한국전자현미경학회지 제14권 제 2 호 1984
Korean J. Electron Microscopy, Vol. 14, No. 2, 1984

Ultrastructural Study on the Luminal Epithelium of the Ovariectomized Rat Uterus after Hormonal Treatment

Lee, J.H. and H.J. Lee*

난소를 절제한 흰쥐 자궁상피의 호르몬투여에 대한 전자현미경적 연구

이 재 현 · 이 헌 주*

(Received Oct. 4, 1984)

국문초록

난소절제한 흰쥐에서 17β-estradiol과 progesterone을 장기간 주사하여 자궁상피의 형태적 변화를 전자현미경으로 관찰하였다. 난소절제후 자궁상피는 막성구조물과 지방구 기타의 구조물을 포함하는 vacuole의 출현이 특이적으로 나타났다. 상피는 낮은 입방형이며 세포 유리면에 소수의 짧은 microvilli를 볼 수 있었다.

Estradiol투여시 상피는 높은 원주형을 나타내며, 분비계는 비교적 잘 발달되고, 세포 유리면에 소수의 긴 microvilli도 관찰 되었다. Progesterone 처리시의 상피는 세포 첨단부에 다수의 vacuole과 핵하부에 다수의 지방구의 집적이 관찰되었다.

이상의 결과로 보아 자궁상피는 형태학적 및 기능적인 상태에서 estrogen과 progesterone 양자에 의해 변화를 받으며 이들 호르몬은 상피세포에 대해 독특한 영향을 나타낸다고 생각된다.

Introduction

The morphological and functional states of the mammalian uterus are known to be controlled by the ovarian steroids (Boomsma *et al.*, 1982). Although the entire uterus is affected by ovarian activity, the most notable and reliable changes occur in the epithelial lining of the endometrium. The uterine epithelium therefore is a dynamic tissue, undergoing predictable cyclic changes as a result of hormonal input (Gordon, 1975).

The columnar epithelial cells that line the uterine lumen play an important role in the interaction between the blastocyst and endometrium during the initial stages of pregnancy. To facilitate the attachment of the blastocyst, the luminal epithelial cells undergo structural changes that are regulated by progesterone and estrogen (Bergstrom and Nilsson, 1972; Hafez,

Department of Veterinary Medicine, College of Agriculture, Kyungpook National University
*Department of Internal Medicine, Yeungnam University Hospital
경북대학교 농과대학 수의학과
*영남대학교 의과대학 병원 내과학교실

1980). However, the distinct effects of estrogen or progesterone on the uterine epithelium can not obtain because the sexual cycle is short (4 days) in the rats.

The present investigation, therefore, was carried out the morphological changes in this uterine epithelium following prolonged treatment of 17β-estradiol and progesterone in ovariectomized rats using the transmission electron microscope.

Materials and Methods

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

Five rats were daily injected subcutaneously with 1μg of 17β-estradiol (Sigma Chemical Co. Ltd.) dissolved in 0.5m*l* propylene glycol for 10 days. Other five rats were similary injected with 2.5mg of progesterone (Nakarai Chemical Ltd.). The remaining five were injected with 0.5m*l* propylene glycol only as controls. Each animal was bled at 8 to 9 hours after the final injection of hormones under chloroform anesthesia.

Uterus on both sides were dissected out, and the short lengths (1~2mm) were fixed in 2% glutaraldehyde-2.5% paraformaldehyde mixture in 0.1M sodium cacodylate buffer solution (pH 7.4) for 48 hours. After post-fixation in 1% osmium tetroxide in 0.1M sodium cacodylate buffer solution (pH 7.4) for 2 hours, the pieces were embedded in epoxy resin (Epon-812). Ultrathin sections were prepares on a (Porter-Blum) MT-1 type ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Hitachi HU-12A electron microscope.

