

## Gingival cytology of children from three to five years of age

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### Abstract

*Collections of exfoliated cells on Millipore filters, which retain the topographical relationships of the cells collected, previously have been used to examine keratinization and inflammation of adult gingivae. In the present study, samples of epithelial and inflammatory cells were obtained from the anterior maxillary gingivae of three- to five-year-old children using this technique. The oral hygiene index (OHI) of these children was recorded and the degree of gingival inflammation clinically assessed. Variation in the width of the band of leukocytes collected from the gingival margin indicated a greater range of gingival inflammation within the sample studied than was apparent from clinical assessment. The degree of leukocytic exudate from the gingival margin appeared to correlate with the degree of keratinization of the adjacent epithelium.*

Brauer,<sup>1</sup> examining the microanatomy of the gingivae of young children, compared it to skin in its ability to resist mechanical forces. The attached gingivae is bound by dense fibrous tissue, and the stratified squamous epithelium has a keratinized or parakeratinized surface associated with stippling of the oral aspect. In 1940, Weinmann<sup>2</sup> described the keratinization of human oral mucosa and in 1959, Weinmann and Meyer<sup>3</sup> listed four types of keratinization: (1) full keratinization, (2) parakeratosis, (3) nonkeratinization, and (4) incomplete keratinization. They also noted that with an increase in gingival inflammation, there was a decrease in the amount and degree of keratinization. Full keratinization occurred primarily in the absence of inflammation and became infrequent when inflammation was present.

The purpose of the present study was to examine the pattern of gingival cytology of children who appeared clinically free of gingival disease. Special attention was given to the level of marginal inflammation, indicated by the amount of leukocytes present on the collected samples.

### Methods and Materials

A group of 10 three- to five-year-old children with intact dentitions were selected for the study from the undergraduate Pedodontic Clinic of the University of Iowa College of Dentistry. These patients were considered to have normal gingivae due to a lack of redness or edema of the gingival margin.

Superficial cells for cytological examination were obtained by the filter imprint technique described by Seavall and Grand<sup>4</sup> as modified by Lainson and Mackenzie.<sup>5</sup> The subjects were instructed not to brush their teeth for at least two hours prior to taking the filter imprints. Confirmation to these instructions was confirmed by the parents. Millipore filters were used, and eight pie-shaped pieces of equal size were cut from each originally round Millipore filter and marked at their apex with India ink.

The gingivae of the anterior teeth were dried briefly with air. The filter piece, apex pointed coronally, was applied to an area in the region of the maxillary right primary central incisor covering the free gingival margin, attached gingivae, and alveolar mucosa into the vestibule. The filters were pressed firmly in place, without sliding the filters on the mucosa and avoiding contact with labial alveolar mucosa. Rubber surgical gloves were used when handling the filters to prevent contamination with exfoliated cells from the examiner's fingers. The filter imprints were removed with cotton pliers, immediately fixed in 95% ethanol, and then stained by a modification of the Papanicolaou method.<sup>6</sup>

After obtaining the filter imprints for each child, an assessment of oral hygiene was made using a modification of the simplified oral hygiene index (OHI-S) described by Greene and Vermilion.<sup>7</sup> This index scores plaque and accumulations of debris from 1 to 3 on the facial and lingual surfaces of six selected teeth after these teeth have been stained with disclosing solution. The possible scores thus ranged from 0 to 36.

### Results

The oral hygiene indices of the children ranged from good (7) to poor (23) but the gingival tissues in all cases

appeared to be within normal limits without typical signs of inflammation.

The staining pattern on the cells collected from the various regions of the gingivae corresponded to the three principal epithelial cell types observed on filters stained by the Papanicolaou method as described by Lainson and Mackenzie.<sup>5</sup> The distribution of various cell types reflected topographical gingival anatomy and, typically, a number of zones could be identified on each filter (Figure 1):

1. A zone of nucleated nonkeratinized cells with the staining characteristics of the alveolar mucosa showing a transition of cell type indicating position of the mucogingival junction
2. An area containing yellow orange staining keratinized cells exfoliated from the attached gingivae
3. A dense yield of epithelial cells with altered staining adjacent to the region of the gingival margin
4. A band of inflammatory cells corresponding to the position of the gingival margin.

The cytological appearance of the mucogingival junction was of keratinized cells of the attached gingivae changing at an abrupt linear zone of transition to the larger nucleated cells exfoliated from the alveolar mucosa.

The region of the attached gingivae varied in degree of keratinization from one specimen to the next. In the majority of specimens the attached gingivae was orthokeratinized with nuclei absent from the exfoliated cells. However, some specimens showed retention of cell nuclei, a cytological appearance corresponding to parakeratosis as observed in histological sections. At the gingival margin a mass of inflammatory cells typically was present. The nuclear morphology of these cells was typically of polymorphonuclear leukocytes. These inflammatory cells surrounded nucleated eosinophilic epithelial cells. A large variation in the width of the band of polymorphs and altered epithelial cells was seen from one specimen to the next.

