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Surface features of exfoliated vaginal epithelial cells during the oestrous cycle of the rat examined by scanning electron microscopy*

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INTRODUCTION

The cyclical changes in the vaginal wall during the oestrous and menstrual cycles have been extensively studied by means of light and transmission electron microscopy (Long & Evans, 1922; Burgos & Wislocki, 1958; Barker & Walker, 1966; Eddy & Walker, 1969; Parakkal & Gregoire, 1972; Christie, Bailey & Bell, 1972), but few studies have been made with the scanning electron microscope (Kanagawa *et al.*, 1972; Rubio, 1976; Parakkal, 1974), and in particular, the surface morphology of exfoliated vaginal epithelial cells during the oestrous cycle appear to have been neglected. The present paper describes the fine structural surface features of rat vaginal epithelial cells, both exfoliated and *in situ*, during the classical four stages of the oestrous cycle.

MATERIALS AND METHODS

Animal preparation

Twenty eight days old immature female rats of the Lewis/fmai strain (Microbiological Associates, Walkersville, Maryland) were given a single subcutaneous injection of 5 i.u. pregnant mares' serum gonadotrophin (NIAMDD-PMSG-2, 2200 i.u./mg) in order to induce the first oestrous cycle. This was followed 50 hours later by a single injection of 100 μg of bovine luteinizing hormone (NIH-LH-B9). This procedure was found necessary in this strain of rat to ensure induction of ovulation and subsequent formation of corpora lutea (Crisp & Denys, 1975). All hormones were kindly supplied by Dr A. F. Parlow through the NIAMDD rat pituitary hormone distribution programme.

Observation of vaginal smears indicated pro-oestrus 7–9 hours after injection of luteinizing hormone, oestrus after 18–20 hours, metoestrus after 28–34 hours, and dioestrus after 60–64 hours.

Preparation of Millipore filters

Millipore filters (GSWPO 1300, 0·22 μm or VSWPO 1300, 0·025 μm), obtained from Millipore Corporation, Bedford, Massachusetts, were prepared according to the method of Shelton & Orenstein (1975). Briefly, the filters were boiled for 15 minutes in three changes of distilled water to remove a surfactant toxic to cells, after which the filters were dried and stored in a sterile glass Petri dish.

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Falcon (No. 3010) organ tissue culture dishes (60 × 15 mm) with outer absorbent filter rings were used for incubating plated vaginal epithelial cells. Fisher filter paper (5·5 cm, No. 9-801-B) was cut into 25 mm discs. After sterilization the individual discs were placed upon 1 ml of 90 % Medium 199 with Hepes' buffer (GIBCO), 10 % human male serum (with low hormone levels) and 50 units of penicillin plus 50 μg streptomycin/ml, in the centre well of the culture dish. Approximately 3 ml of a 0·1 % bovine serum albumin (BSA) in 0·9 % phosphate buffered saline (PBS) solution was placed in the outer ring of the culture dish in order to maintain the humidity of the culture chamber.

Cell culture

Three Millipore filters, prepared as above, were then placed upon the filter paper disc in the centre well of the culture chamber. The dishes were covered, placed in a humidified chamber, and gassed with 95 % air/5 % CO_2 before sealing. The filters were equilibrated at 36·5 °C for ½ hour in a Roller Therm tissue culture incubator.

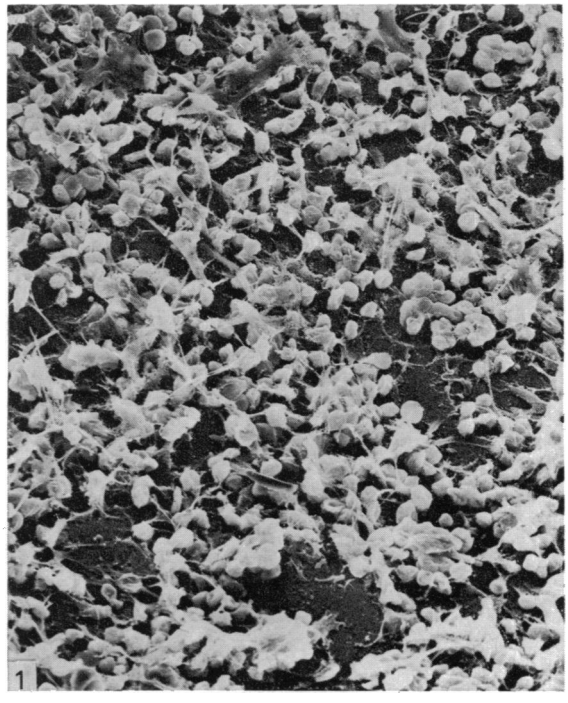
Exfoliated epithelial cells were harvested by vaginal lavage using sterile round-tipped Pasteur pipettes containing 100 μl of culture medium. Approximately 20 μl of the cell suspension was plated onto each of the preincubated, moistened Millipore filters. The culture dishes were covered and placed in the humidified chamber. The chamber was gassed again with 95 % air/5 % CO_2 before sealing. The plated cells were incubated at 36·5 °C for an additional ½ hour.