Results

After ovariectomy, the uterine epithelium was characterized by the appearance of a number of vacuoles which was contained with the membraneous structures, lipid droplets, dense bodies and the others. The epithelium was low cuboidal, and a few short microvilli were present at the cell surface. Indentated nucleus and relatively clear nuclei are present. Dense bodies are appeared in the cytoplasm and secretory granules are rarely found. In addition, rER and Golgi apparatus were somewhat developed, and mitochondria were irregular in shape and size. A few small vesicles and lipid droplets were often found.

After estradiol treatment, the uterine epithelium was high columnar in shape. The mitochondria were appeared throughout the cytoplasm, however, long or swelling mitochondria were often found. A few multivesicular bodies and dense bodies were also found. Golgi apparatus and rER were relatively well-developed, often dilated especially the cisternae of rER. Relatively long and sparse microvilli were present at the cell surface. A few small vesicle swere found at the apical region. No lipid droplets were found.

After progesterone treatment, the uterine epithelium was characterized by the appearance of numerous vesicles at the apical region and numerous lipid droplets at the subnuclear region. At the cell surface a number of short and blunt microvilli were found. A few various mitochondria in shape were also found. Well-developed Golgi apparatus, a few rER and multivesicular bodies were shown. Golgi vesicles were severely dilated in some cells. No secretory granules

were found.

Discussion

In this study of ovariectomy, the appearance of a number of vacuoles which was contained with the membraneous structures, lipid droplets, dense bodies and the others were characteristic. The epithelium was low cuboidal, and a few short microvilli were present at the cell surface. Dense body and secretory granules are rarely present. Golgi apparatus and rER were somewhat developed. After estradiol treatment, the high columnar epithelium was characterized by the appearance of swelling mitochondria, a few multivesicular bodies, dense body, relatively well-developed Golgi apparatus and rER are relatively long sparse microvilli at the cell surface. And after progesterone treatment, the appearance of numerous vesicles at the apical region and numerous lipid droplets at the subnuclear region was characteristics. At the cell surface a number of short and blunt microvilli were found. Well-developed Golgi apparatus, a few rER and multivesicular bodies were also shown.

The investigations reported in the present study indicate that the luminal epithelium of the rat endometrium undergoes the effects of steroid hormones as reflected by the varying structure of the cell organelles. Furthermore, it has been shown that the epithelium was suppressed by the ovariectomy, and estradiol was activated the activity of cells whereas progesterone was induced the pinocytosis and the retention of the lipid.

It has been suggested that the estradiol was induced hypertrophy and hyperplasia of the uterine epithelium and the synthesis of a secretory product in various animals (Bareither and Verhage, 1980; Boomsma *et al.*, 1982; Eroschenko, 1982; Koseki and Fujimoto, 1974), including human (Johannisson and Nilsson, 1972, West *et al.*, 1976), whereas progesterone was suppressed of these (Martin and Finn, 1970). However, there are considerable disagreements depend upon the animals (Martin and Finn, 1970; Meyer, 1970; Nilsson, 1972; Parr and Parr, 1974, 1977).

With regard to the development of the cilia and microvilli in the uterine epithelium, Johannisson and Nilsson(1972) reported that it was decreased in size and number by progesterone, however, Finn and Porter(1975) reported that it was increased in length and number by estrogen. Gordon(1975) also reported that the differentiation of microvilli was probably under the influence of estrogen stimulation. On the other hand, Barberini *et al.* (1978) reported that the uterine luminal epithelium of the rabbit was composed of two cell types; ciliated and non-ciliated cell. In this study, non-ciliated cell was found, and the length of microvilli was increased by the estradiol whereas the microvilli was short and blunt by progesterone, but the number of it was more by progesterone than by estradiol.

It was reported that the height of the luminal epithelium of the endometrium and uterine gland was increased by both of estrogen and progesterone(Barberini *et al.*, 1978; Finn and Porter, 1975). In this study, the low cuboidal epithelium in ovariectomy was increased in height by the estradiol and progesterone. However, the cell height was more increased by the estradiol than the progesterone.

