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Scanning Electron Microscopy of the Effect of Short-term Hormonal Therapy on Postmenopausal Endometrium

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ABSTRACT

A scanning electron microscopic study of the endometrial surface was made in postmenopausal women, both untreated and after short-term hormonal therapy. In the untreated women the endometrial surface was characterized by slightly bulging cell surfaces with few, short microvilli. The surface area of the cells varied. Often a single kinocilium was observed.

After administration of estrogen alone the epithelial cells showed a uniform surface area. The cell surfaces were bulging and possessed numerous fairly long microvilli, which often formed tuft-like structures. The length of the microvilli seemed to depend upon the potency of the estrogen used.

After sequential administration of estrogen followed by a gestagen, the uterine surface was characterized by microvilli and apical protrusions. The microvilli were long and numerous when the priming estrogen was estradiol + estriol, while they were shorter when estradiol valerate was given. The apical protrusions were larger and more numerous after administration of progesterone than after treatment with norgestrel or noretisterone. The type of estrogen used also influenced the development of the protrusions: generally, they were largest and more numerous after treatment with estradiol and estriol followed by progesterone.

It is concluded that by appropriate hormone administration the surface ultrastructure of the menopausal endometrium can be altered to correspond to that of the normal cycle and that the surface morphology obtained is closely correlated to the type of hormone treatment used.

INTRODUCTION

In view of the frequent use of estrogens and estrogen/gestagen combinations in the treatment of climacteric disturbances it is important that a knowledge of the hormonal effect on the resting endometrium is obtained and also specifically to what extent the hormonal treatment results in a morphology representative of the cycling endometrium with the doses normally used in the substitutional therapy of menopausal women.

Further, it would be of interest to know whether the changes produced by different genuine estrogens differ quantitatively or qualitatively, and whether the use of synthetic gestagens, as compared with progesterone, is reflected in the structure of the endometrium after sequential therapy.

The surface of the human uterine epithelium, as observed by scanning electron microscopy (SEM), responds markedly to the hormonal changes both during the normal menstrual cycle (4–7, 9–12, 14, 19) and during a hormonal therapy (4, 15). To find out to what extent the SEM technique can be used to indicate the hormonal responses of the types mentioned, a study was performed on endometrial biopsies from postmenopausal women treated with female sex hormones.

MATERIAL AND METHODS

Thirteen women aged 49–69 years with genital prolapse were studied (Table I). The menopause had occurred 6 months to 29 years prior to the hormonal treatment in question, and none of the patients had received steroids within the last year.

Therapy groups

3 patients received no treatment (controls), 3 received estrogens only, and 7 were given estrogen/gestagen combinations in the form of sequential therapy (Table I). The assignment to the different groups was random. In the group treated with estrogens alone, biopsies were obtained in the third week of the artificial cycle. In the group receiving estrogen/gestagen combinations biopsies were taken between day 19 and day 22 of the artificial cycle (Table I).

The estrogens given were genuine estrogens (estradiol valerate or estradiol + estriol, ratio 2 : 1). The gestagens

Table I. *Data on patients and hormone schedules*

Case no.	Fig. no.	Age (years)	Years of menopause	Types of hormone given	Day of biopsy (Day of artificial cycle)
Controls					
1	1, 2	59	8		
2	3	60	8		
3		69	30		
Estrogen treatment					
4	4	69	17	Estradiol + Estriol 4 mg + 2 mg. Days 1–14	15
5	5	65	22	Estradiol valerate 4 mg. Days 1–17	18
6	6	53	4	Estradiol valerate 2 mg. Days 1–20	21
Sequential treatment (estrogen–gestagen)					
7	7	49	0.5	Estradiol valerate 2 mg. Days 1–19 Norgestrel 0.5 mg. Days 12–19	20
8		54	3	Estradiol valerate 2 mg. Days 1–19. Norgestrel 0.5 mg. Days 12–19	20
9		56	5	Estradiol valerate 2 mg. Days 1–22. Noretisterone 5 mg. Days 15–22	23
10		59	10	Estradiol + Estriol 2 mg + 1 mg. Days 1–22. Noretisterone 5 mg. Days 15–22	23
11	8	52	6	Estradiol + Estriol 2 mg + 1 mg. Days 1–21. Progesterone 0.4 g. Days 15–21	22
12		59	20	Estradiol valerate 2 mg. Days 1–21. Progesterone 0.2 g. Days 15–21	22
13	9	62	9	Estradiol valerate 2 mg. Days 1–21. Progesterone 0.4 g. Days 15–21	22

comprised progesterone or 19-nor-testosterone derivates (norgestrel and noretisterone). The doses used were within the therapeutic range (Table I). Progesterone was administered rectally, and all other compounds orally in tablet form.