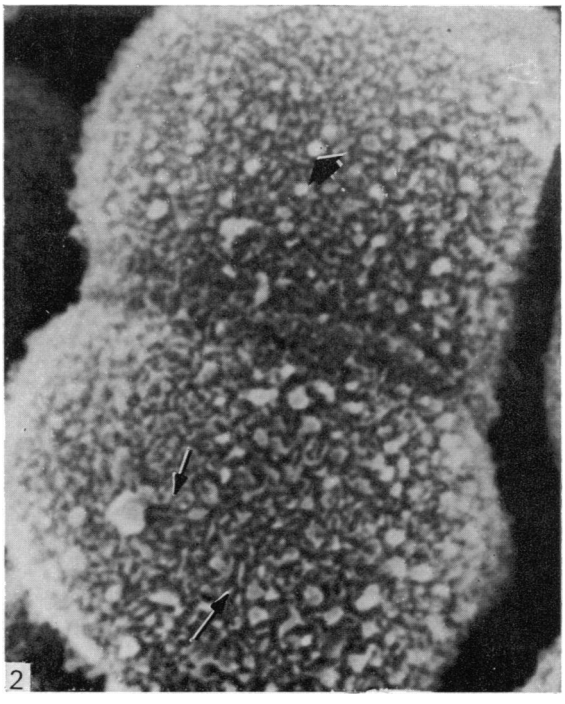
SEM preparation

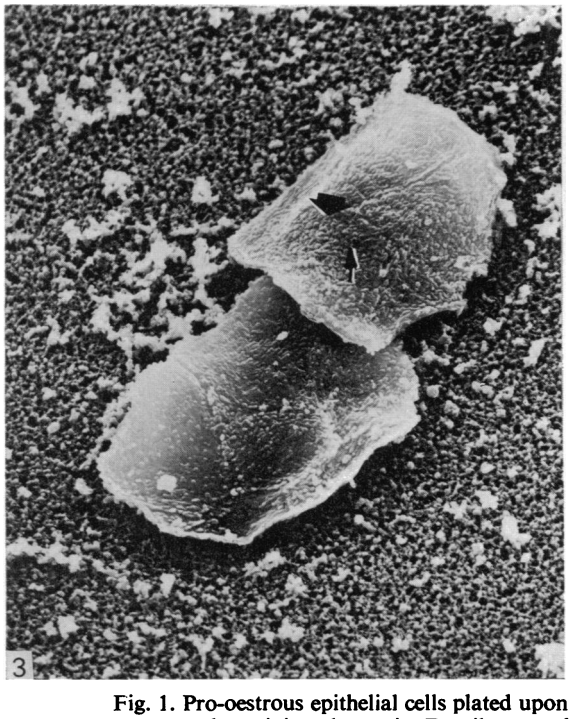
Plated epithelial cells. At least three plated specimens from each stage of the cycle were fixed for 2 hours in either (1) 6 % glutaraldehyde in 0·1 M phosphate buffer, (2) 3 % glutaraldehyde in Medium 199 with either Hepes' or bicarbonate buffer without serum, or (3) 2 % paraformaldehyde and 3 % glutaraldehyde containing picric acid (Ito & Karnovsky, 1968).