With regard to the mitochondria in the endometrium, Finn and Porter(1975) reported that the prominent mitochondria and dilated cristae were found during follicular phase, and the giant mitochondria was also appeared during

luteal phase in human endometrium. In this study, the present writer was found that the long mitochondria appeared after ovariectomy, and swelling mitochondria after estradiol treatment.

It was shown that the abundant vesicles were appeared in the apical surface during the luteal phase (Finn and Porter, 1975), and he suggested that it was common feature which presence of vesicles just under the clear cytoplasmic area of the terminal web. Parr and Parr(1974, 1977) suggested that it was endocytotic profile in mouse and rat. On the other hand, Gordon (1975) was reported that the vesicles were found during the follicular phase, and it was indicative of a high functional state, the hormone was considered to affect the state of its activity directly. In this study, the abundant vesicles were found after the progesterone treatment. Therefore, our present data is consistent with the Finn and Porter(1975). However, the authors could not conclude that whether this profile is merocrine or endocytosis and secretion mode of secretory products.

It was reported that the accumulation of lipid droplets at subnuclear region was shown during luteal phase (Finn and Porter, 1975) whereas the estrogen was caused reduction of it (Finn and Porter, 1975). On the other hand, the phospholipid on the rat endometrium was increased by the estradiol injection, whereas neutral and unsaturated lipid was increased by the progesterone injection (Boshier and Holloway, 1973). In this study, the numerous lipid droplets were shown after the progesterone treatment at the subnuclear region. The present data could not obtain the distinct component of lipid showing here. Additional studies by histochemistry are required to confirm the component of lipid on the ovariectomized rat endometrium.

In this study, the vacuoles were appeared after ovariectomy. It contained with the lipid droplets, membranous structures and the others. It was reported that the vacuoles were found during the late of luteal phase (Finn and Porter, 1975). Their functional significance remains undetermined.

These data indicated that the endometrium was dependent on estrogen and progesterone for changes in both its morphological and functional state and suggested that each hormone exerted a unique effect on the epithelial cells.

Summary

Morphological changes of the epithelium of the endometrium by prolonged treatment of 17β-estradiol or progesterone in ovariectomized rats was studied at the ultrastructural level. The epithelium of the endometrium in ovariectomized rats was characterized by the appearance of a number of vacuoles which was contained with the membraneous structures, lipid droplets and the others. The epithelium was low cuboidal, and a few short microvilli were present at the cell surface. Secretory granules are rarely found. After estradiol treatment, the epithelium was high columnar in shape. The mitochondria was appeared throughout the cytoplasm, however, long or swelling mitochondria was often found. Golgi apparatus and rER were relatively well-developed. Relatively long and sparse microvilli were present at the cell surface. After progesterone treatment, the epithelium was characterized by the appearance of numerous vesicles at the apical region and numerous lipid droplets at the subnuclear region. At the cell surface a number of short and blunt microvilli were found.

These data indicated that the endometrium was dependent on estrogen and progesterone for

changes in both its morphological and functional state and suggested that each hormone exerted a unique effect on the epithelial cells.

References

Barberini, F., S. Sartori. and P. Motta. 1978. Changes in the surface morphology of the rabbit endometrium related to the estrous and progestational stages of the reproductive cycle. Cell Tiss. Res. 190, 207～222.

Bareither, M.L. and H.G. Verhage. 1980. Effect of estrogen and progesterone on secretory granule formation and release in the endometrium of the ovariectomized cat. Biol. Reprod. 22, 635～643.

Bergstrom, S. and O. Nilsson. 1972. Ultrastructural response of blastocysts and uterine epithelium to progesterone deprivation during delayed implantation in mice. J. Endocrinol. 55, 217～218.

Boomsma, R.A., R.C. Jaffe and H.G. Verhage. 1982. The uterine progestational response in cats: Changes in morphology and progesterone receptors during chronic administration of progesterone to estradiol-primed animals. Biol. Reprod. 26, 511～521.

Boshier, D.P. and H. Holloway. 1973. Effects of ovarian steroid hormones on histochemically demonstrable lipids in the rat uterine epithelium. J. Endocrinol. 56, 59～67.

