

ESTROGEN AND ANTAGONIST-INDUCED CHANGES IN ENDOMETRIAL TOPOGRAPHY OF IMMATURE AND CYCLING RATS

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ABSTRACT

The topographical changes of the luminal surface of the endometrium of immature and ovariectomized rats treated with estrogen, antagonists to estrogen, and progesterone, and during various stages of the estrous cycle and in pregnancy were examined by scanning electron microscopy. Massive increases in numbers and length of endometrial cell microvilli were observed at estrus, after injection of estradiol-17 β , diethylstilbestrol, estrogen plus progesterone, or the inhibitor CI628 to immature and ovariectomized rats. Withdrawal of the estrogen stimulus results in diminution of microvilli, producing a state identical to diestrus, during pregnancy, and after injection of progesterone. The estrogen antagonist appears to have both estrogenic and progestogenic properties, stimulating endometrial cell hypertrophy, secretion of protein, and production of numerous apical microvilli.

Changes in the proliferative and secretory activity of the uterine endometrium occur in response to the rhythmical variations in the secretion of ovarian hormones. Primed by a period of estrogen dominance at ovulation and a subsequent shift to progestogen dominance and permissive estrogenic action, the endometrial epithelium undergoes the structural and physiological changes necessary for blastocyst attachment and implantation *in utero*. This report presents details of the endometrial topography during periods known to be under cyclical control by ovarian hormones, in pregnancy, and in immature rats treated with estrogen and antagonists to estrogen.

MATERIALS AND METHODS

Administration of Hormones and Antagonists

48 immature female albino rats (21–23 days old) were used in this study. The rats, separated in groups of six,

¹ Figs. 1–17 appear at the conclusion of the text.

were given daily subcutaneous injections of: estrogen, (a) diethylstilbestrol (Sigma Chemical Co., St. Louis, Mo.), or (b) estradiol-17 β (Sigma), at concentrations of 0.1–0.4 μ g dissolved in 0.1 ml glycerol; estrogen antagonist, (c) nafoxidine (Upjohn 11,100), or (d) Parke Davis CI628, at a concentration of 500 μ g in 0.1 ml glycerol; antagonist plus estrogen (e) CI628 (500 μ g), followed by estradiol-17 β (0.4 μ g) after 30 min; estrogen plus progestogen (f) estradiol-17 β (0.4 μ g), plus 1 mg progesterone (Sigma), dissolved in 0.1 ml glycerol; progestogen (g) 1–2 mg progesterone in 0.1 ml glycerol; and glycerol (h) 0.1 ml glycerol.

In some experiments spayed rats were injected with estrogen, antagonists, and combinations of both, at doses indicated above. Both immature and ovariectomized rats were given daily injections of hormones, antagonists, or vehicle, and were sacrificed at 12-h intervals for a period of 96 h. The glycerol-treated rats served as controls.

The Mature Cycling Rat

Mature female rats at different stages of the estrous cycle were also used for studies of the uterine topography. The specific stages (proestrus, estrus, diestrus) were

selected after vaginal smears were examined. The endometrial surface of pregnant animals (14–21 days of gestation) was also examined.

Animal Preparation and Electron

Microscope Techniques

Rats under light ether anesthesia were perfused via the dorsal aorta with formaldehyde-glutaraldehyde fixative (Karnovsky, 1965) for 30 min. The uteri were excised and immersed in cold fixative for an additional 15–30 min. Uteri were then carefully cut into small squares (2–4 mm²), rinsed in cold 0.1 M cacodylate buffer at pH 7.4, and postfixed in cold 2% osmium tetroxide. Subsequent to alcohol-water dehydration, the tissues either were embedded in Epon for transmission electron microscopy, or were dried by the CO₂ critical point method. The dried tissues were attached to metal stubs with silver paint, subsequently coated with gold-palladium in a vacuum evaporator, and examined with Hitachi HFS-2 and Stereoscan (ETEC) scanning electron microscopes operating at 10–20 kV. The photographs were taken on Polaroid 55/PN film at varying scanning angles and magnifications.