Hormone analysis

FSH and LH analysis were performed prior to treatment in 5 patients—one from the control group 2 from the estrogen group and 2 from the group treated sequentially. The results corresponded to postmenopausal values.

Biopsies

Biopsies were obtained during operation for genital prolapse, with the patient fully anesthetized. No biopsies were taken before commencement of the treatment.

The specimens were taken with a Reifferscheid's curette. In each case, two biopsies were taken from the anterior wall of the fundus close to the midline. One biopsy was taken for light microscopy and another for electron microscopy. The latter specimen was washed for less than one minute in 0.1 M cacodylate buffer, then fixed at pH 7.3 in 2.5% glutaraldehyde in the same buffer, rinsed in distilled water, dehydrated in acetone and prepared in carbon dioxide by the critical point drying technique. The specimens were coated with gold–palladium and studied in a JEOL-JSM-U3 scanning electron microscope.

RESULTS

Control group

The uterine luminal epithelium displayed cells with slightly bulging surfaces with few, short microvilli. The surface area of the cells differed markedly, the width of the cells ranging from 3–15 μm (Fig. 1). A single kinocilium was often present (Fig. 2). Some cells appeared to protrude slightly and were almost devoid of microvilli (Fig. 3). Occasionally, areas containing both cells with fully developed protrusions of a secretory type and ciliated cells were encountered. Such areas were most common at the gland openings.

Ciliated cells were rarely found. However, considering the rather frequent occurrence of a single kinocilium, there is some difficulty in defining the term ciliated cell.

Estrogen treated group

Two marked changes appeared on the luminal surface of the endometrium on administration of estrogen—an increasing number of long microvilli and the development of ciliated cells.