Vaginal wall. Intact vaginal walls were prepared as follows: both 'smeared and non-smeared' animals were killed by cervical dislocation, and the entire vagina and lower uterine horns were carefully excised. Within 2 minutes of killing, the vagina was immersed in 2 % paraformaldehyde and 3 % glutaraldehyde containing picric acid (Ito & Karnovsky, 1968). After an initial 1–2 hours fixation the anterior wall of the vagina was cut longitudinally and folded back to expose the posterior wall. Fixation was then continued for at least an additional 6–7 hours.

Following fixation, all specimens were washed twice (10 minutes each) in 0·1 M phosphate buffer with 3 % sucrose. The plated cells and intact vaginal walls were post-fixed in 1 % osmium tetroxide in 0·1 M phosphate buffer for 1½ hours, and then washed and stored in 0·1 M phosphate buffer with 3 % sucrose. Specimens were dehydrated first in ascending grades of cold ethanol solutions, then in ethanol–chloroform solutions at room temperature, and finally in pure dry (silica gel) chloroform. The specimens were critical point dried from CO_2 in a Samdri apparatus (Tousimis), mounted on specimen stubs with double-sided sticky tape, and coated with gold/palladium (60/40) in a sputter coater (Technics), or with platinum–carbon in a vacuum evaporator (Denton Vacuums, Inc.). The specimens were viewed in an ETEC Autoscan SEM at 20 kV and photographed with Polaroid Type 55 P/N film.







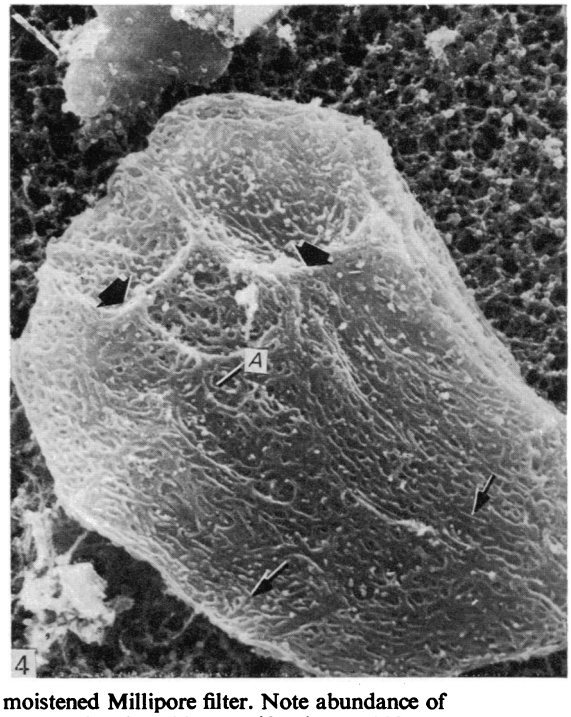


Fig. 1. Pro-oestrous epithelial cells plated upon moistened Millipore filter. Note abundance of mucus and precipitated protein. Detail cannot be appreciated at this magnification. × 200.

Fig. 2. High power micrograph of the pro-oestrous exfoliated cells. Note the microvilli (arrows), and the smooth-surfaced blebs (arrowhead). × 6000.

Fig. 3. High power scanning electron micrograph of two cornified cells at oestrus. Note the microridges (small arrow) covering the cell surfaces, and elongated furrows bordered by raised ridges (arrowhead). These may represent intercellular boundaries, and attachment loci for overlying cells. × 1350.

Fig. 4. High power scanning electron micrograph of cornified cell at oestrus. Note the abundance of microridges (arrows) frequently surrounding a pit or hole (A), and raised ridges (arrowheads). × 2800.