Eroschenko, V.P. 1982. Surface changes in oviduct, uterus and vaginal cells of neonatal mice after estradiol-17β and the insecticide chlordecone(Kepone®) treatment: A scanning electron micrnscopic study. Biol. Reprod. 26, 707～720.

Finn, C.A. and D.G. Porter. 1975. The uterus: Handbooks in reproductive biology. Vol. 1. pp. 21～54. Elek Science, London.

Gordon, M. 1975. Cyclic changes in the fine structure of the epithelial cells of human endometrium. Int. Rev. Cytol. 42, 127～172.

Hafez, E.S.E. 1980. Reproduction in farm animals. 4th Ed. pp. 72～74. Lea & Febiger, Philadelphia.

Johannisson, E. and L. Nilsson. 1972. Scanning electron microscopic study of the human endometrium. Fert. Steril. 23, 613～625.

Koseki, Y. and G.I. Fujimoto. 1974. Progesterone effects contrasted with 17-estradiol on DNA synthesis and epithelial nuclear proliferation in the castrate rabbit uterus. Biol. Reprod. 10, 596～604.

Martin, L. and C.A. Finn. 1970. Interactions of estradiol and progestins in the mouse uterus. J. Endocrinol. 48, 109～115.

Meyer, J.M. 1970. Studies on the ultrastructure of the uterine mucosa of the rabbit. Arch. Anat. Histol. Fmbryol. 53, 1～40.

Nilsson, O. 1972. Ultrastructure of the process of secretion in the rat uterine epithelium at preimplantation. J. Ultrastr. Res. 40, 572～580.

Parr, M.B. and E.L. Parr. 1974. Uterine luminal epithelium: Protrusions mediate endocytosis, not apocrine secretion, in the rat. Biol. Reprod. 11, 220～233.

Parr, M.B. and E.L. Parr. 1977. Endocytosis in the uterine epithelium of the mouse. J. Reprod. Fertil. 50, 151～153.

West, N.N., H.G. Verhage and R.M. Brenner. 1976. Suppression of the estradiol receptor system by progesterone in the oviduct and uterus of the cat. Endocrinol. 99, 1010～1016.

Figure Legends

Fig. 1. Endometrial epithelium in ovariectomized rat. The low cuboidal epithelium and a number of vacuoles(V) are seen. ×3,500

Fig. 2a. Endometrial epithelium in ovariectomized rat. The vacuole(V) and dense bodies (head of arrow) are seen in the low cuboidal epithelium. ×3,500

