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Citation	Japanese Journal of Veterinary Research, 32(2), 43-57
Issue Date	1984-04-28
DOI	https://doi.org/10.14943/jjvr.32.2.43
Doc URL	https://hdl.handle.net/2115/4692
Type	departmental bulletin paper
File Information	KJ00002374205.pdf



MORPHOLOGICAL STUDY ON THE JUNCTURA CELLS OF THE RAT OVIDUCT AFTER HORMONAL TREATMENT¹

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(Received for publication February 21, 1984)

Morphological changes of the epithelium of the uterotubal junction (UTJ) by prolonged treatment of 17 β -estradiol, progesterone or oxytocin in ovariectomized rats was studied at the ultrastructural level. The epithelium of the UTJ in ovariectomized and oxytocin treated rats was characterized by the appearance of a number of immature epithelial cells and a few ciliated cells. Sometimes a few secretory granules and lysosome-like structures were found. After estradiol treatment, the epithelial cells were characterized by a rough surfaced endoplasmic reticulum (rER) with remarkably dilated cisternae and well developed Golgi apparatus. These epithelial cells showed active formation of the secretory granules but no evidence of their secretion. After progesterone treatment, the epithelial cells were shown to have numerous cytoplasmic protrusions of various shapes and sizes. A few peg-cells and a lamellar structure in the secretory granules were shown.

These data suggest that the formation of the secretory granules in the epithelial cells of the UTJ became active by estradiol treatment, whereas their active release exclusively due to the progesterone treatment.

Key words: uterotubal junction, epithelial cells, estradiol, progesterone.

INTRODUCTION

The uterotubal junction may present a barrier to sperm or gamete transport in the female reproductive tract of mammals; however, understanding of its role in such transport is still far from complete.^{3,4,9)} Although there is general agreement that estrogen is essential for maturation of secretory cells, formation of secretory granules and ciliogenesis of ciliated cells in the oviductal epithelium of various animals, including man,^{2,5,6,12-15,18,19)} most of these have devoted attention primarily to the changes of the oviductal epithelium, whereas relatively little attention has been paid to the epithelium

¹ This work was supported in part by a grant from the Association of International Education, Japan, for which grateful acknowledgement is made.

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of the UTJ in steroid hormone treated rats.

The present investigation was focussed mainly on the morphological changes in this epithelium following prolonged treatment of 17 β -estradiol, progesterone or oxytocin in ovariectomized rats using the electron microscope.

MATERIALS AND METHODS

Twenty virgin Wistar rats (approx. 3 to 4 months of age) were bilaterally ovariectomized under chloroform anesthesia two weeks before use. The ovariectomized rats were subcutaneously injected with antibiotics 500 i.u. (Ampiclox, Chongkeun Dang Co. Ltd., Korea) for five days to prevent bacterial infections, and vaginal smears were taken for analysis for 14 days.

Each experiential animal received a subcutaneous injection of either 1 μ g 17 β -estradiol (Sigma Chem. Co., Ltd.) or 2.5 mg progesterone (Nakarai Chem. Ltd.) in 0.5 ml propylene glycol, or 0.25 i.u. of oxytocin (oxyvet, Bayer Vetchem Ltd., Korea). The control animals received a subcutaneous injection of 0.5 ml propylene glycol alone. Each animal was injected for 10 days respectively and bled at 8 to 9 hours after the final injection of hormones under chloroform anesthesia.

UTJ on both sides were dissected out, and the short lengths (1–2mm) were fixed in 2% glutaraldehyde-2.5% paraformaldehyde mixture in 0.1 M sodium cacodylate buffer solution (pH 7.5) for 48 hours. After post-fixation in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer solution (pH 7.5) for 2 hours, the pieces were embedded in epoxy resin (Epon-812). Ultrathin sections were prepared on a porterblum MT-I type ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Hitachi HU-12A electron microscope. Semithin sections (1 μ m) were sliced from the tissue blocks, stained with 1% toluidine blue in 0.1 M phosphate buffer solution (pH 7.2) and used for determining the segments.

RESULTS

After ovariectomy, the epithelium of the UTJ was characterized by the appearance of a number of immature epithelial cells, relatively long microvilli at the cell surface and a number of apical secretory granules in the secretory cells (fig. 1). The secretory granules were frequently observed to have a core profile (fig. 2). Moderately developed Golgi apparatus, which was located at the supranuclear region, was observed. The apical membrane of the secretory cells was relatively bulged; however, no evidence of secretory release was present (figs. 1 & 2). Sometimes a few multivesicular bodies were present. A few rough surfaced endoplasmic reticulum (rER) were slightly dilated (fig. 2). In addition, a small Golgi apparatus, a few mitochondria, ribosomes, multivesicular bodies and short profiles of rER were characteristic of the immature epithelial cells of UTJ. Ciliated cells were often found, and the rootlets and ciliae were present in their apical cytoplasm (fig. 1).

