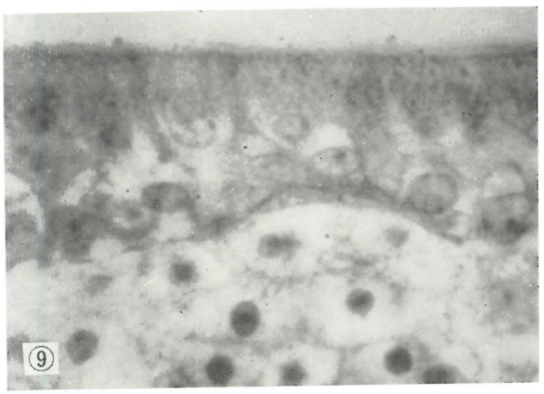
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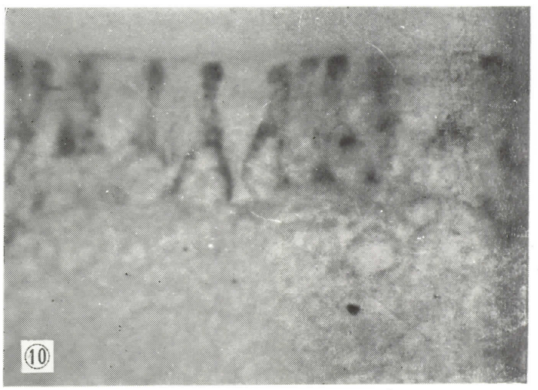
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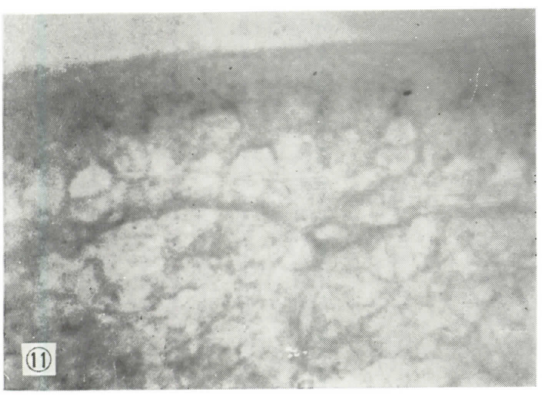
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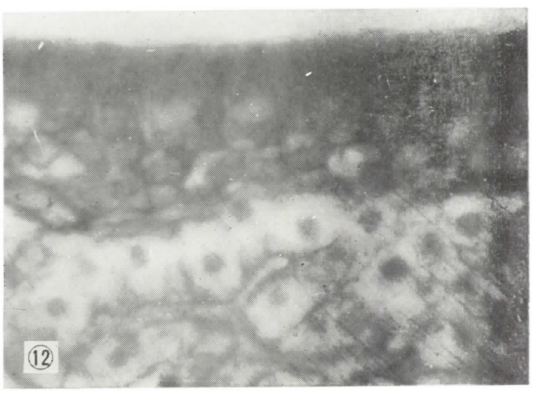
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Plate 3

Fig. 13. The epithelium in Stage 2. The basal cells fill the mucin, especially in the apical cytoplasm. Some of them swell to the uterine lumen. AB stain. ×1,000.

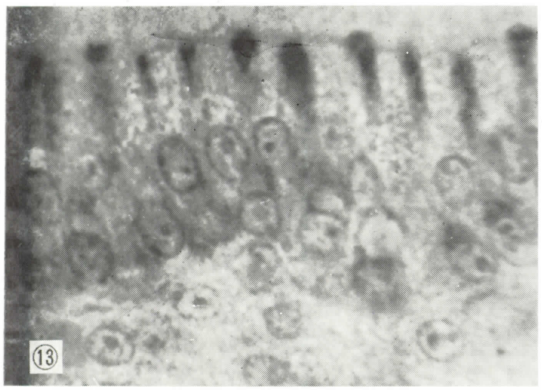
Fig. 14. The epithelium in Stage 2. The PAS-positive materials (PA) are found in the supranuclear region and the unstained pigment granules (P) are in the apical region. PAS method. ×1,000.