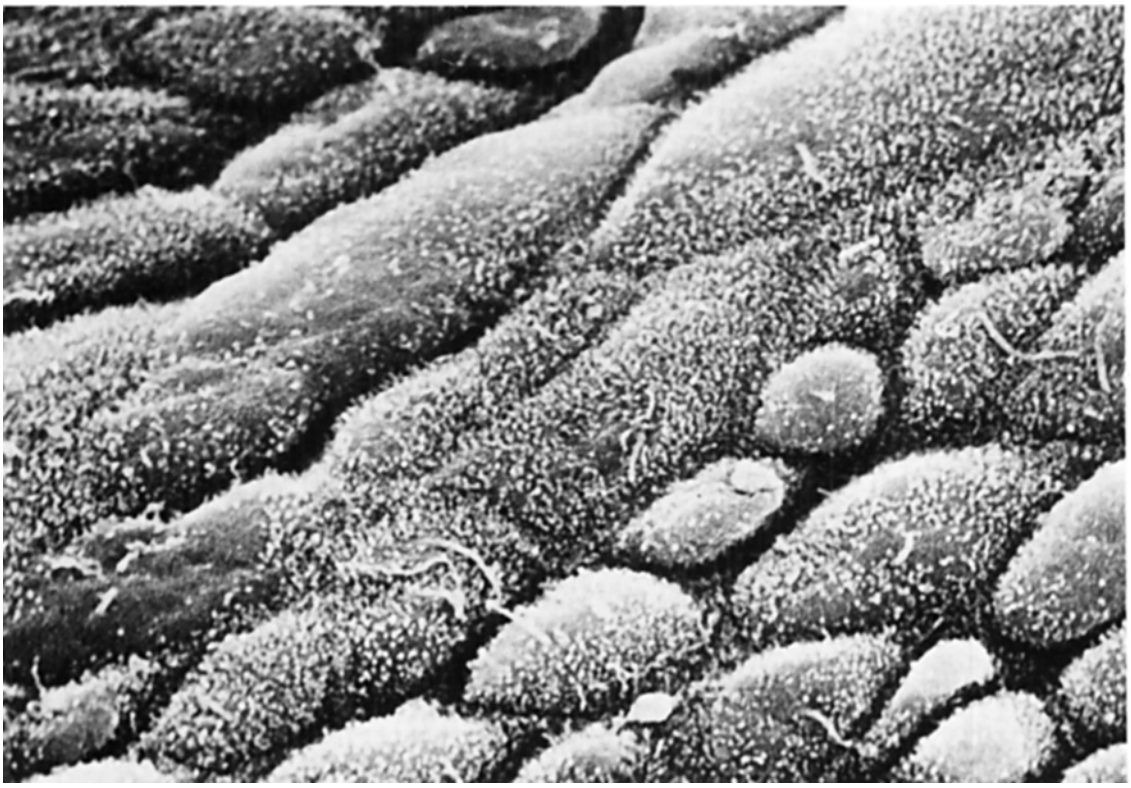

Fig. 1. Postmenopausal endometrial surface (Case 1). The luminal epithelial cells have slightly bulging surfaces with a varying number of short microvilli. The luminal surface area differs appreciably between the cells. A single kinocilium is often observed. ×3 150.

The surface areas of the non-ciliated cells became more uniform. The apical surfaces were bulging and possessed many fairly long microvilli. A few apical protrusions were occasionally found.

The microvilli were seen to respond differentially to the amounts of estrogen administered. Thus, the most pronounced effect was observed after administration of estradiol–estriol. The microvilli then were long and often grouped together in a tuft-like structure (Fig. 4). Further, a dose of estradiol valerate of 4 mg per day for 17 days (Case 5) gave rise to longer microvilli (Fig. 5) than did half this dose for 20 days (Case 6) (Fig. 6). This occurred despite the fact that the postmenopausal period was considerably longer in the former patient than in the latter.

Estrogen–gestagen treated group

The luminal surface of the uterus was characterized by the presence of many ciliated cells and of large

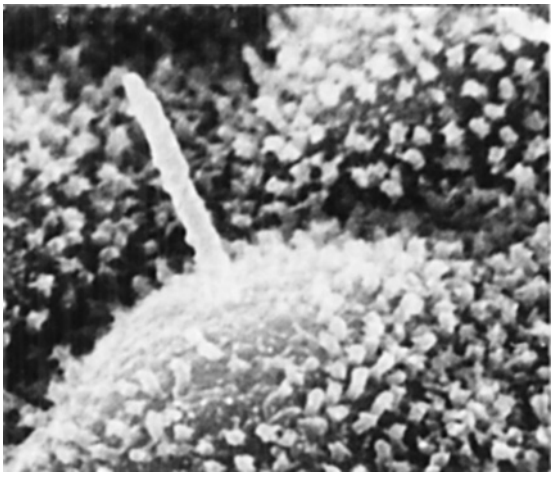

Fig. 2. Postmenopausal endometrial surface (Case 1). A single kinocilium is seen on a luminal epithelial cell. ×13 200.

Fig. 3. Postmenopausal endometrial surface (Case 2). The microvilli are irregularly distributed on the cell surface, some cells being almost devoid of microvilli. ×6 300.

apical protrusions on the non-ciliated cells. The number of kinocilia on each cell was 80–100. The surface of the cilia had a slightly granular appearance. Between the cilia, small microvilli were observed.

The apical protrusions varied in appearance from slightly raised areas on the apical cell surfaces (Figs. 7, 9) to large morel-like structures which almost obscured the underlying surface of the cell (Fig. 8). Sometimes the apical protrusions had a finely granular surface. Often the base of the protrusions bore a few microvilli. In addition to the protrusions, the non-ciliated cells also possessed microvilli.

The appearance of both the microvilli and the apical protrusions changed according to the hormonal treatment. Since the apical protrusions can be rather irregularly distributed, an evaluation of their shape and frequency necessitates scrutiny of several endometrial areas of more than one specimen.