RESULTS

The early response to estrogen and antiestrogen included cellular hypertrophy and increased formation of granular endoplasmic reticulum. This pattern is in marked contrast to the epithelium from uteri of ovariectomized or immature females, which was composed of low cuboidal cells with poorly developed Golgi and granular endoplasmic reticulum cisternae (Figs. 2–4). These unstimulated cells had few blunt microvilli and rounded cytoplasmic processes that intruded into the lumen (Fig. 1). Few glands opened to the uterine lumen (Fig. 3).

Early topographical changes in the lining epithelium were apparent 12–24 h after injection of estrogen or inhibitor. The most prominent change was in the increase in number and height of microvilli per cell (Fig. 5). Increase in microvilli occurred on most cells throughout the uterine surface. Glands opening to the lumen were now relatively numerous, but not deep, and seemed to form by the involution of the lining cells (Fig. 6). These gland-openings were surrounded by cells possessing numerous elongate microvilli.

Subsequent changes include further production of microvilli, hypertrophy of the lining cells, and bulging of the apical cell surface into the lumen. These pseudopod-like structures were generally devoid of organelles and resembled somewhat the pinopods described by Enders and Nelson (1973).

At 72 h after injection of estradiol or diethylstilbestrol, the glands were now very numerous (Fig. 6 A), the pits of which contained clusters of microvilli or secretory product. No extensive pinocytotic activity was observed at the base of the microvilli of lining and glandular epithelial cells. The topography of the lining cells remained in this state as long as estrogen was administered to the animal. Withdrawal of estrogen for prolonged periods (24–48 h) was associated with a decrease in number of microvilli.

The endometrial lining of progesterone-treated immature rats was somewhat flattened. These roughly hexagonal cells possessed sparse numbers of microvilli and displayed a topography resembling the endometrium of the immature or spayed state. Estrogen plus progestogen treatment induced an endometrial pattern similar to that of estrus.

The inhibitors nafoxidine and CI628 also elicited massive formation of microvilli within a 72-h period after injection into immature rats. The cells were two to three times as high as those treated with estradiol-17 β , and their apices bulged into the lumen (Figs. 7 and 8). Several narrow glands opened between the lining cells (Fig. 9).

Topography of the Endometrium During the Estrous Cycle

The uterine surface at all phases of the estrous cycle was thrown into longitudinal folds, the folds being more pronounced near the cervix. At proestrus, the cells lining these folds bulged into the lumen and displayed morphological similarity to the endometrium of inhibitor-treated rats. These cells, displaying very uneven heights, possessed numerous microvilli (Fig. 10). Few glands opened to the lumen at this time.

The uterine surface was most uneven at estrus, in spite of the ballooning effect of the luminal fluid. The endometrial cells displayed the most highly varied morphologies. Most cells possessed massive numbers of microvilli (Fig. 11). Interspersed between these were cells displaying smooth apical topographies and with few microvilli. Examined with the transmission electron microscope, these smooth cells possessed a highly vesiculated apical surface. Deposits of secretory material adhered to the lining cell surfaces.

Glands were most numerous at estrus. The lining cells often formed pronounced clusters around the glandular pits (Fig. 12). Deposits of

secretory material often occluded the pits (Fig. 12, inset).

At diestrus, the epithelial cells were somewhat flattened with a roughly hexagonal appearance (Fig. 13). Most cells possessed sparse numbers of microvilli. Others were either laden with microvilli or entirely smooth. Few cells having clusters of elongate microvilli were interspersed between the lining cells. These elongate microvilli displayed some blebbing of their surfaces (Figs. 14, 15). Few glands were present in the lumen.

The Pregnant Uterus

The uterine surface of the pregnant rat was extremely irregular, the flattened apical surface of the epithelial cells having a few microvilli (Figs. 16, 17).

DISCUSSION

Cyclic morphological changes in the fallopian tube and the endometrium reflect specific physiological effects of the ovarian hormones (Schueller, 1968; Brenner, 1969; Ferenczy et al., 1972; Patek et al., 1972 *a, b*; 1973; Patek and Nilsson, 1973).