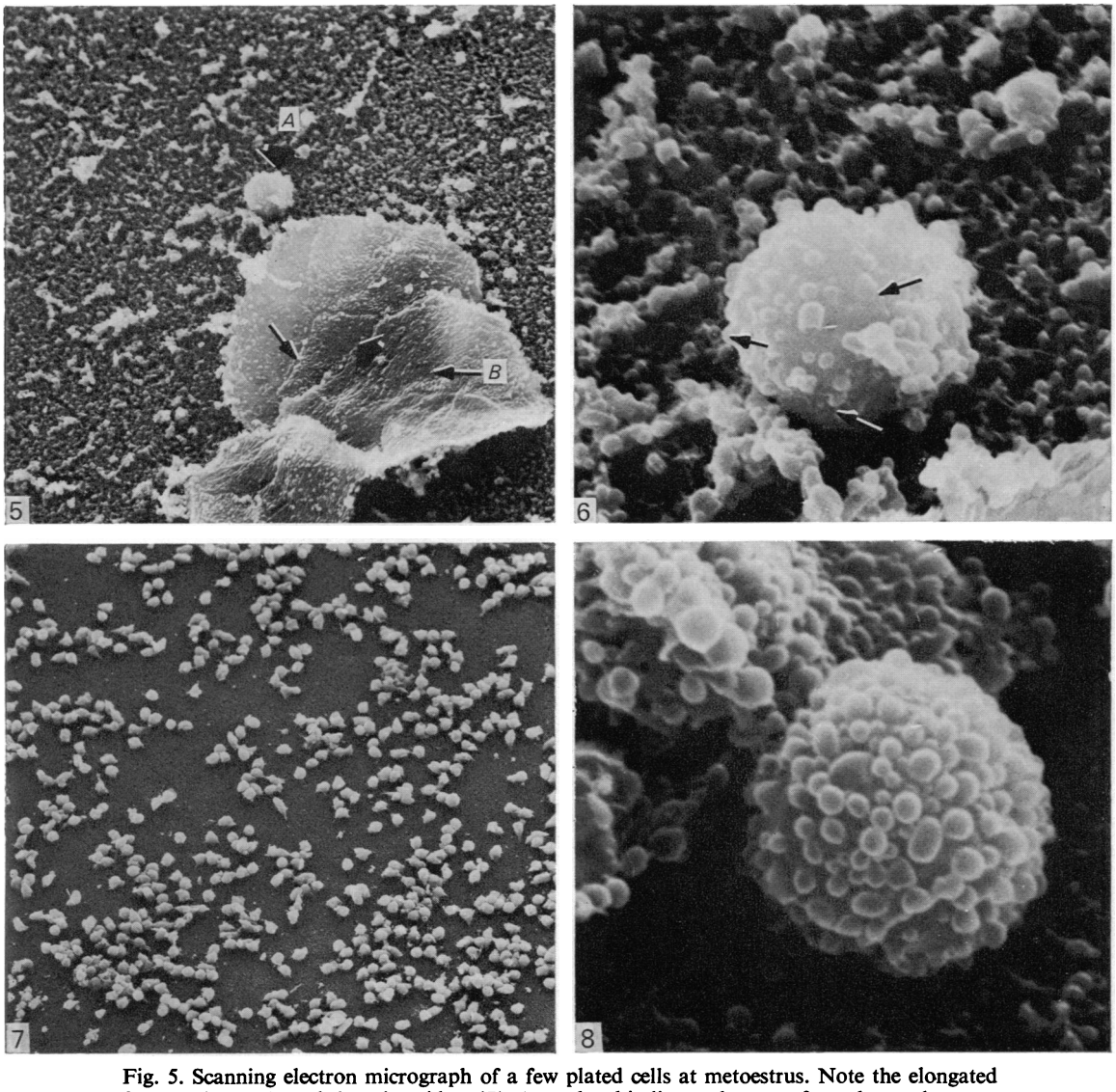


Fig. 5. Scanning electron micrograph of a few plated cells at metoestrus. Note the elongated furrows (arrows), and the microridges (*B*). Arrowhead indicates the area of attachment between two cornified cells. The arrowhead at (*A*) indicates a leucocyte. × 1080.

Fig. 6. Higher power micrograph of the leucocyte seen in Fig. 5. Arrows indicate cell surface blebs which are continuous with the cell membrane. × 5400.

Fig. 7. Low power scanning electron micrograph of a dioestrous vaginal smear showing numerous leucocytes. × 260.

Fig. 8. High power micrograph of a leucocyte at dioestrus. Note the cell surface blebs of varying sizes, some of which are continuous with the cell membrane, while others may represent adhering mucous droplets. × 8800.

RESULTS

Effect of fixative and size of Millipore filter

Surface features of plated cells were unaffected by the three fixatives employed. Unless otherwise indicated, the observations presented here are from specimens fixed in 2 % paraformaldehyde and 3 % glutaraldehyde with picric acid. Considerably

more exfoliated cells remained attached throughout the preparative procedures to the surface of Millipore filter type GSWPO 1300 (pore size 0·22 μm) than to filter type VSWPO 1300 (pore size 0·025 μm). Hence, the data presented are those obtained from the use of the 0·22 μm (GSWPO 1300) Millipore filter.