The microvilli were long and numerous when the priming estrogen consisted of estradiol + estriol (Cases 10–11) (Fig. 8), while they were shorter when estradiol valerate (Cases7–9 and 12–13) was given (Figs. 7, 9). Whether the type of gestagen also influences the microvilli is an open question. Thus when norgestrel (Cases 7–8) was added sequentially to estradiol valerate, the microvilli appeared shorter than was the case when noretisterone (Cases 9–10) was added. However, the latter patients were estrogen-primed for 3 days longer than the former ones.

The apical protrusions were larger and more numerous after administration of progesterone (Cases 11–13) (Fig. 8) than after administration of

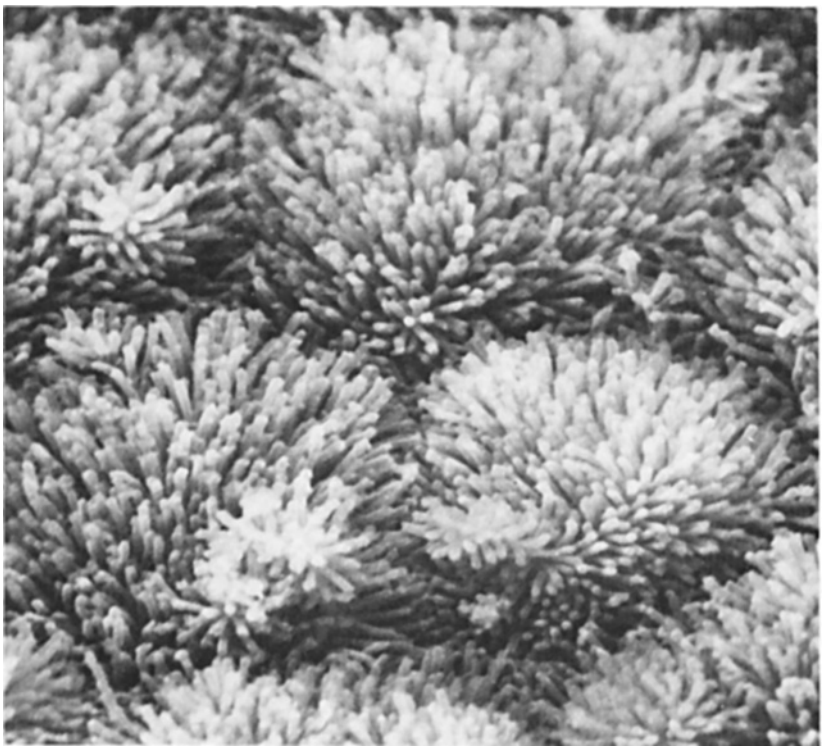

Fig. 4. Endometrial surface after treatment with estrogen (estradiol + estriol) (Case 4). The luminal surfaces possess numerous fairly long microvilli. Often some microvilli are grouped together, forming a tuft-like structure. ×12 500.