Studies by Nilsson (1970) and Ljungkvist and Nilsson (1971) have reported significant changes in the luminal surface of the human endometrium that are related to hormonal levels. Decrease in size of microvilli and increased protrusion of the apical cell surface into the luminal cavity characterize the topography of the endometrial surface during the secretory phase (Johannisson and Nilsson, 1972). Cilia that degenerate during the secretory phase regenerate during the proliferative phase. It appears, therefore, that the surface of the lining cells undergoes cyclic changes correlated to variations in ovarian hormonal levels.

In nonprimate mammals, several studies have established that the uterine surface epithelium undergoes pronounced morphological changes in response to estrogenic influences (Nilsson, 1958 *a, b*; 1959 *a, b*). In response to estrogen, microvilli increased in numbers and height to effect a significant augmentation of luminal surface membrane.

Other studies have shown that changes in the luminal epithelium are correlated with periods of preimplantation and implantation (Warren and Enders, 1964; Ljungkvist and Nilsson, 1971; Psychoyos and Mandon, 1971). In a recent scanning/transmission electron microscope study, Enders and Nelson (1973) have described the formation of ectoplasmic projections (called pinopods) during

periods before implantation and in delayed implantation. These pinopods seem to function in the endocytosis of luminal fluid and in transport into the endoplasm.

Our report draws attention to the rat uterine endometrium as a model where structural changes in the luminal topography reflect the physiological effect of various ovarian hormones. Indeed, the morphological state of the lining epithelium can be modulated by treating immature and ovariectomized rats with estrogen and antagonists to estrogen. In addition, the uterine lining cells display morphologies characteristic for the various phases of the normal estrous cycle. The massive increase in numbers and length of uterine cell microvilli observed at estrus, after injection of estradiol, diethylstilbestrol, estrogen plus progesterone, or of inhibitor to immature or spayed rats, reflects the extensive amplification of the uterine lining in response to ovarian hormones. Since cell surface amplification appears not to be associated with extensive pinocytotic activity of luminal proteins, other physiologically significant functions may be performed by this membranous interface. It is possible, for instance, that these microvilli may be functioning in the absorption and transport of electrolytes involved in uterine metabolism. Indeed, the presence of alkaline phosphatase activity on the lining cell membrane supports this suggestion (Borell et al., 1959).

The highly developed microvillar surfaces may even be related to sperm transport at estrus and/or to blastocyst-uterine binding subsequent to coitus and fertilization. The estrogen-stimulated formation of cell surface membrane may be related therefore to the production of specific binding sites that make the uterus receptive to the blastocyst.

Withdrawal of the estrogenic stimulus leads to diminution of microvilli at the cell surfaces, and to an apparent retardation of intracellular protein synthesis and of secretory activity of the endometrial cells. Estrogen withdrawal usually leads to surface membrane withdrawal, producing a state identical to that of diestrus and during pregnancy.

The estrogen antagonist CI628 appears to have estrogenic and progestogenic properties, a suggestion previously made by Heuson et al., (1972) for the antagonist U11-100A. The inhibitor stimulates endometrial hypertrophy, secretion of proteins, and production of numerous microvilli, reminiscent of proestrus of the normally cycling rat.

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FIGURE LEGENDS

FIGURE 1 Scanning micrograph of the luminal surface of the uterus of the immature rat. Several small blunt microvilli and large rounded processes (star) project into the lumen. $\times 34,000$.

FIGURE 2 A longitudinal section through the endometrium of the immature female rat is illustrated in this light micrograph. Nuclei are located centrally and basally; lipid droplets accumulate at their bases. $1\text{-}\mu\text{m}$ thick section stained with toluidine blue. $\times 1,560$.

FIGURE 3 A low magnification scanning micrograph of the uterine surface of the immature rat shows gland openings that are widely dispersed over the surface. $\times 600$.

FIGURE 4 A transmission electron micrograph of the epithelium of the immature rat uterus is shown here. A poorly developed Golgi apparatus, few cisternae of the granular endoplasmic reticulum, many polysomes, and few mitochondria are illustrated in the cytoplasm of these cells. Few short microvilli project into the lumen (arrows). $\times 3,400$.