Exfoliated cells

Pro-oestrus. At this stage of the cycle the Millipore filter was covered with an apparently homogenous population of cells which were round to ovoid in shape, measuring approximately 12–14 μm in diameter (Fig. 1). The cells occurred singly, or in clumps, or plaques. In addition, many cells were seen entangled in and distorted by mucus and precipitated protein. Under high power pro-oestrous cells typically exhibited both small smooth-surfaced blebs and short microvillus-like protrusions (Fig. 2).

Oestrus. The Millipore filter at oestrus was covered by large angular cornified cells of about 32 μm average diameter. Cells occurred either singly or in groups of two or three attached cells.

Under high power some cornified cells were covered with randomly oriented and branching microridges approximately 0·4 μm wide, and of variable length (Fig. 3); elongated furrows, bordered by raised ridges, passed obliquely across the cell surface. Other cells exhibited both microridges, and pits of about 0·8 μm diameter which were frequently surrounded by microridges (Fig. 4).

Metoestrus. At metoestrus the filter was covered by large, angular cells, like those at oestrus. Leucocytes were occasionally seen among the cornified cells. The latter possessed microridges, as well as furrows bordered by raised ridges (Fig. 5). Leucocytes during metoestrus exhibited variable numbers of smooth-surfaced blebs (Fig. 6). Some of these appeared to be continuous with the cell surface, while others were probably adhering mucous droplets.

Dioestrus. The Millipore filter was covered predominantly by leucocytes with a diameter of about 7 μm (Fig. 7). Giemsa-stained vaginal smears indicated the leucocytes consisted of both small lymphocytes and neutrophils. They were generally round and fairly uniform in size. Some cells were distorted by mucus and precipitated protein.

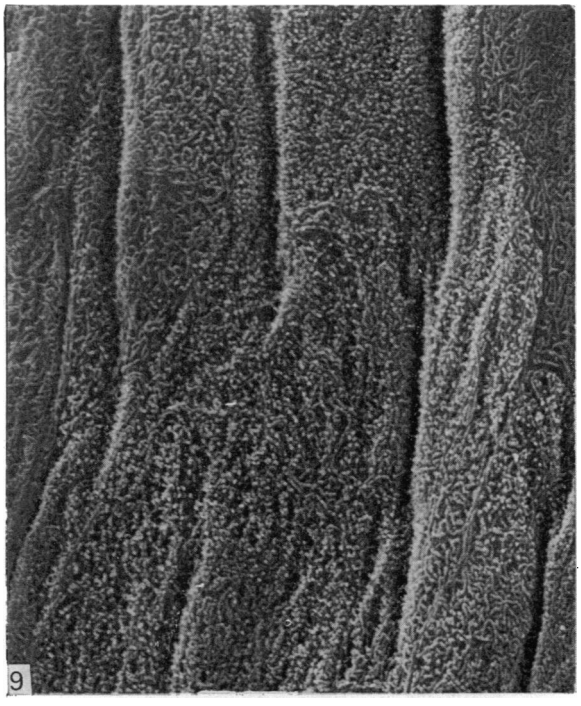
At high power the leucocytes of dioestrus were seen to be covered by smooth-surfaced round blebs of varying size (Fig. 8).