Several specimens demonstrated an appearance thought to represent a severe degree of inflammation. These showed a wide and dense band of polymorphs next to a band of eosinophilic epithelial cells from the free gingival margin. For some distance from the gingival margin, a heavy yield of inflammatory cells was observed.

The cytological appearance of the gingivae of the 3 to 5 year olds in this study generally was similar to that observed by Lainson and Mackenzie<sup>8</sup> in adults, with a pattern of nonkeratinized alveolar mucosa and of para- or orthokeratinized attached gingivae. However, comparison of the cytological appearance of children with that of adults suggests a greater degree of orthokeratinization in the younger age group. The changes observed at the gingival margin were similar to those seen in adults,

but the clinical degree of inflammation seen in the children was not marked.

The pattern observed on the filter specimens showed an association between increasing leukocytic exudate and altered staining and heavier yields of epithelial cells from the gingival margin. This suggests that increasing inflammation leads to reduced keratinization and a higher rate of cell turnover of the epithelium adjacent to the gingival margin. Similar suggestions have been made by Demetriou, Ramfjord, and Ash,<sup>9</sup> who described a reduction in keratinization of the gingivae and an increased cell labeling associated with increased inflammation in rhesus monkeys.

## Discussion

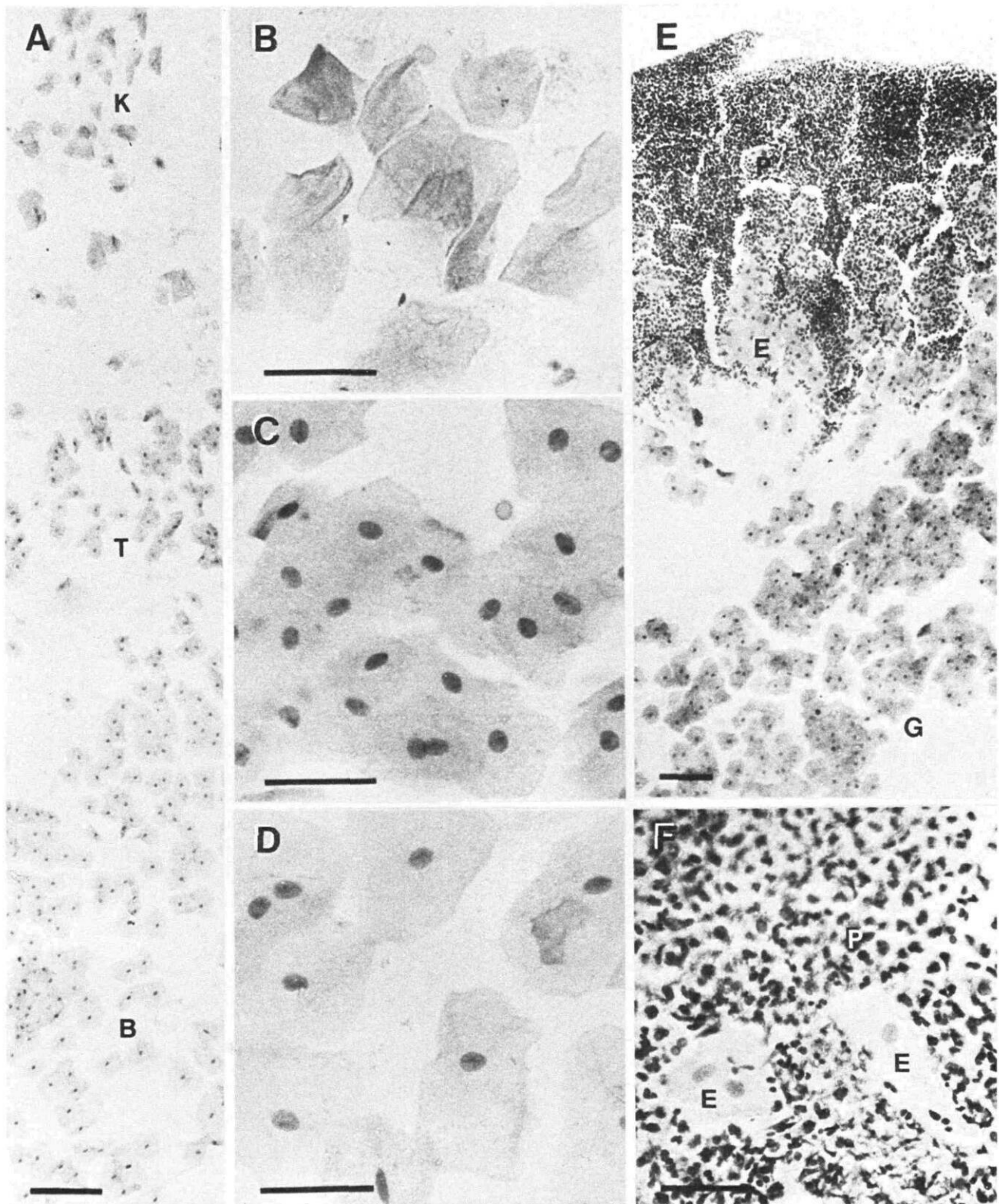
Several studies have demonstrated the widespread occurrence of periodontal pathology in children but, due to its typically incipient nature, periodontal disease in children has not received the attention given to dental caries.<sup>10-18</sup>