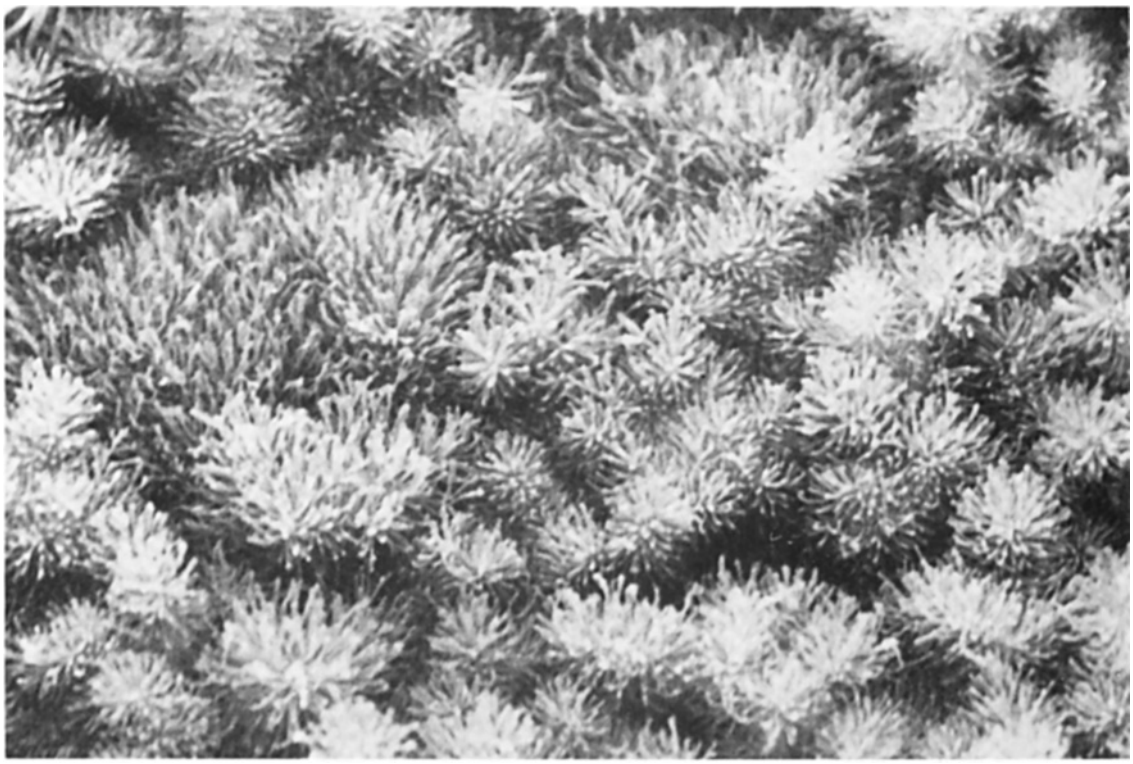

Fig. 5. Endometrial surface after treatment with estrogen (estradiol valerate, high dose) (Case 5). The microvilli are numerous, long, and often arranged in tufts on the cell surface. ×3 150.

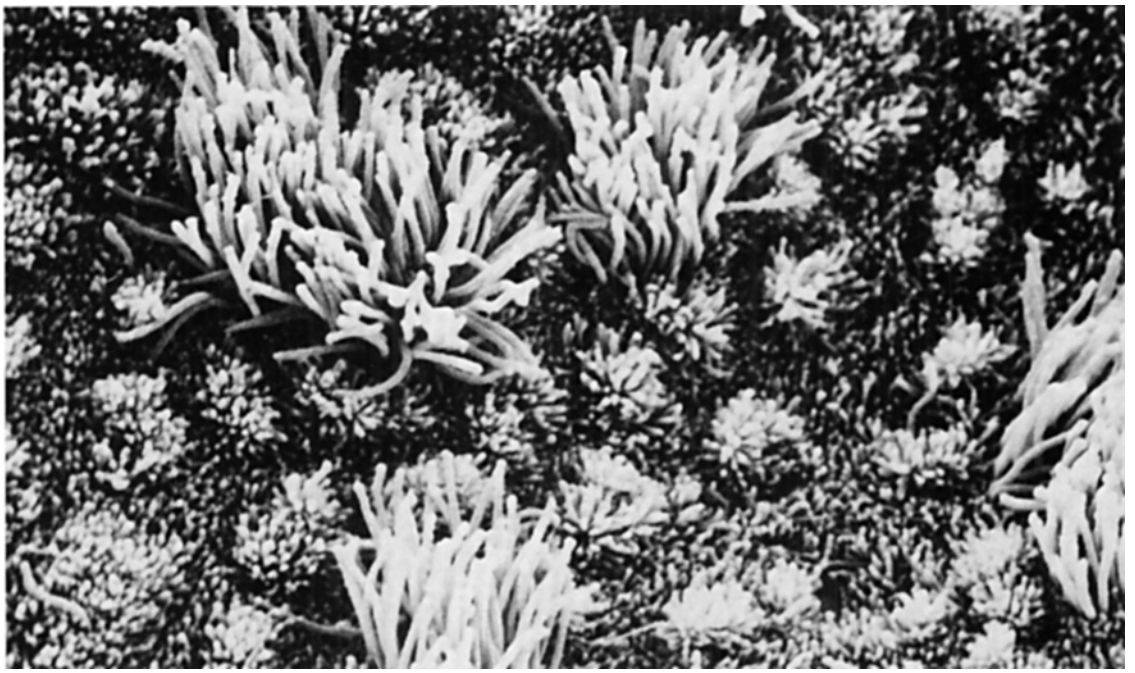

Fig. 6. Endometrial surface after treatment with estrogen (estradiol valerate, low dose) (Case 6). The microvilli are less well developed than after treatment with a higher dose of estradiol valerate (cf. Fig. 5). Tufts of microvilli are still found. Several ciliated cells are seen. ×3 150.