Fig. 15. The epithelium in Stage 3. The basal cells present releasing features of the mucin. Also a large apocrine projection is seen. AB stain. ×1,000.

Fig. 16. The epithelium in Stage 3. Many releasing features of basal cells are presented, AB stain. ×1,000.

Fig. 17. The epithelium in Stage 3. The pigment granules (P) and the PAS-positive granules (PA) increase in the apical cells. PAS method. ×1,000.

Fig. 18. The released materials from the cells in Stage 3 are consisted of fine granules and grobular bodies. These materials are PAS-positive, but do not contain the pigment granules. PAS method. ×1,000.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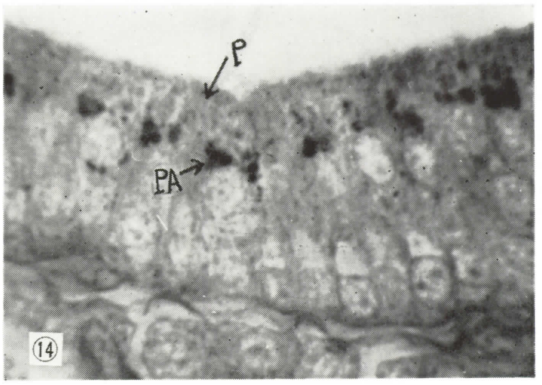
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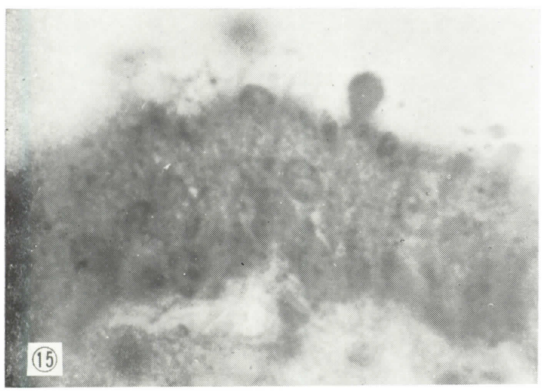
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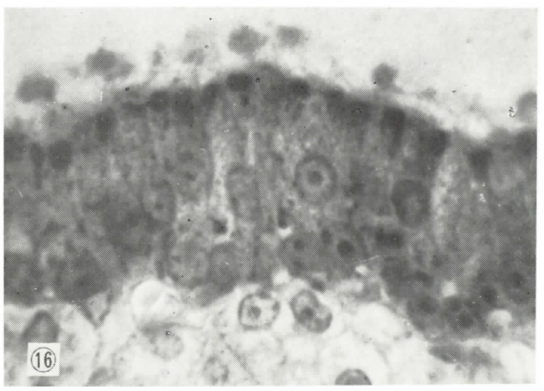
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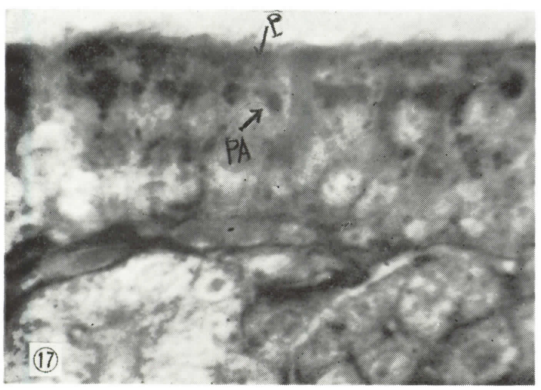