After oxytocin treatment for 10 days of the ovariectomized rats, the epithelium of the UTJ generally showed the same findings as those after ovariectomy; however, a large number of ciliated cells and lysosome-like structures than these observed in the ovariectomized rat UTJ were found (figs. 3 & 4).

After estradiol treatment, there was no ciliated cells and appeared secretory cells alone. These cells were relatively voluminous and had a smooth apical surface as a whole, but in some cells a few cytoplasmic protrusions were present. The supranuclear cytoplasm contained many long profiles of remarkably distended rER, which were filled with flocculent materials (figs. 5 & 6). There was no distinctive ultrastructural evidence for secretion in the secretory cells: however, active formation of the secretory granules was observed (fig. 6). The epithelial cells also showed the well-developed Golgi apparatus. On the maturing face of the Golgi apparatus, many membrane-bound secretory granules in different sizes and densities were found (fig. 6). A relatively few secretory granules and small vesicles were present at the apical surface (fig. 5). Also, a few mitochondria and a number of ribosomes were found. Microvilli were relatively long, and a few multivesicular bodies were often found in the supranuclear region.

After progesterone treatment, the epithelium of the UTJ was characterized by the appearance of numerous cytoplasmic protrusions of various shapes and sizes (figs. 7 & 9). In the cytoplasmic protrusions a few rER and small vesicles, and a number of ribosomes were present; however, no other cell organelles were found (fig. 9). Relatively short and sparse microvilli were present at the apical surface (fig. 7). Many multivesicular bodies were also shown (figs. 8, 9 & 11). Golgi apparatus was moderately developed, and rather short profiles of rER were found in the cytoplasm. In the apical portion of the secretory cells, no secretory granules were present (figs. 7, 9 & 10). Peg-cells (so-called dark cells) were often found (fig. 10). In the cytoplasm of the peg-cells, a few scattered mitochondria, a few short rER, a moderate number of multivesicular bodies and Golgi apparatus were found. Secretory granules adjacent to the Golgi apparatus of the secretory cells were shown to be circular membrane-bound granules of various sizes and densities, and the characteristic lamellar structures within the secretory granules were encountered (fig. 11). The profiles of cell division were also frequently found.

DISCUSSION

In this study of estradiol treatment, frequent appearance of remarkably dilated cisternae of rER, well-developed Golgi apparatus and many membrane-bound secretory granules of various sizes and densities indicates that the formation of secretory granules is activated by estrogen. And after progesterone treatment, the appearance of numerous cytoplasmic protrusions at the apical surface of the epithelium is morphological evidence for the release of the secretory granules by the progesterone.

It has been suggested that the formation and release of secretory granules of the oviductal epithelium in various animals, including man, were accelerated by artificial estrogen treatment and/or estrogen secretion at the estrus stage in the physiological estrous cycle.^{2,12,13,18,19)}

Recently, it was described that the nonciliated cells of the cat oviduct were clearly dependent on both the presence of estradiol and the absence of progesterone for their functional differentiation into and maintenance of the secretory state.²⁾

Apical cytoplasmic protrusion has been reported as an apocrine type of secretion in the human,⁵⁾ ewe¹²⁾ and bovine¹³⁾ oviduct during the luteal phase of the estrous cycle, whereas other investigators have described these during the estrus phase.^{2,16)}

On the other hand, some investigators have also reported the appearance of these structures as an apocrine secretion on the uterine surface during the luteal phase and by progesterone treatment.^{1,8,10,17)} In this study, the cytoplasmic protrusions were seen only after the progesterone treatment, and the present authors agree with NAYAK et al. (1976) and NAKAK & ELLINGTON (1977) on the cellular responses of the oviductal epithelium to the steroid hormones, even though the estimated oviductal segment is different.

It was reported that no ciliated cells exist in the UTJ of the rat oviduct.⁷⁾ However, the present investigation showed the presence of a few ciliated cells after ovariectomy and oxytocin treatment.

It was shown that the vascular system of the UTJ were furnished with a peculiar potential for a response to progesterone, whereas that of the oviduct shows a potential response to estrogen.³⁾ The present authors also suggested that the junctura cells differ from the cells in the other segment of the oviduct in morphologic profiles.¹¹⁾

The results of the present study show that the effects of prolonged hormone treatment on the UTJ in ovariectomized rats are clearly different from those of the oviductal epithelium; namely, the estrogen treatment exclusively activates a secretory function. In contrast to the response which occurs in the oviductal epithelium, the present investigation suggests that the epithelium of the UTJ becomes active in the formation of secretory granules by estradiol treatment, whereas the release of secretory granules is activated by progesterone treatment.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 Epithelium of UTG in ovariectomized rat
Note one ciliated cell (CC), a number of immature cells (IC) and secretory cells (SC) A number of secretory granules (arrow) are also seen. $\times 4,000$
- Fig. 2 Electron micrograph of the epithelium of UTJ after ovariectomy showing moderately developed Golgi apparatus (G), a number of secretory granules at the apical surface (arrow) and core profiles of secretory granules (double arrow) $\times 6,000$