FIGURES 5 AND 6 These scanning micrographs show the luminal surface of uterine epithelial cells at 24 h after the injection of immature rats with estradiol- 17β . Slender, elongate microvilli now project from the apices of the epithelial cells; the microvilli show a marked heterogeneity in length, but are longest near gland openings. $\times 17,000$, $\times 8,500$. A low-magnification scanning micrograph (Fig. 6 A) shows numerous gland openings into the uterine lumen. $\times 600$.

FIGURES 7-9 The uterine surface of immature rats given three injections of inhibitor (CI 628) over a 72-h period is shown in these scanning micrographs. Numerous microvilli extend into the lumen from the lining cells (Figs. 7, 8), and a narrow gland opening is shown (Fig. 9). $\times 3,600$, $\times 6,000$, $\times 6,000$.

FIGURES 10-15 The following scanning micrographs show the luminal surface of the uterus of mature rats with vaginal smears displaying various phases of the estrous cycle.

FIGURE 10 At proestrus, the protruding apical surfaces of the lining cells possess numerous elongate microvilli. Few uterine glands open into the lumen. $\times 3,400$.

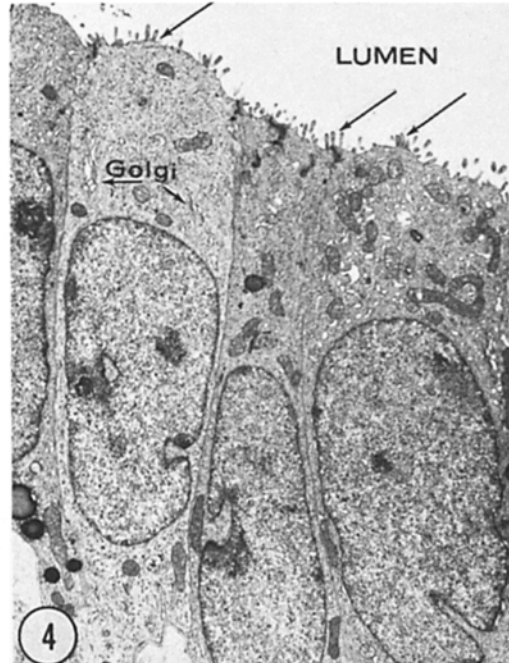
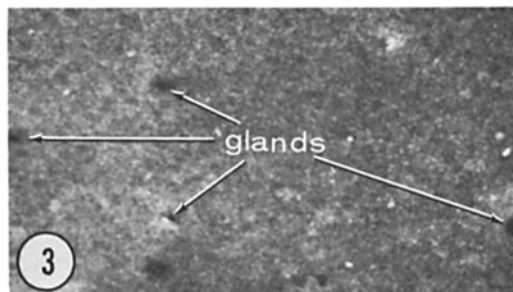
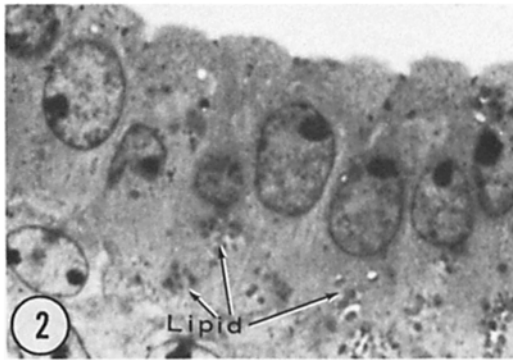
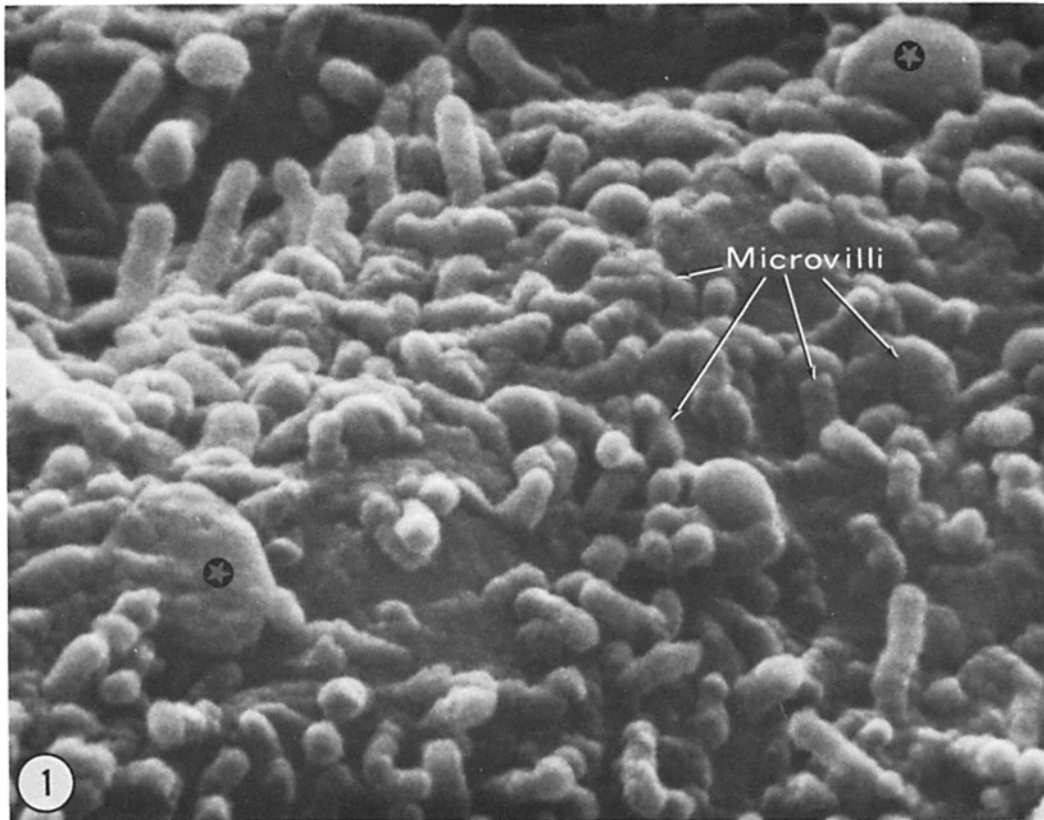
FIGURE 11 Interspersed between lining cells possessing numerous microvilli are smooth-surfaced cells (arrows) that bulge into the uterine lumen of a rat in estrus. The microvilli of some cells are clumped and appear to be coated with extracellular material. $\times 3,500$.