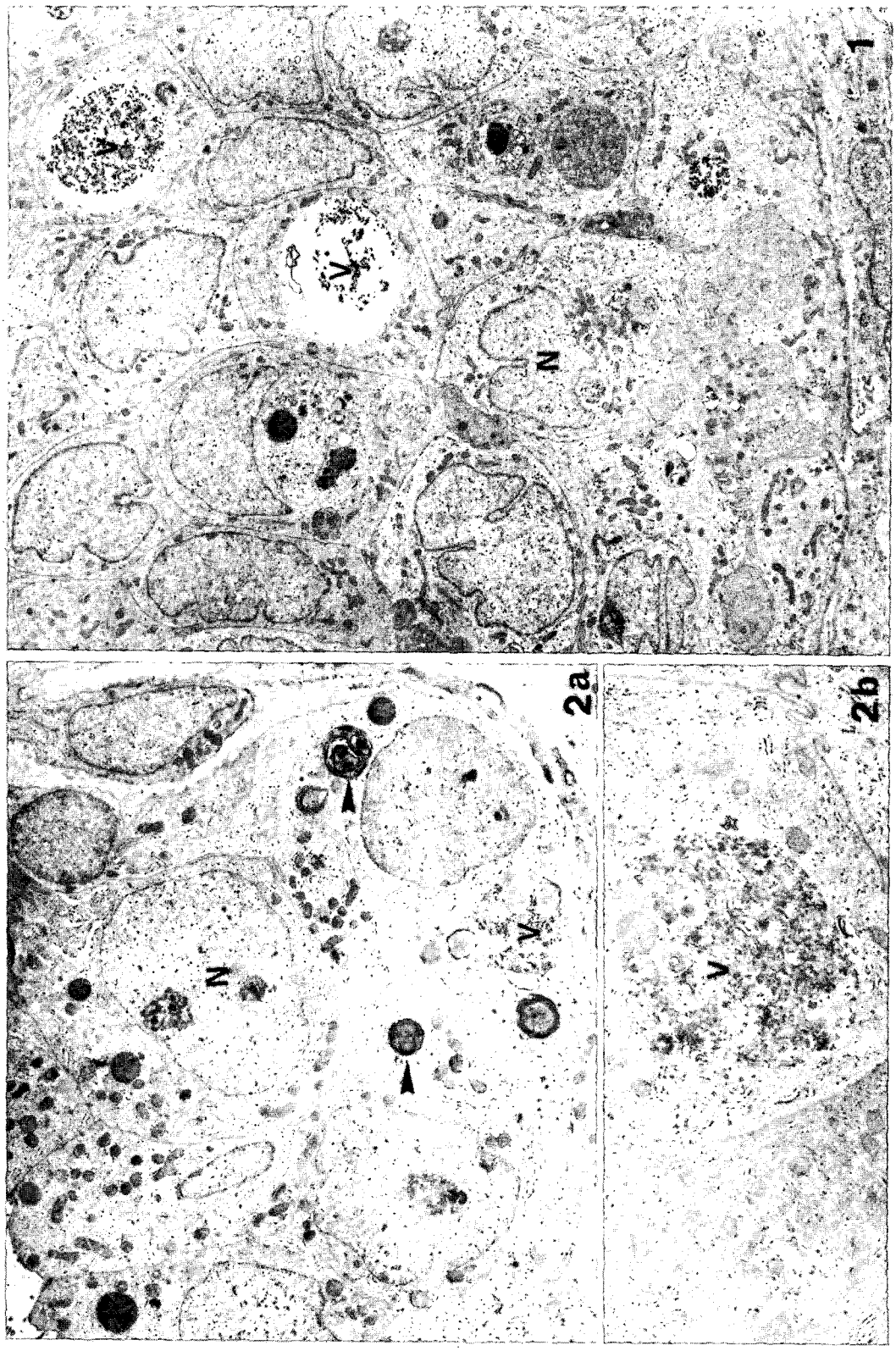
Fig. 2b. The vacuole(V) contain with the lipid droplet, membranous structures and the others. ×8,000