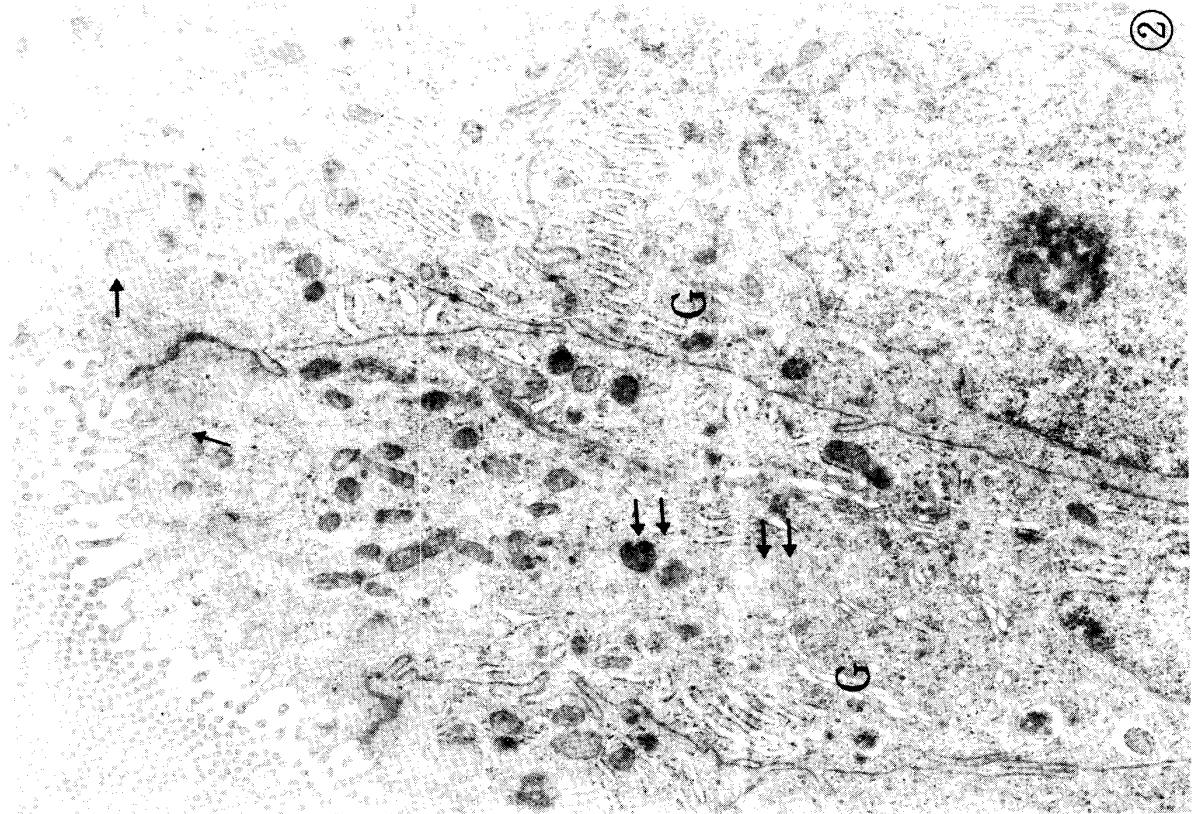