FIGURE 12 This low-magnification scanning micrograph (Fig. 12, *inset*) displays the uneven surface of the uterine lining and the presence of numerous gland openings of a rat in estrus. Clumps of extracellular material (presumably secretory product) lie adjacent to the gland openings (*sp. inset*). Several large rounded cells surround the narrow gland openings (arrow). *Inset*, $\times 600$. $\times 18,500$.

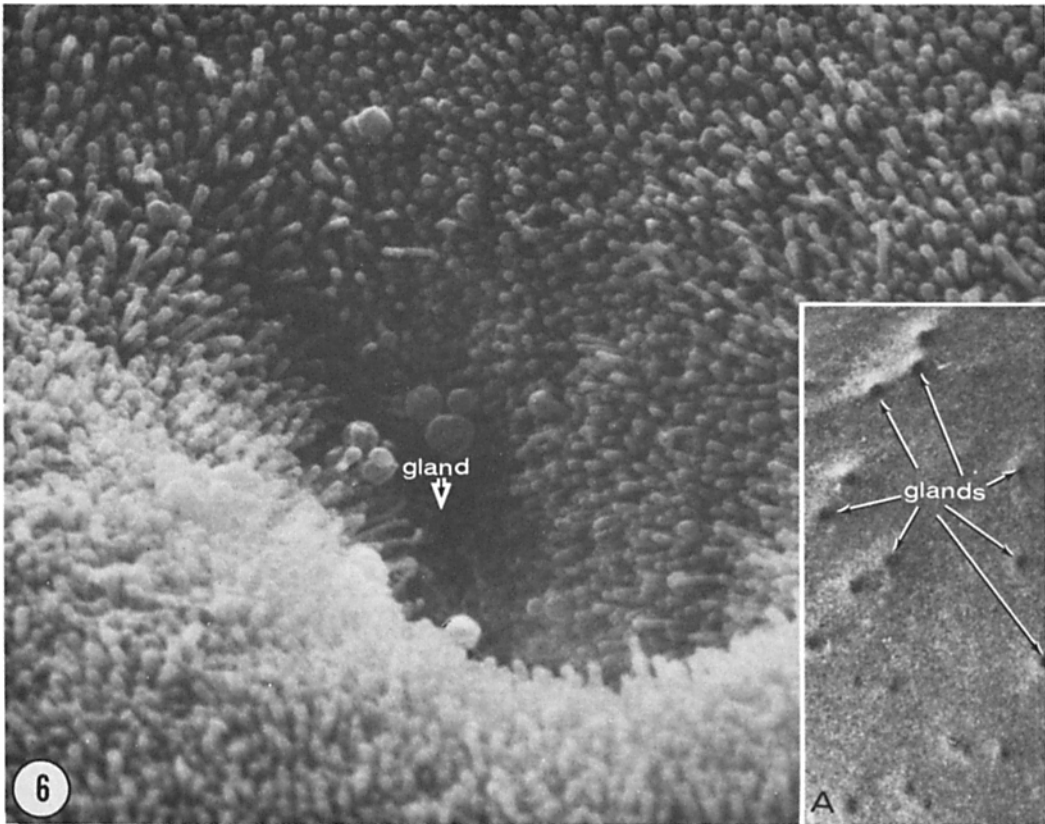
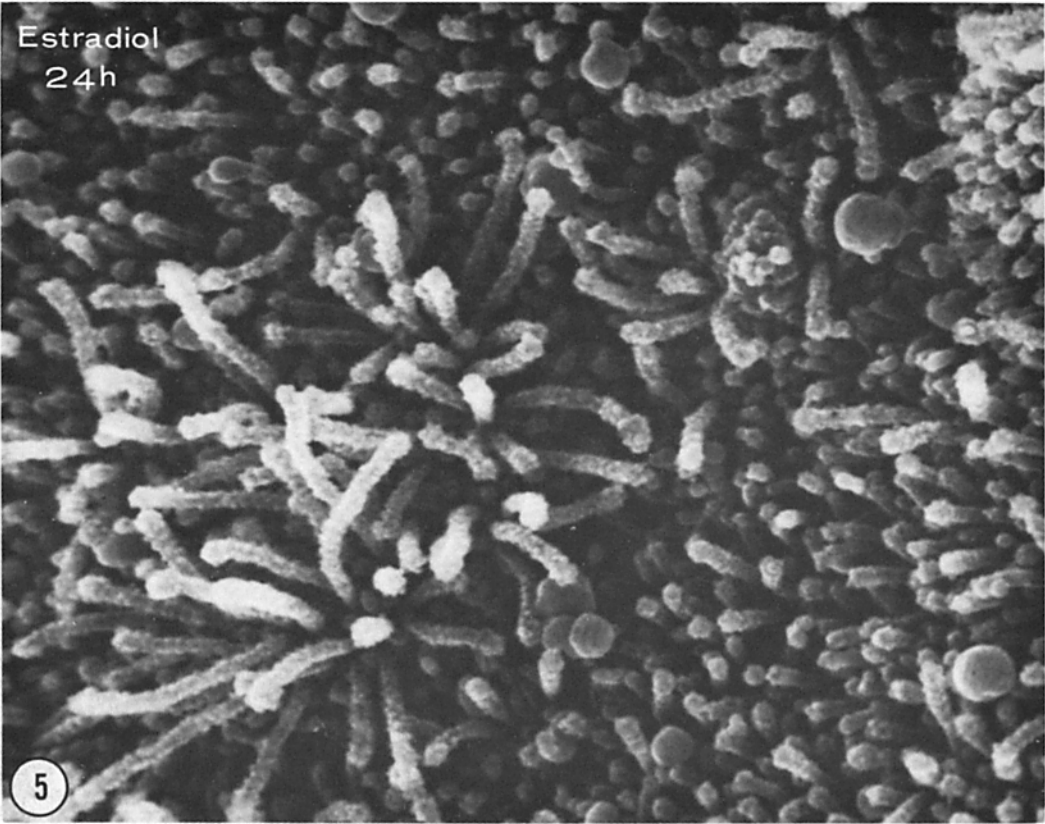
FIGURE 13 At diestrus, the roughly hexagonal cells are flattened and they display in this scanning micrograph luminal surfaces with tufts of microvilli, sparse distribution of microvilli, and smooth surfaces. $\times 3,400$.