An association between the inflammatory exudate and plaque has been demonstrated by others; Lindhe and Axelsson<sup>19</sup> looked at the effects of various preventive measures on the degree of plaque and gingivitis in Swedish school children. With professionally administered oral prophylaxis and fluoride treatments both the gingival and plaque indices decreased significantly over 12 months. Using the gingival index of L oe and Sillness, Poulson et al.<sup>20</sup> demonstrated that Danish school children had less gingival bleeding with increased professional oral hygiene. At the termination of the study, 70.3% of the children receiving professional cleanings every two weeks had a score of zero (no bleeding) compared to 44.7% at the beginning of this investigation. Mackenzie, et al.<sup>21</sup> demonstrated a rapid increase of leukocytes with cessation of oral hygiene and a decrease of leukocytes during periods of increased oral hygiene in adults.

All of the children selected for this study had normal-appearing gingivae, but the OHI was found to range from 7 to 23. No correlation was found between the OHIs obtained in either the amount of inflammatory exudate microscopically visible on examination, or the percentage of nucleated and non-nucleated cells found in an area adjacent to the attached gingivae as reported in adults.<sup>8</sup>

The purpose of this study was to describe the cytology of the normal gingivae, and the OHI was taken only to rule out major differences in the specimens that might have resulted from increased plaque. Given the subjectivity which is present inherently in clinical observations, the sample size may not have been large enough to investigate differences in tissues and to compare these with the OHI. Further, the OHI is a reflection of the area of tooth covered by plaque and not a reliable index of the quantity or composition of plaque.

As yet, the Millipore technique has not been standardized with sufficient reliability to provide more than a



**Figure 1.** Keratinized and nonkeratinized zones of oral epithelium are readily identifiable with the Millipore technique of sampling and a modification of the Papanicolaou stain: (A) low magnification photomicrograph of the various zones of the mucogingival junction showing keratinized cells of the attached gingivae [K], the transitional zone [T], and the cells of the nonkeratinized alveolar mucosa (Scale = 200  $\mu\text{m}$ ); (B) seen at higher power, the orthokeratinized cells of the attached gingivae are non-nucleated and stain orange; (C) cells of the transitional zone are of intermediate size, nucleated, and stain

reddish; (D) the nucleated cells of the buccal mucosa are larger and stain blue (Scale for B, C, D = 40  $\mu\text{m}$ ); (E) the gingival margin region shows polymorphonuclear leukocytes [P] intermixed with epithelial cells which are eosinophilic and nucleated. A high yield of nucleated orange cells from the attached gingivae [G] lies adjacent to the exudate (Scale = 200  $\mu\text{m}$ ); (F) a higher magnification of polymorphonuclear leukocytes [P] and eosinophilic epithelial cells [E] from the marginal region (Scale = 40  $\mu\text{m}$ ).

descriptive analysis of the cellular topography of the gingivae. For such descriptive purposes it would appear that the Millipore technique is a useful, noninvasive method for obtaining cellular material. The reproducibility of this technique as a method for examining experimentally induced changes in gingival inflammation and the correlation between tissue changes and inflammatory status is untested at present. For example, the effects of variation in the amount of pressure applied to obtain the cells and the changes in the cell yields resulting from prior oral hygiene procedures requires further investigation. However, it would appear that the method has the potential for detecting finer changes in the gingival condition than may be detected clinically and thus may be of value in monitoring the effects of various therapeutic agents on gingival health.

## Conclusion

The Millipore technique was used to investigate the cytology of the gingivae in young children. The appearance of the gingival tissues was similar to that of adults but a lesser amount of inflammation was apparent clinically in the children in the presence of cytologically detectable inflammatory exudate. Also, a greater degree of orthokeratinization was found in children. The potential usefulness of the Millipore technique for measuring slight changes in inflammation and the gingival tissues in small sample studies is encouraging. Further standardization of this technique could provide a noninvasive method for measuring subtle changes in the gingivae of subjects when modifications of oral hygiene are employed.

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