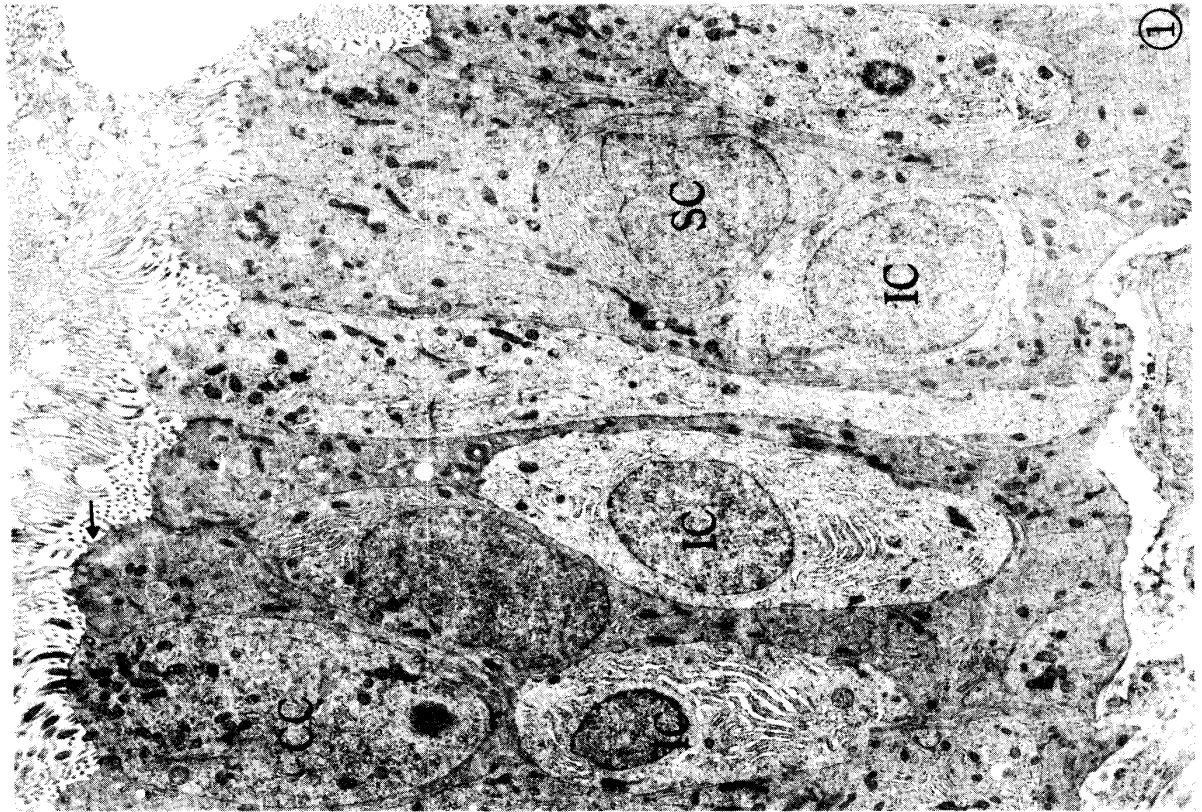
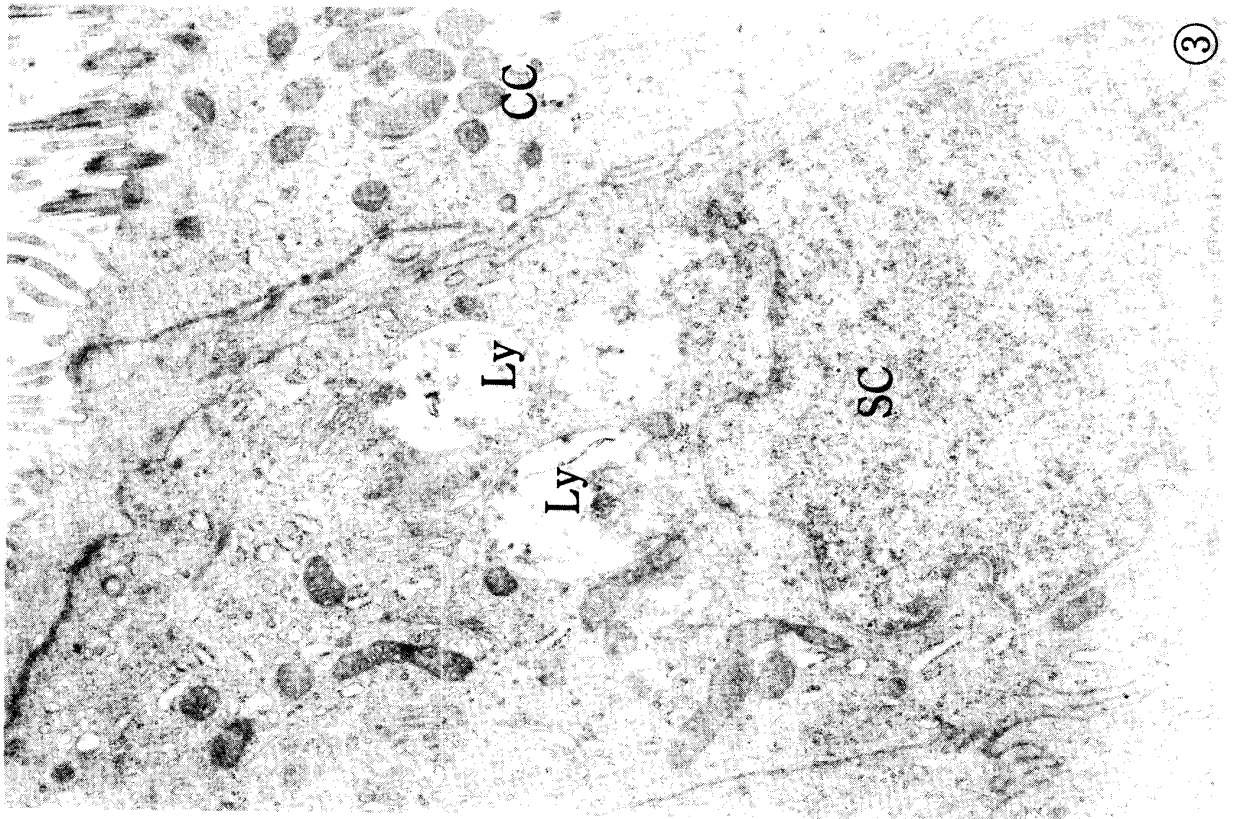