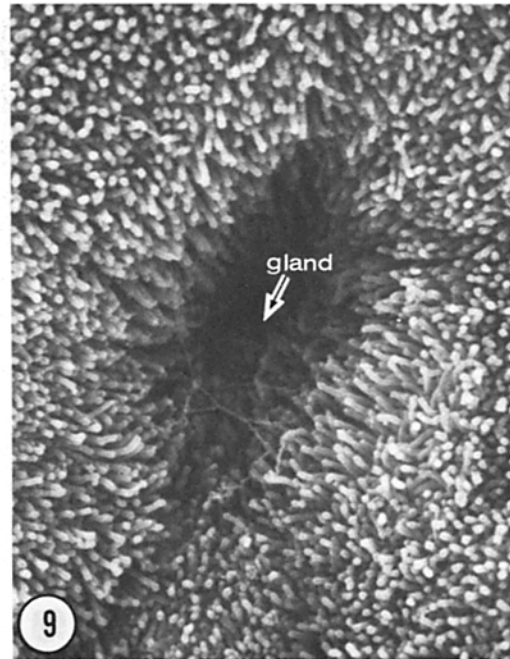
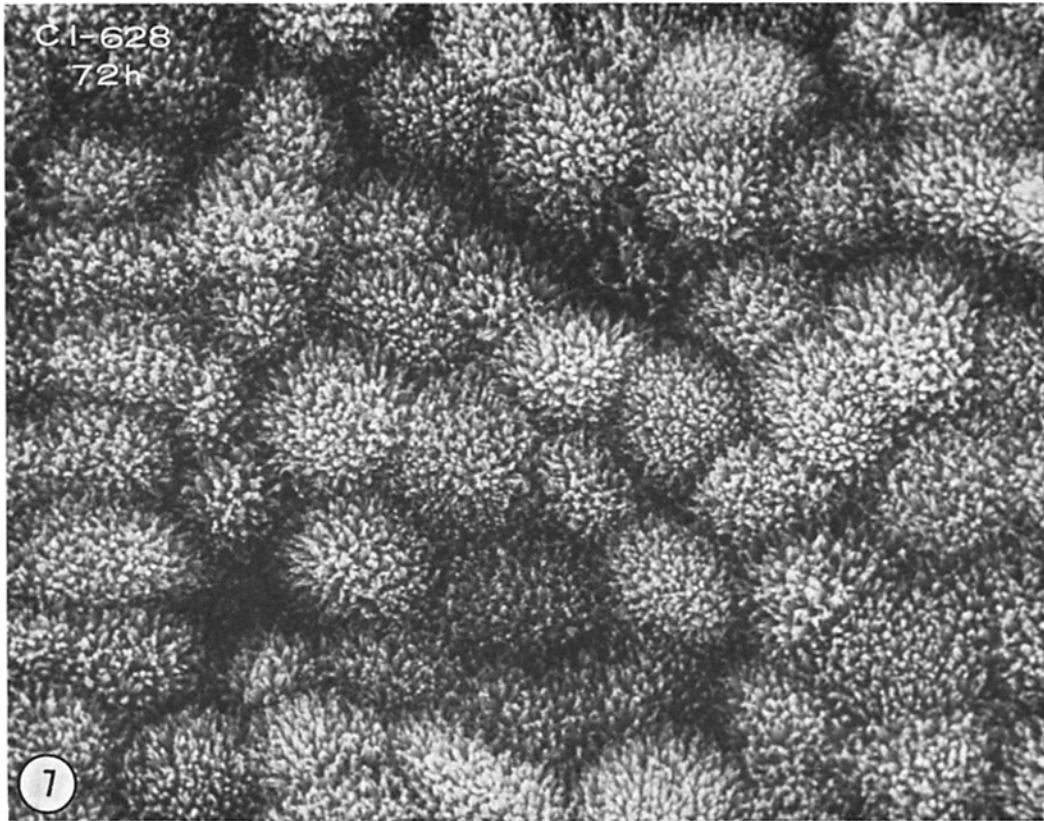
FIGURES 14 AND 15 At higher magnification some blebbing of the microvillar surface is apparent. $\times 32,000$; $\times 50,000$.