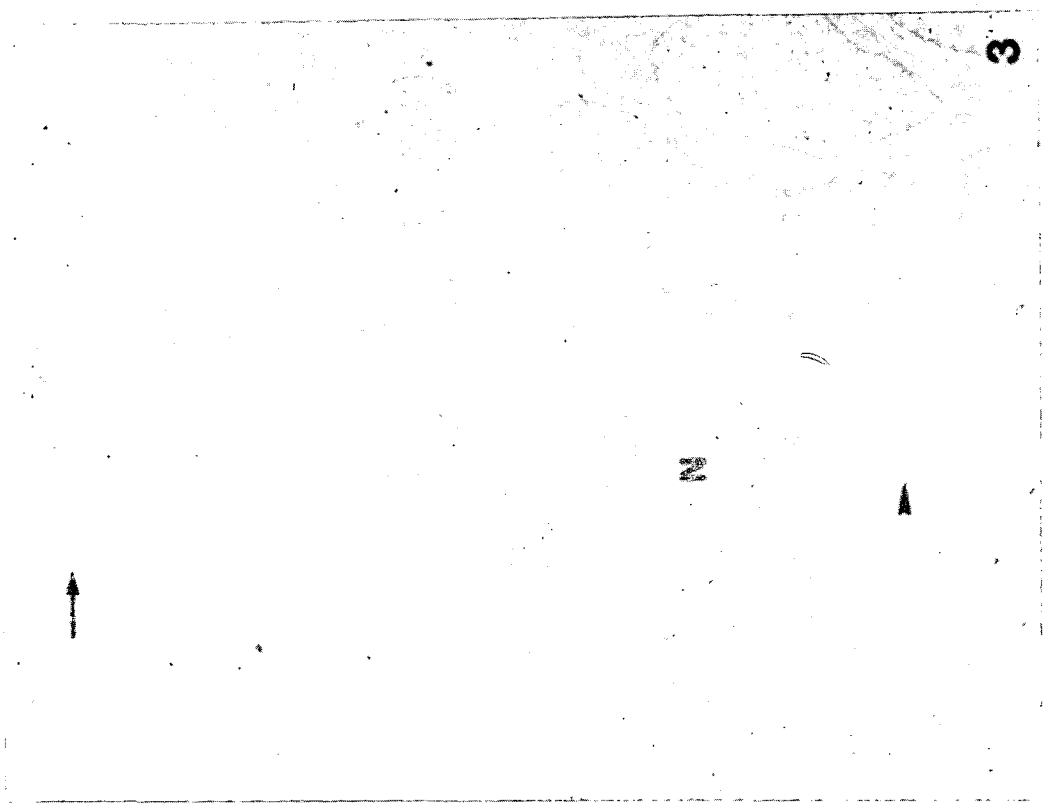
Fig. 3. Endometrial epithelium after estradiol treatment. A number of high columnar epithelium are seen. Relatively long sparse microvilli at the cell surface and a few multivesicular body(arrow) are seen. Long mitochondria (head of arrow) is also seen. ×5,000

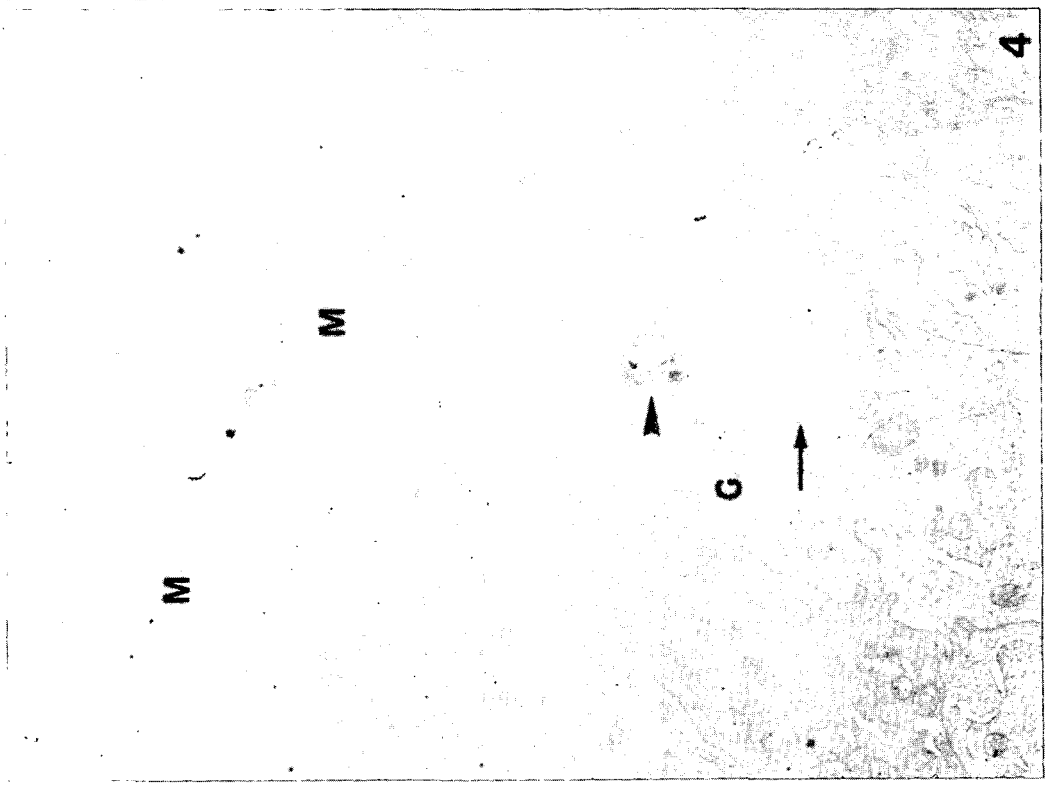
Fig. 4. Endometrial epithelium after estradiol treatment. Note the cytoplasmic bulging, swelling, swelling mitochondria(M), well-developed Golgi apparatus(G), dilated rER, dense body (head of arrow) and multivesicular body (arrow). ×7,000