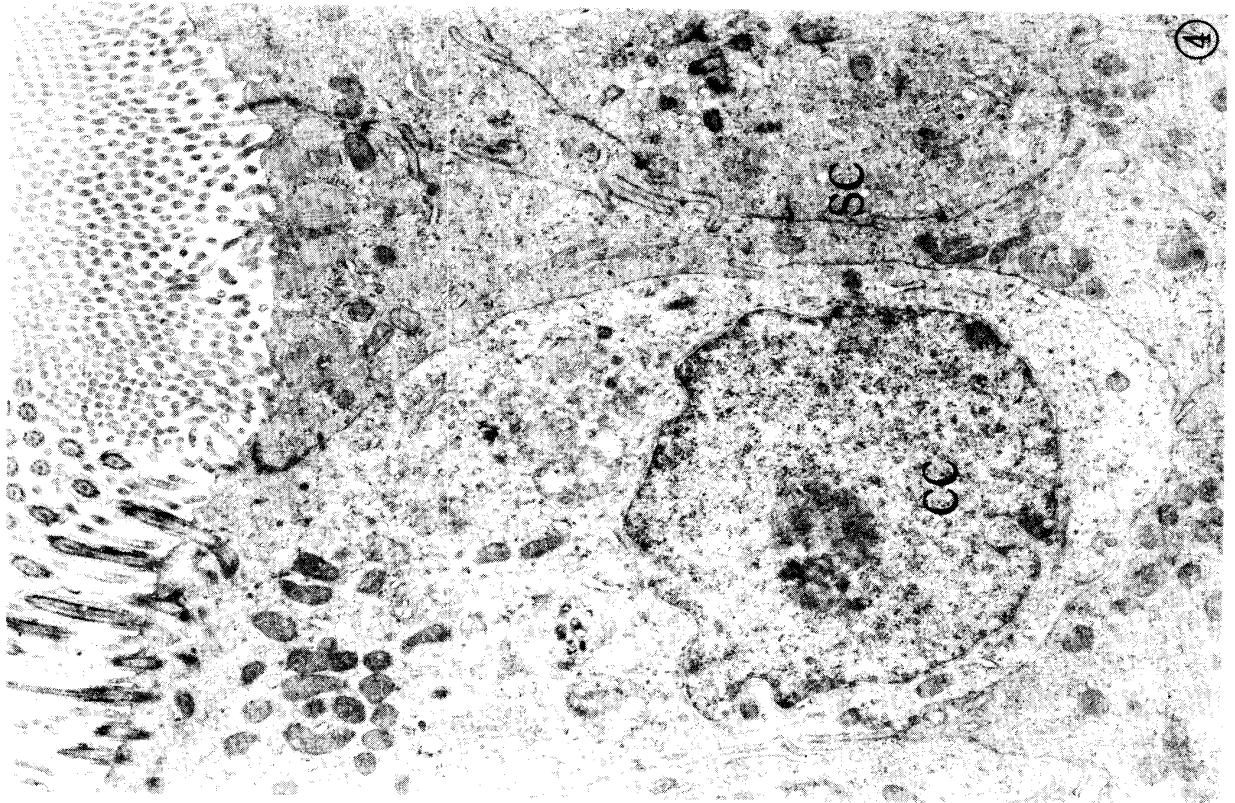