P
PA
⑰

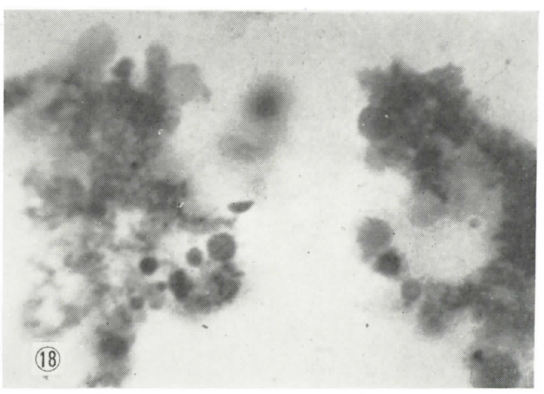

⑱

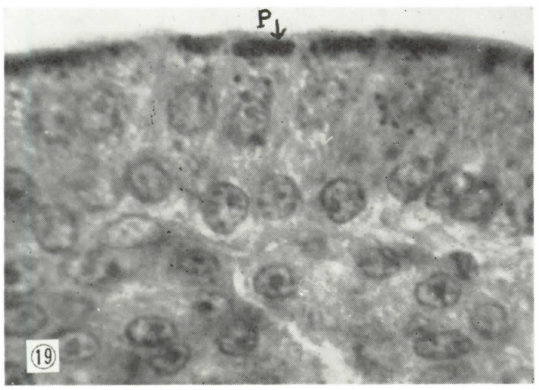
Plate 4

Fig. 19. The epithelium in Stage 4. The mucin is not found in the basal cells. In the apical cells, the pigment granules (P) increase and congregate in the apical cytoplasm. AB stain. ×1,000.

Fig. 20. The epithelium in Stage 4. Especially, the typical feature of the apical cells is presented. The PAS-positive granules (PA) situate near the nucleus (N) and the pigment granules (P) in the apical cytoplasm. PAS method. ×2,000.

Fig. 21. The epithelium in Stage 4. The mucin is not found in the basal cells. The pigment granules (P) are found in tips of the apical cells. AB stain. ×1,000.

Fig. 22. In the apical cytoplasm of the apical cells, both types of granules mingle each other, and these materials are released from some of the cells. The released materials are consisted of the yellow pigment granules and the PAS-positive grobular bodies. PAS method. ×1,000.


P
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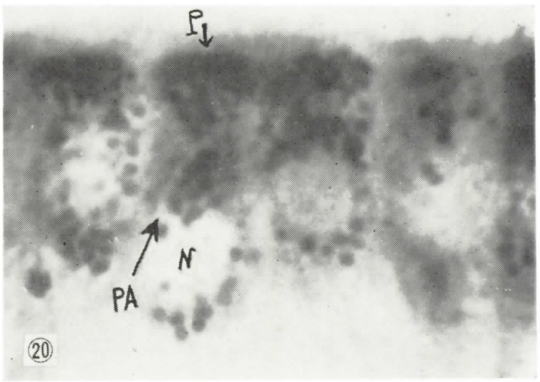
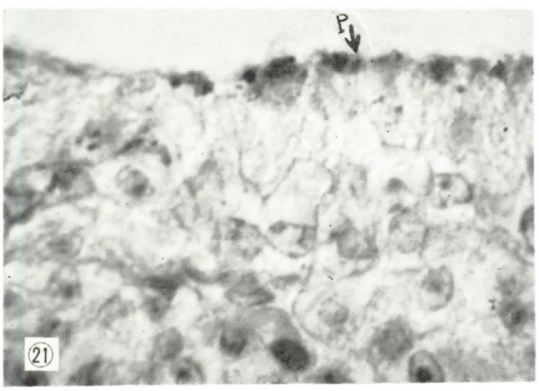

P
N
PA
20


P
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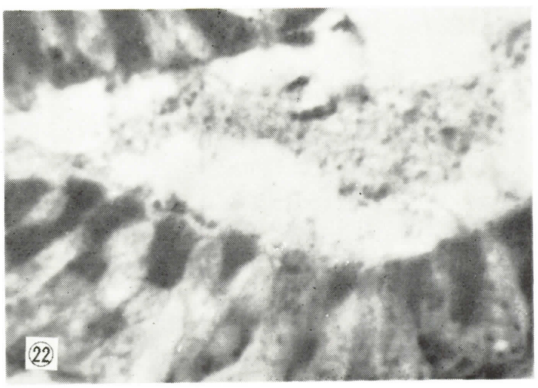
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