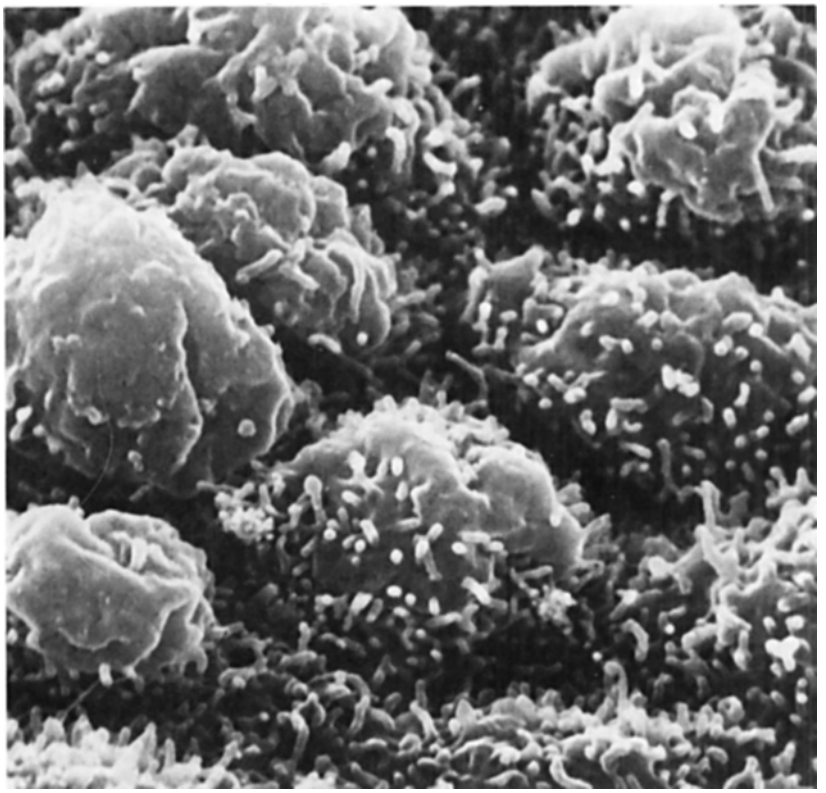

Fig. 7. Endometrial surface after sequential treatment with estrogen–gestagen (estradiol valerate–norgestrel) (Case 7). The apical protrusions are moderately large, while the microvilli are short and poorly developed. ×12 000.