PLATE II

- Fig. 3 Epithelium of UJT after oxytocin treatment
Part of a ciliated cell (CC) and a secretory cell (SC) are seen.
In the cytoplasm of the secretory cell (SC), numerous lysosome-like structures (Ly) are observed. $\times 12,000$
- Fig. 4 Epithelium of UTJ after oxytocin treatment
A ciliated cell (CC) and part of a secretory cell (SC) are observed.
A number of secretory granules are seen at the apical surface of the secretory cell. $\times 8,000$



3



4

PLATE III

Fig. 5 Epithelium of UTJ after estradiol treatment

Note the remarkably dilated rER and well-developed Golgi apparatus (G)

A few small secretory granules (arrow) are also seen. $\times 10,000$

Fig. 6 Epithelium of UTJ after estradiol treatment

Note the remarkably dilated rER and well-developed Golgi apparatus (G)

On the maturing face of the Golgi apparatus, many membrane-bound secretory granules (SG) in different sizes and various densities are seen. $\times 20,000$

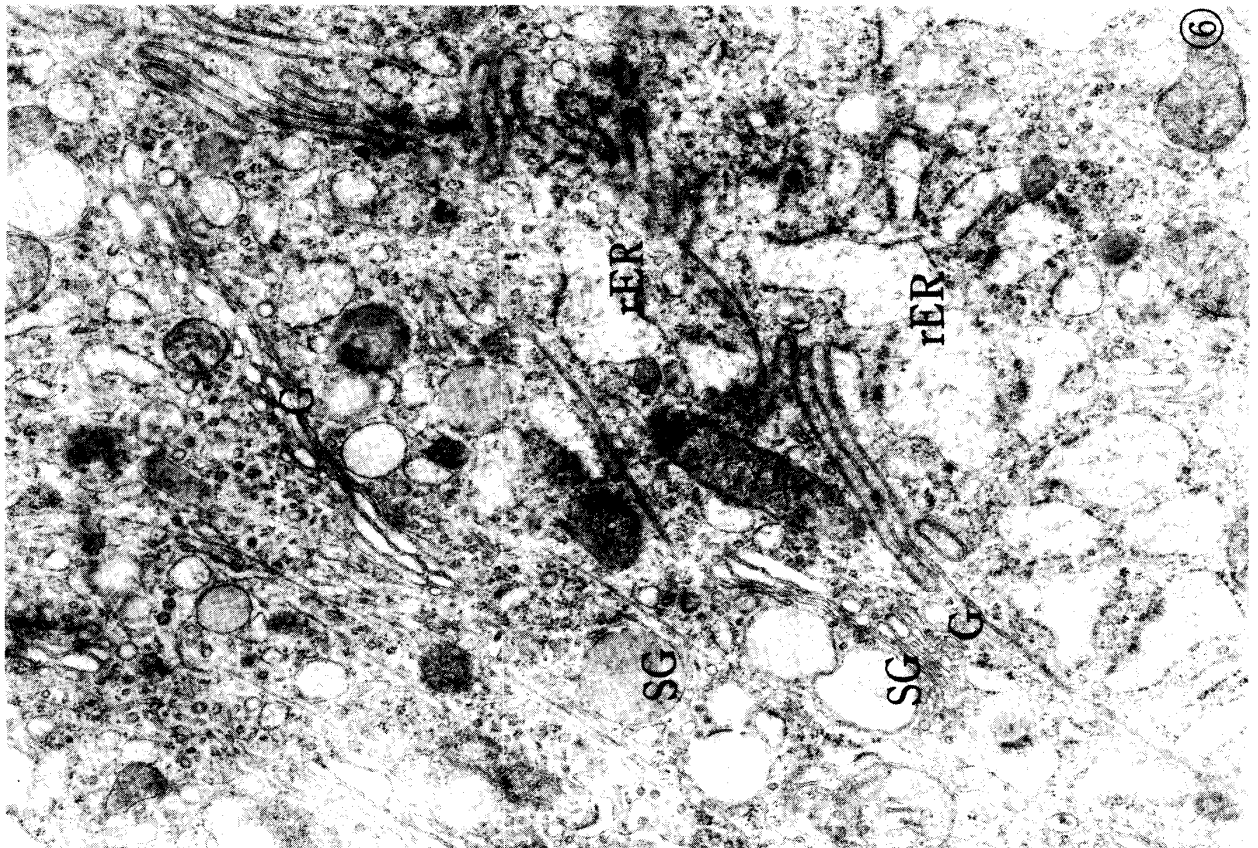
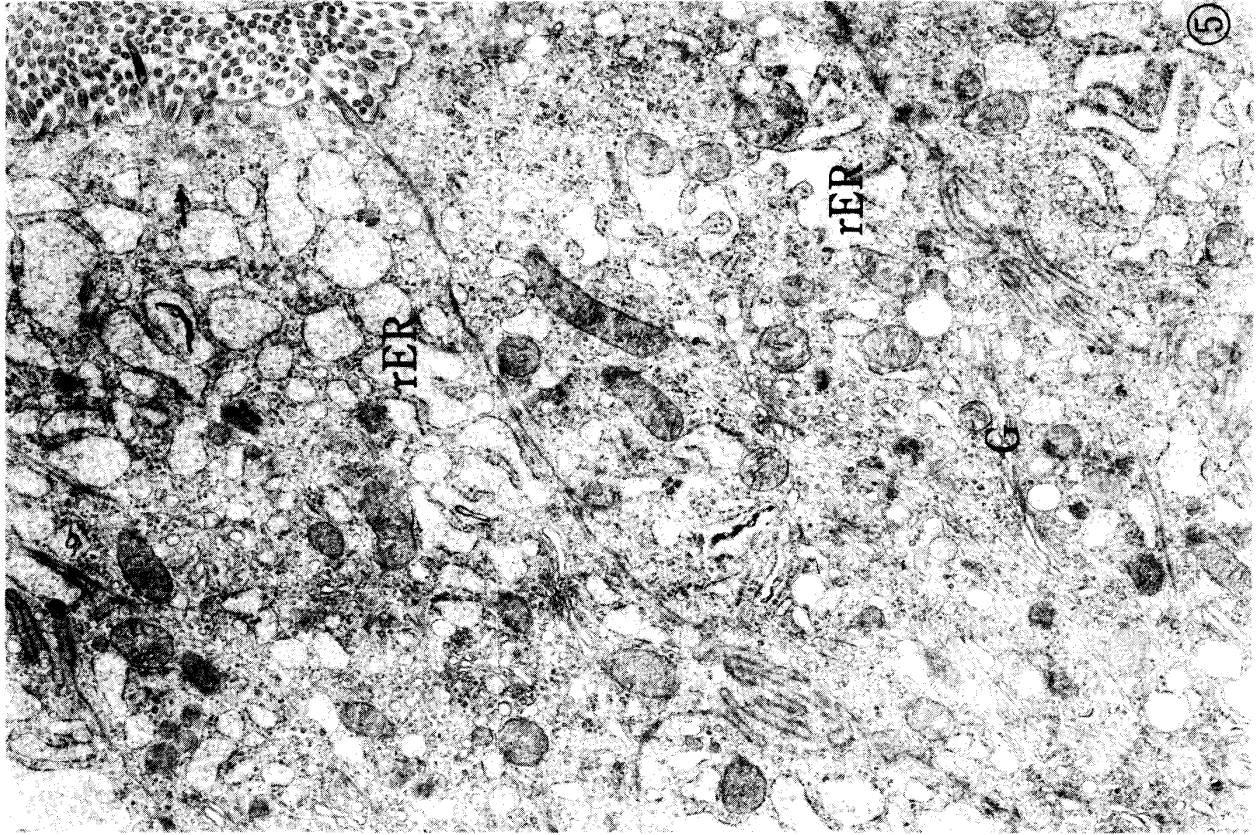