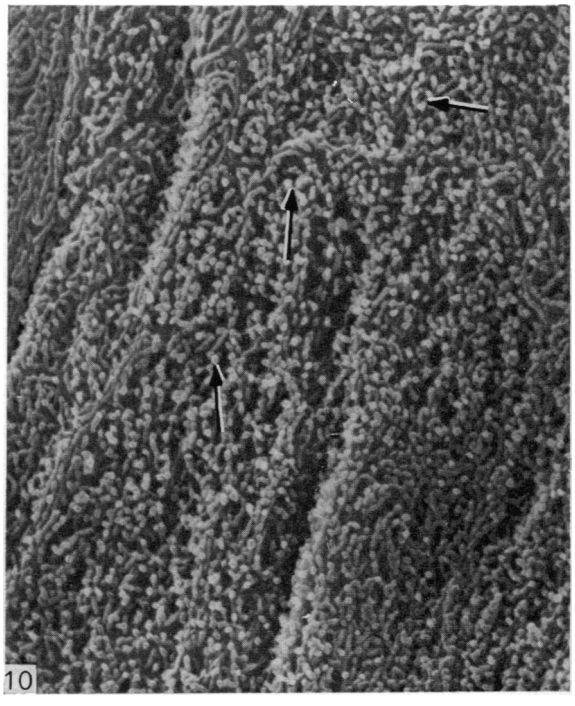
Vaginal wall

Pro-oestrus. The entire posterior vaginal wall at pro-oestrus exhibited longitudinal folds (Fig. 9). The surface cells were covered with bulbous-tipped microvilli (Fig. 10) which extended over the folds and into the furrows of the wall, and masked the shape and size of the cells.

Oestrus. The wall at oestrus presented a mosaic of flattened cells of varying sizes (Fig. 11). At low magnification, slightly raised, irregular lines demarcated cell boundaries. Some cells appeared to be sloughing. The surface cells possessed both microridges and pits (Fig. 12).

Metoestrus. The wall at metoestrus very closely resembled that at oestrus (Fig. 13). Cell boundaries were clearly demarcated. Increased sloughing was observed as compared with oestrus. The surface epithelial cells again exhibited pits as well as microridges (Fig. 14). Occasionally, microridges were seen encircling a central pit or hole.





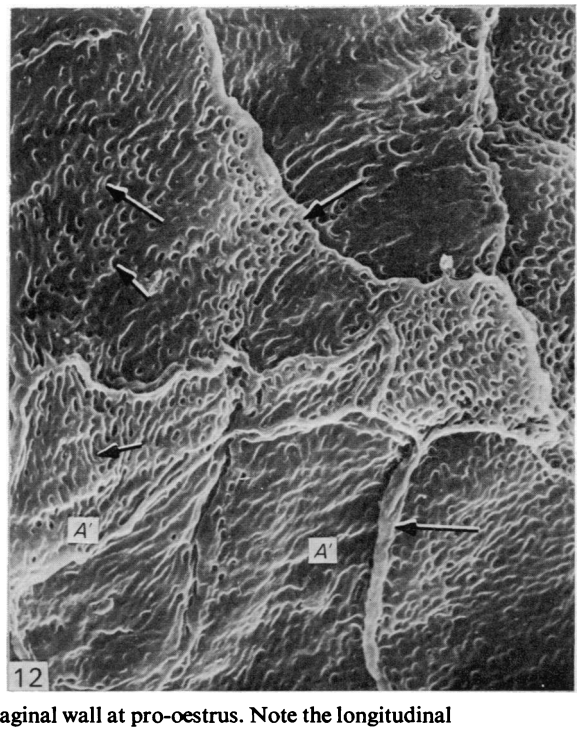
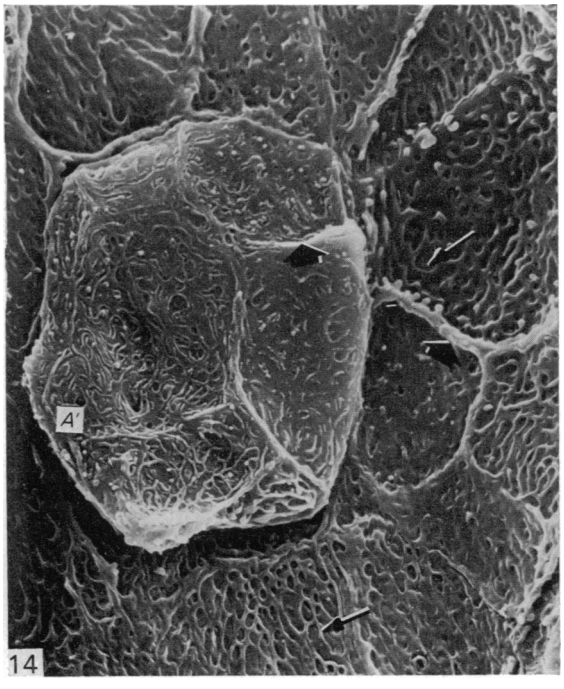