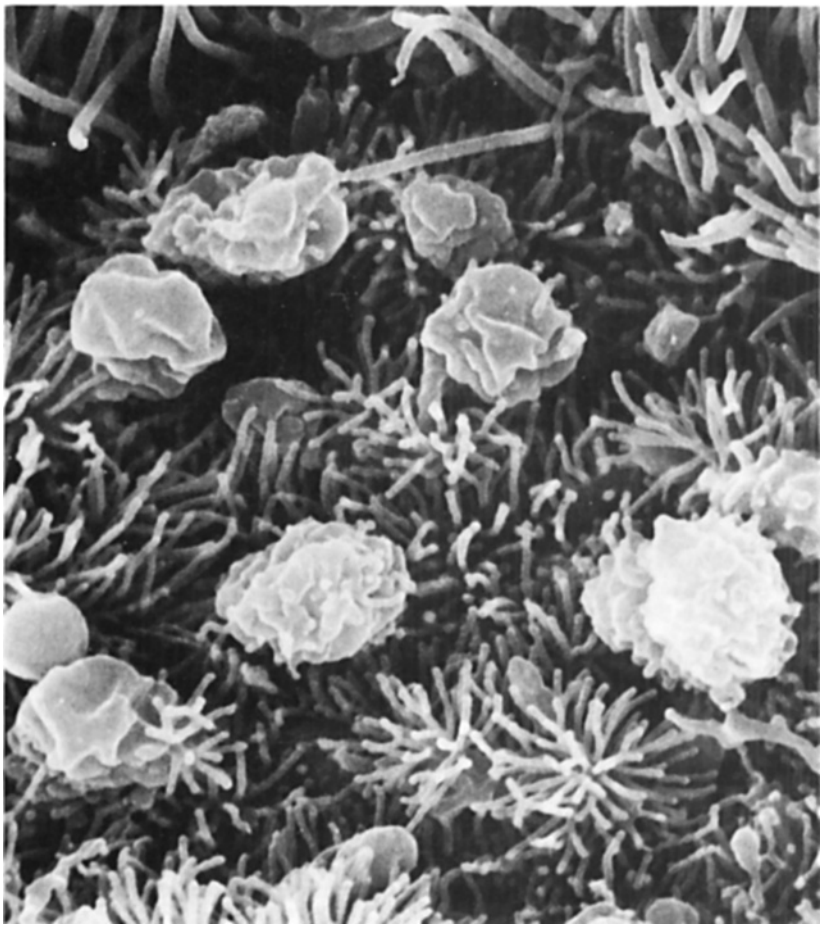

Fig. 8. Endometrial surface after sequential treatment with estrogen–gestagen (estradiol+estriol–progesterone) (Case 11). Apical protrusions and microvilli are observed on the luminal surface of the non-ciliated cells. The protrusions are well developed, forming morel-like structures. The microvilli are long and slender. ×9600.

norgestrel (Cases 7–8) (Fig. 7) or noretisterone (Cases 9–10). However, the type of estrogen used also has to be considered. Thus, when estradiol+ estriol (Case 11) was given before progesterone, the protrusions were well developed (Fig. 8), whereas they had grown less when estradiol valerate (Cases 12–13) was used for priming (Fig. 9). The protrusions were largest and most numerous after treatment with estradiol + estriol and progesterone (Case 11) (Fig. 8).

DISCUSSION

Generally the production of ovarian steroids in the post-climacteric woman is low, as documented by hormonal analysis (8). Although areas of differentiated endometrium are sometimes found (16), the present material was considered characteristic of an inactive (atrophic) endometrium. This is based on the FSH–LH analysis performed and the light microscopical morphology which is similar to findings from biopsies of senile endometrium (12).

The mucosa at the top of the fundus is regarded as the site that best reflects the hormonal status (3). Since the aim of the study was to make a general assessment of the value of SEM of human endometrium for classifying hormonal effects, only subjective judgements were made. However, if the technique is found to deserve application, some morphometric method for objective quantification will be desirable.

The present dose schedule of 30–60 mg estradiol over a 3-week period is in accordance with the schedule used in treating menopausal women with genuine estrogens (2). This dose is sufficient in some cases to provoke bleeding from a proliferative endometrium. The cyclic sequential treatment in the doses used in this study causes regular bleeding

