Scanning Electron Microscopy of Normal Uterine Cervix, Carcinoma in situ and Squamous Cell Carcinoma

Preliminary Findings

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SUMMARY

Colposcopically directed punch biopsy specimens of the uterine cervix were taken from women in whom Papanicolaou smears indicated cytological abnormalities. Half of each specimen was processed for scanning electron microscopy and half for light microscopy. The surface morphology of normal cervical, metaplastic and frankly carcinomatous epithelia and epithelia of carcinoma in situ, as seen under the scanning electron microscope, was compared with the appearance revealed by Papanicolaou smears, and haematoxylin- and eosin-stained sections.


The human uterine cervix is a very accessible organ for the study of epithelial neoplasia. Cervical carcinoma has been studied by means of exfoliative cytology, colposcopy, light microscopy and transmission electron microscopy. It has been shown that the surface of the epithelial cells of the cervix is altered as the epithelium undergoes various physiological and pathological changes. Light and transmission electron microscopy allow one to examine the tissue in cross-section only and thus very little detail of the surface can be seen. With the aid of the coloscope one can examine the surface of the cervix at magnifications of 6-40x. The scanning electron microscope has been used to study the surface of cervical biopsy specimens and exfoliated cervical cells in greater detail. The present study was undertaken to relate clinical observations and findings of light microscopy to the ultrastructure of the surface of normal and abnormal cervical epithelium. Particular attention was paid to carcinoma in situ and frank carcinoma.

MATERIALS AND METHODS

Thirty-seven patients with abnormal exfoliated cells, revealed by Papanicolaou smears, were studied. Under colposcopic direction a punch biopsy specimen was taken from the affected area of the cervix, using the saline technique of Koller as recommended by Jordan et al. Half of the biopsy specimen was fixed and stained for light microscopy and the other half was fixed for scanning electron microscopy. This specimen was first fixed in 2.5% glutaraldehyde in buffer for 24 hours, washed in buffer plus 2% glucose for 24 hours, and then postfixed in 1% osmium tetroxide in buffer for 1½ hours. Thereafter the specimen was dehydrated through serial dilutions of alcohol, followed by ether and finally amyl acetate. From amyl acetate the specimen was dried using the critical point technique. The specimens were then coated with silver in a vacuum and viewed in a Cambridge Stereoscan electron microscope.

RESULTS

Representative samples from 37 patients studied will be discussed in this paper. All cell measurements given are only approximate, since they were taken directly off the scanning electron microscope prints.

Squamous Epithelium

The squamous epithelium of the ectocervix consisted of 'paving-stone'-like cells which had a flat surface (Fig. 1).
Individual cells were surrounded by raised ridges, or terminal bars. The cell surfaces were covered by small ridges, or microridges which were about half as thick as the terminal bars. These microridges showed polarity in that they were usually parallel to one another. This polarity may spread into adjacent cells, but other cells showed shorter microridges with very little polarity. In these latter cells some of the microridges were so short as to be more like micropegs. In Fig. 1 there appears to be a greater variation in size of cells. The average diameter of the squamous cell was 40 - 50 μm.

Columnar Epithelium

The endocervical epithelium was a simple columnar epithelium which consisted of small, round to hexagonal cells (Fig. 2). There were no terminal bars and the cells were separated by grooves. The cell surfaces, which were convex, were covered by numerous short microvilli. The average diameter of these cells was 6 μm.

Squamous Metaplasia

The scanning electron microscopic appearance of typical squamous metaplasia is shown in Fig. 3. This patient’s cervical cytology demonstrated atypical cells, while colposcopy indicated typical metaplasia. This photomicrograph shows the tip of one endocervical villus, the base of which (not shown) revealed normal columnar epithelium. Fig. 3 demonstrates that the cells were hexagonal or polygonal. The most striking feature was the prominent terminal bar surrounding each cell. These terminal bars differed from those in squamous epithelium in that they resembled very closely packed microvilli. The surface of the cells was covered by numerous microvilli. The tip of the adjacent villus showed flattening of cell surfaces and apparent fusing of these cells. The base of this villus also showed normal columnar epithelium.

Carcinoma in situ

Fig. 4 shows the scanning electron microscopic appearance of the cervix in a patient with a positive Papanicolaou smear. Colposcopy showed an extensive carcinoma in situ lesion of the cervix, but light microscopy of the biopsy specimen showed only an early carcinoma in situ. Scanning electron microscopy showed rounded cells which appeared to be coming away from the rest of the epithelium. There were no terminal bars between the cells and
the cell surfaces were 'granular'. This appearance was due to pits between which there were short microvilli or irregular microridges. These cells were about 18 μm in diameter and similar in size to parabasal cells.

In another patient, in whom a Papanicolaou smear showed carcinoma in situ, colposcopic examination of the cervix revealed atypical metaplasia. The histology of this biopsy specimen showed extensive carcinoma in situ. The scanning electron microscope showed very bizarre cells which resembled normal squamous cells in that they looked like paving stones and were separated from one another by terminal bars. The terminal bars, however, were thickened and irregular in nature compared with those in Fig. 1. The surfaces of these cells were pitted and irregular. There were no surface structures that resembled either microridges or microvilli. The average diameter of these cells was 20 - 25 μm.

**Squamous Carcinoma**

This specimen was taken from a patient who had an extensive keratinising squamous carcinoma of the vagina which extended onto the cervix (Fig. 5). The cells were irregular in outline and cell borders were difficult to establish because terminal bars were present only in some places. The most notable feature was the smooth appearance of the cell surface compared with the carcinoma in situ cells. The cell surfaces were also pitted and the openings to these pits were surrounded by 'flattened microridges'.

DISCUSSION

The features of normal and metaplastic squamous and columnar epithelial tissue seen by us on scanning electron microscopy resembled those described previously. However, some significant differences were observed in the squamous cells. Previous workers described squamous cells which showed microridges that tended to run concentrically around the centre of the cell. They also observed more microridges per cell and surface bulges in the cells, probably owing to the nuclei. These differences can be explained by the fact that in the previous studies, the specimens were air-dried from acetone before they were coated for viewing in the scanning electron microscope. Air-drying of specimens causes marked shrinkage of the tissue, and therefore we have used the critical point method of drying to minimise this artefact. Previous transmission electron microscopy of the ectocervix showed the surface of the squamous cells to be covered by numerous microvilli. It is apparent from scanning electron micrographs of squamous epithelium that the microvilli seen by transmission electron microscopy were in fact microridges seen in cross-section. The previously published scanning electron micrographs of carcinoma in situ show features which differ from those of the early or late lesions of carcinoma in situ described in this study. Jordan et al. described rounded cells, arranged irregularly, and covered by closely packed microvilli, with occasional pitting. The present study demonstrates that there is much similarity between the appearances of early and late carcinoma in situ and squamous cell carcinoma. This similarity is mainly related to the pitted surface of the cells, and could support the idea of progression of a lesion from early carcinoma in situ to frank carcinoma.

Scanning electron microscopy allows for identification of the different types of normal and abnormal cervical epithelia from the surface appearance alone. These are only preliminary findings, and a long-term study is envisaged to try to correlate the ultrastructure of the cervix with the pathogenesis of cervical disease.

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REFERENCES