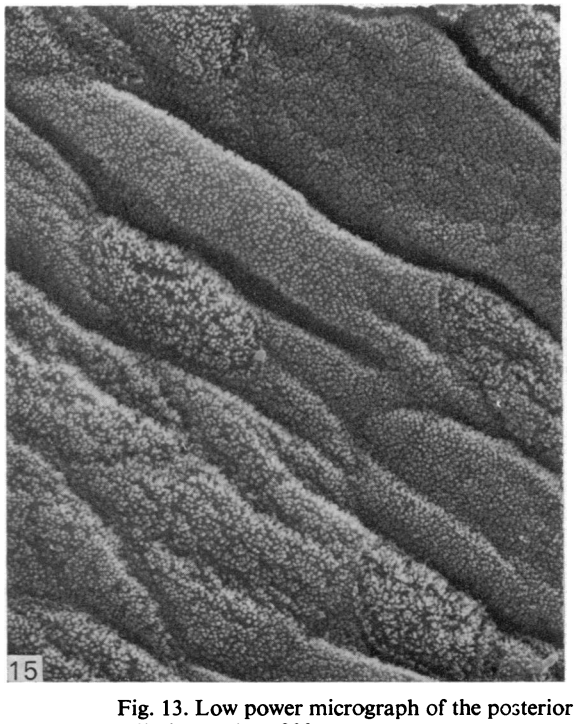
Fig. 9. Low power micrograph of the posterior vaginal wall at pro-oestrus. Note the longitudinal folds and rugae of this part of the wall. × 2800.

Fig. 10. High power micrograph of the posterior vaginal wall at pro-oestrus. Note the club-tipped microvilli (arrows). × 5600.

Fig. 11. Low power scanning electron micrograph of the oestrous vaginal wall. Note what appear to be cells in the process of sloughing (arrows). × 280.

Fig. 12. High power micrograph of vaginal wall at oestrus. Note the pits (small arrows), the ridges at (*A'*), and the cell to cell attachment sites represented by the raised ridges (long arrows). × 2800.





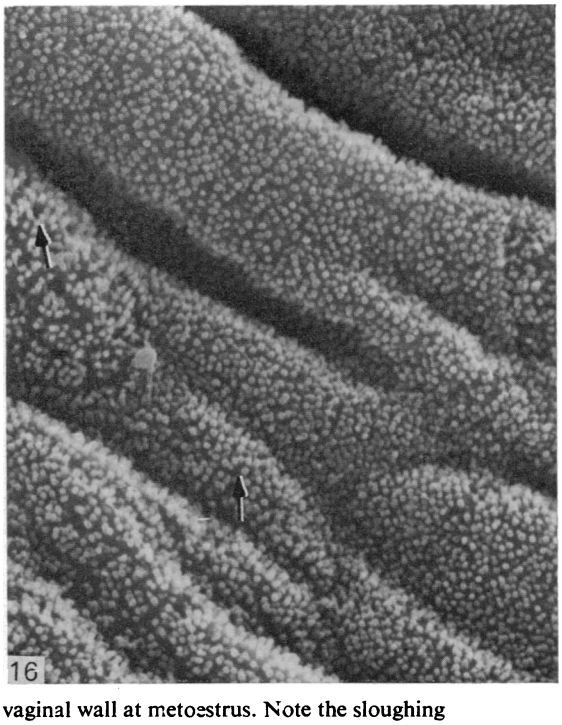


Fig. 13. Low power micrograph of the posterior vaginal wall at metoestrus. Note the sloughing cells (arrows). × 200.

Fig. 14. High power scanning electron micrograph of the vaginal wall at metoestrus. Note the pits (arrows), microridges (*A'*), and the raised ridges (arrowheads). The cell in the field seems to be in the process of sloughing. × 2800.

Fig. 15. Low power micrograph of the posterior vaginal wall at dioestrus. Note the longitudinal folding of the wall, and the 'cobblestone' appearance of the surface of the wall. × 2800.

Fig. 16. High power micrograph of the wall at dioestrus. Note the club-tipped short microvilli (arrows) covering the entire free surface of the surface epithelial cells. × 5600.