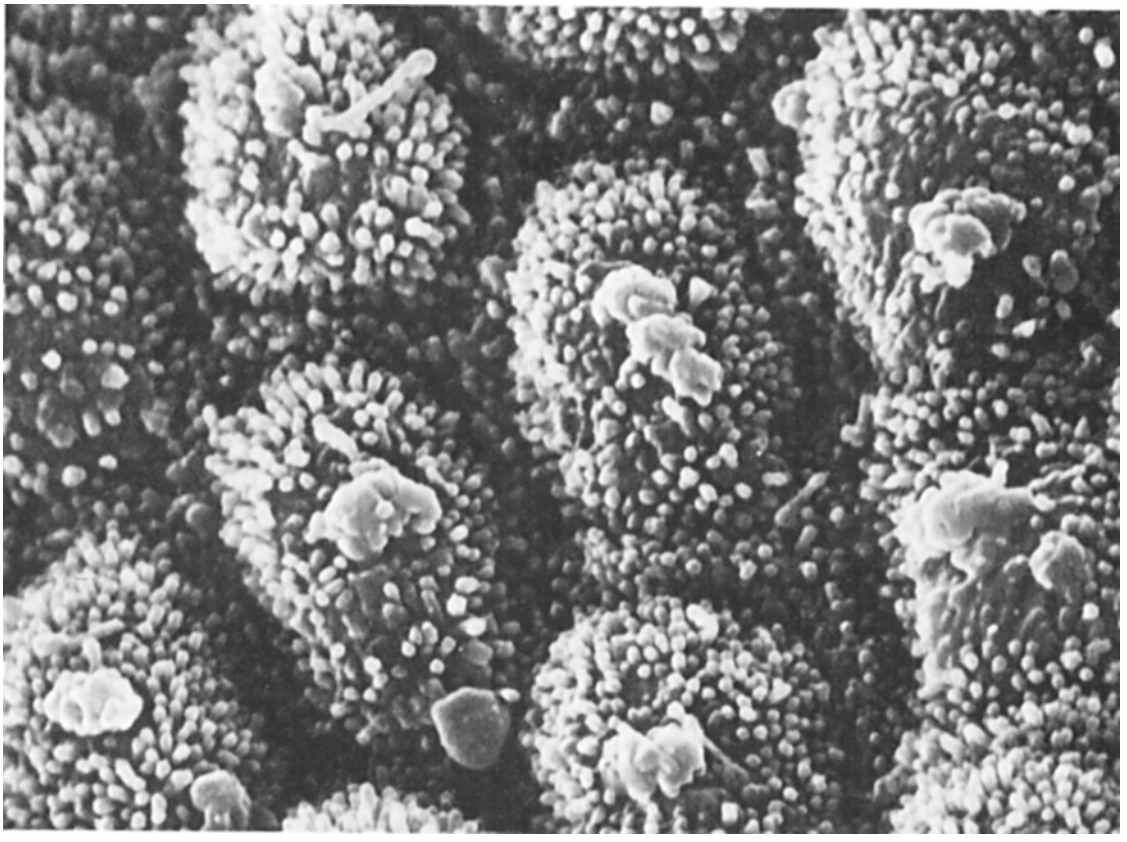

Fig. 9. Endometrial surface after sequential treatment with estrogen–gestagen (estradiol valerate–progesterone) (Case 13). The epithelial cells are bulging and hedge-like structures are running in the grooves between the cells. The microvilli are short. Several of the cells possess small apical protrusions. ×6300.

from a secretory endometrium. Since the surface morphology of the endometrium of some of our patients was rather similar to the morphology of normal follicular and luteal phases, it may be concluded that the hormone schedules used restore at least some normal functional and ultrastructural characteristics of the endometrium in menopausal women.

SEM of normal cyclic endometrium has demonstrated that ciliogenesis is estrogen-dependent (6, 13). This observation was confirmed in the present study. A concentration of ciliated cells around the gland openings was also noted (4, 6, 9, 19). However, the finding of a decrease in the number of ciliated cells during the luteal phase of the normal cycle (13) was not supported in our examinations of the corresponding phase in women on sequential treatment.

During the normal menstrual cycle the microvilli change in appearance from long and slender structures in the preovulatory phase to shorter, thicker, sometimes interbranching ones in the postovulatory phase (5, 10, 14). These findings were also reproduced in the present study. However, the tuft-like arrangement of the microvilli after the estrogen treatment has not been described in the preovulatory endometrium of the fertile woman, but seems to be caused by a graded response to estrogen. The same interpretation might explain also the apparently weaker effect of 2 mg estradiol valerate, containing 1.6 mg estradiol, on ciliogenesis and the development of microvilli as compared with the effect of estradiol + estriol in a dose of 2 mg + 1 mg.

Siiteri et al. (18) suggested that estriol has some protective effect against malignant changes in the endometrium by competitive receptor binding, although it seems to have little or no effect upon the

endometrial light microscopical morphology (1, 17). However, an effect on the electron microscopical level might occur and could be revealed with the present technique.

The predominant surface structure during the postovulatory phase is the apical protrusion (4, 7, 9–12, 14, 19). In the material from the sequentially treated patients it was confirmed that this picture was attributable to the gestagens after priming of the endometrium with estrogens. However, the endometrial response to the different gestagens differs both qualitatively and quantitatively, progresterone being the most potent compound.

In conclusion, our study has shown that by administration of female sex hormones changes can be produced in the surface ultrastructure of the menopausal endometrium similar to those of the physiological cycle and that the morphology produced is closely correlated to the type of hormone used.

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