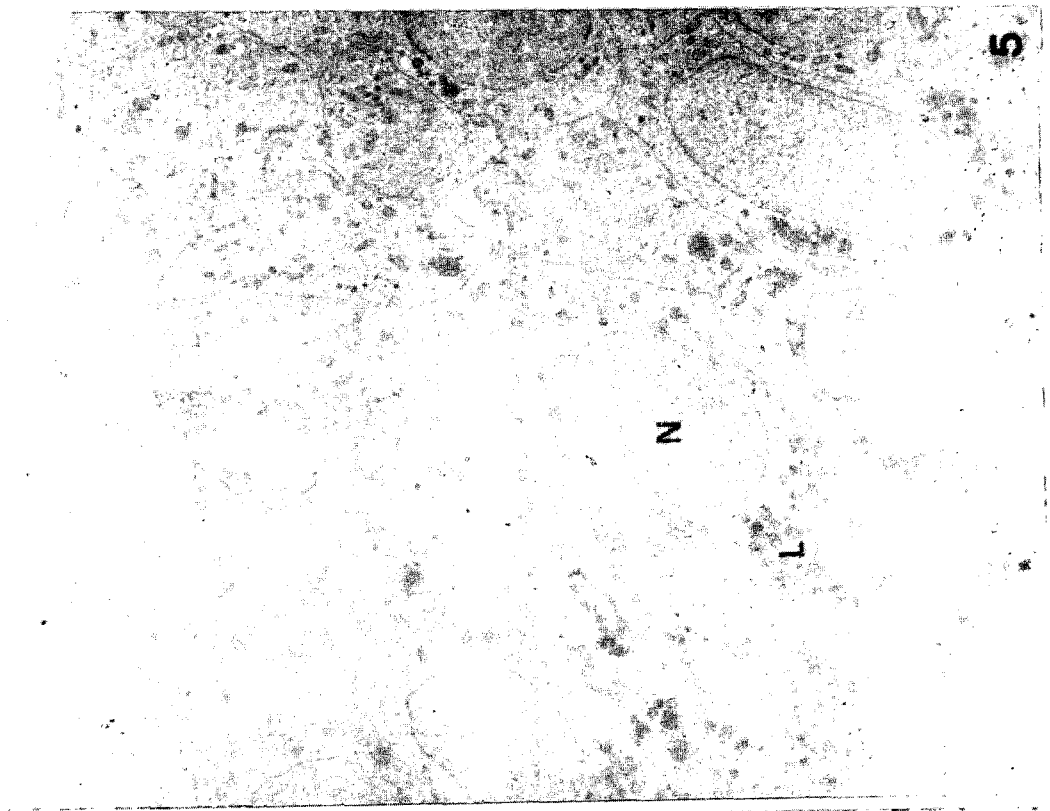
Fig. 5. Endometrial epithelium after progesterone treatment. Note numerous vesicles at the apical region and numerous lipid droplets(L) at the subnuclear region. ×3,500

Fig. 6. Endometrial epithelium after progesterone treatment. Well-developed Golgi apparatus(G), numerous vesicles (arrow) and a few multivesicular bodies(head of arrow) are seen. A numer of microvilli at the cell surface are short and blunt. ×7,500


1
N
V
2a
N
V
2b
V


3
M


4
M
M
C


5
N
L


6
G