Dioestrus. The dioestrous vaginal wall resembled a 'cobblestone' pavement with stones of varying sizes and shapes (Fig. 15). The surface was thrown into longitudinal folds and shallow furrows. Cells which appeared to be leucocytes were seen occasionally between the epithelial cells. The surface cells were covered with numerous short microvilli with bulbous endings (Fig. 16). The microvilli covered the entire cell surface and extended into the furrows of the wall.

DISCUSSION

Plating exfoliated cells onto moistened Millipore filters is an easy way to attach cell suspensions and maintain them during short periods of incubations (Shelton & Orenstein, 1975). Manipulations of the filter and cells during processing for SEM observations is reasonably easy, and few cells are lost.

The surface morphology of the exfoliated vaginal epithelial cells resembles very closely the surface features of the intact wall in the non-lavaged rat as presented here, and as seen by others in the rat (Parakkal, 1974), in the mouse (Rubio, 1976), and in the rabbit (Kanagawa *et al.*, 1972).

The plated cells during pro-oestrus comprise a fairly homogeneous population and exhibit short microvillus-like protrusions and a few small rounded blebs like the cells of the intact wall.

In 6–8 hours the surface morphology of the intact vaginal wall and the exfoliated cells has changed. The cells are larger and have become angular and cornified. Both the intact wall and plated cells typically exhibit randomly oriented microridges, pits and furrows bordered by raised ridges. It is possible that the microridges provide channels for the movement of mucus and spermatozoa. The furrows bordered by raised ridges represent intercellular boundaries between cells. It was not until the introduction of the SEM that these features were clearly identified as continuous microridges, rather than microvilli, as had been previously inferred (Parakkal & Gregoire, 1972). Rubio (1976) suggested that the pits function in cell cohesion. The club-tipped microvilli present in overlying cells could fit into these pits and anchor the cell in place. During exfoliation the microvilli may retract, allowing the cells to slough. Pirbazari (1977) suggested that in the gingival mucosa the tips of the microvilli actually break off during sloughing, leaving a cellular remnant in the pit. No such remanants were found in the pits in the present study. Clearly, for a better understanding of the process of exfoliation, and the role of these pits in this process, more work is required.

During metoestrus the epithelial cells resembled those seen during oestrus. A few leucocytes were also seen at this stage. In dioestrus the smear consisted solely of leucocytes (both polymorphonuclear cells and small lymphocytes). A few cells resembling leucocytes seemed to be in the process of passing between the cells of the intact wall. The surface morphology of the leucocytes during dioestrus in this study did not resemble that of typical blood leucocytic elements (Wetzel, 1976), but this is perhaps a consequence of the isolating and preparative procedures, for the surface morphology of leucocytes is known to be extremely sensitive to environmental change (Wetzel, 1976).

Vaginal lavage at pro-oestrus enables one to obtain a homogeneous population of cells which may well possess receptors for both oestrogens and progestins. Plated cells incubated with labelled steroids might be the basis of a radioreceptor assay method for these hormones. They might also be useful for studying hormone-mediated differentiation in culture, and ultrastructural aspects of exfoliation.

SUMMARY

The purpose of this study was to document vaginal exfoliative cytology (smears) as seen with the scanning electron microscope during the first, hormonally induced oestrous cycle of PMS-LH treated immature rats and to compare it with the surface morphology of the intact vaginal wall of similarly treated rats. Exfoliated cells were obtained by vaginal lavage 7–9 hours (pro-oestrus), 18–20 hours (oestrus), 28–34 hours (metoestrus) and 60–64 hours (dioestrus) after administration of luteinizing hormone. Cell suspensions were plated upon moistened Millipore filters according to the method of Shelton & Orenstein (1975). The pro-oestrous stage was characterized by round to ovoid cells with numerous short microvillus-like protrusions and blebs. The vaginal wall exhibited folds lined by cells with many short microvilli. Oestrus was characterized by flattened angular cells with either blebs, microridges or both. The intact wall exhibited 'flattened mosaics' of cells, some in the process of desquamation, with ridges and pits. Metoestrus resembled oestrus, except for the presence of a few leucocytes in the plated specimens. Dioestrus was characterized by an abundance of pleomorphic leucocytes with blebs of varying sizes. The vaginal wall appeared as a 'cobblestone' pavement with cells of varying sizes and shapes possessing numerous short, microvilli with bulbous endings.

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