FIGURES 16 AND 17 The luminal surface of the epithelium from the endometrium of a pregnant rat is illustrated in these scanning micrographs. Except at the borders between adjacent cells, few microvilli are present on these cells. Numerous elongate fibers are present on their surface (arrows). $\times 3,100$; $\times 18,400$.

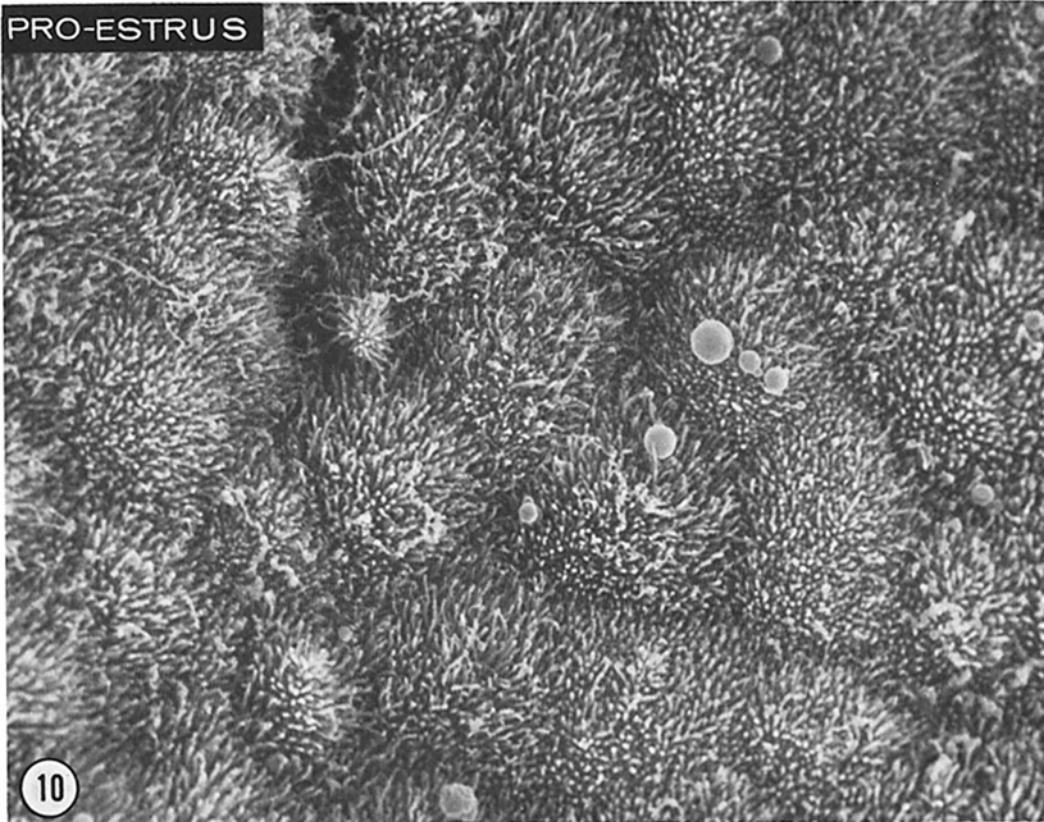


Estradiol
24h





PRO-ESTRUS



ESTRUS