PLATE IV

- Fig. 7 Epithelium of UTJ after progesterone treatment
Note the cytoplasmic protrusion (arrow) and absence of secretory granules on the apical surface
Relatively short sparse microvilli are seen. $\times 4,000$
- Fig. 8 Epithelial cell of UTJ after progesterone treatment
High magnification of multivesicular body. $\times 40,000$
- Fig. 9 Higher magnification of Fig. 7
In the cytoplasmic protrusion, a relatively few cell organelles are seen. One multivesicular body (MB) is also seen. $\times 12,000$

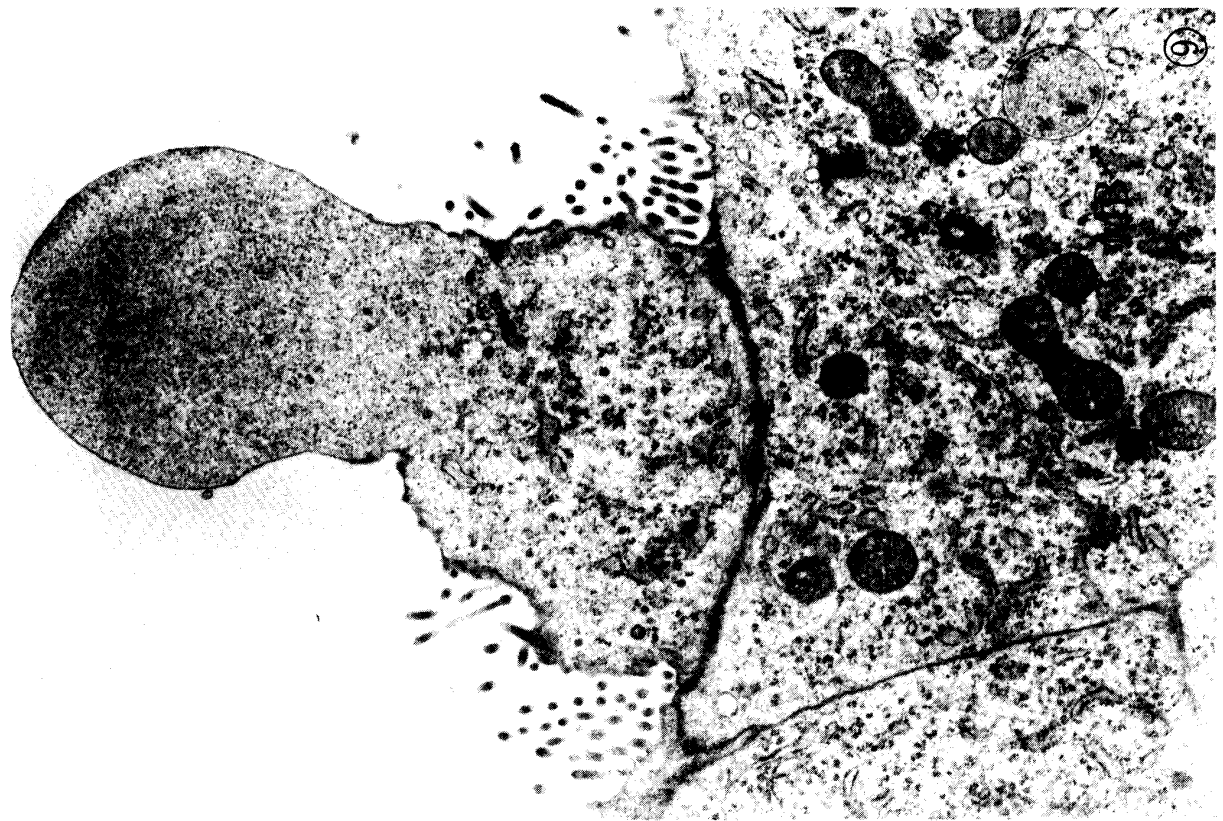
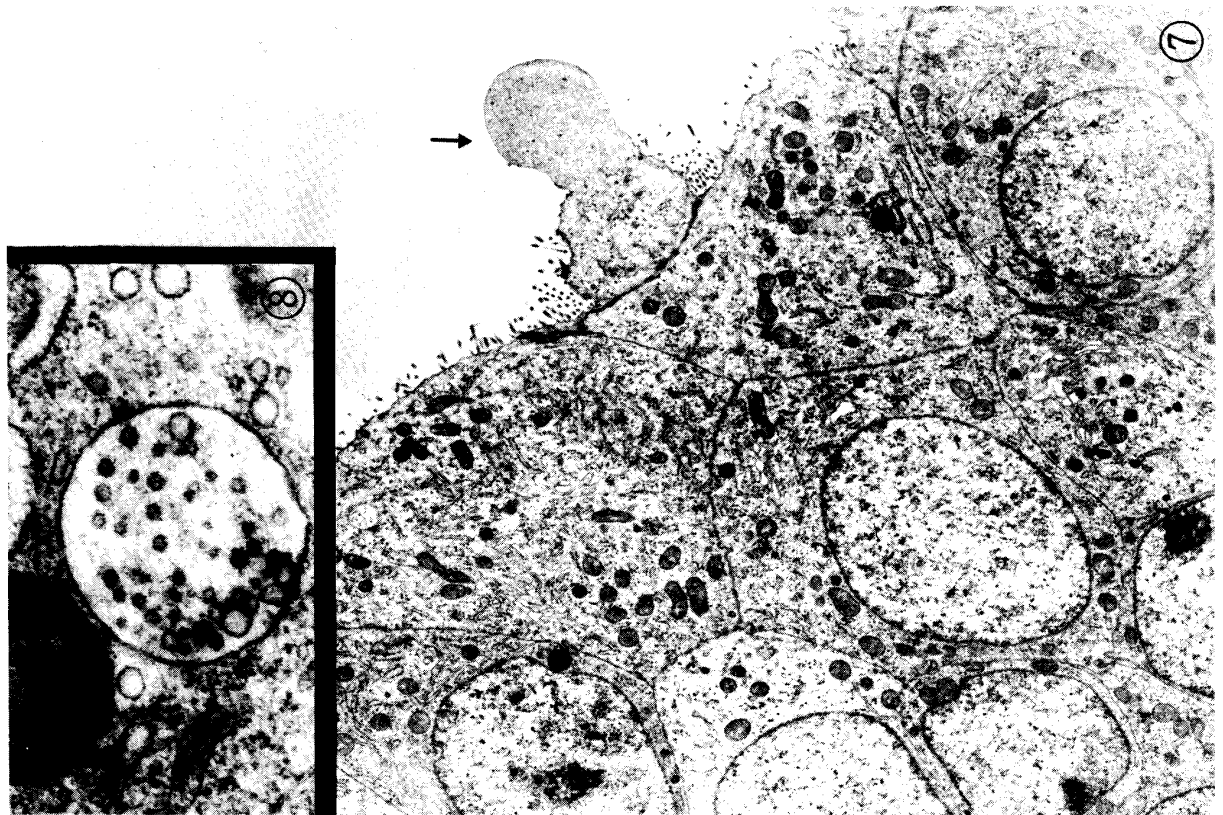


PLATE V

Fig. 10 Epithelium of UTJ after progesterone treatment
A peg-cell (PC) is seen. $\times 6,000$

Fig. 11 Epithelial cell of UTJ after progesterone treatment
Note lamellar structures (arrow) in the granule
One multivesicular body (MB) is also observed. $\times 40,000$

