

Fine structure of the oral epithelial cell surface in the Japanese lizard, *Takydromus tachydromoides*

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Abstract: Scanning electron microscopy was employed to investigate the ultrastructure of oral epithelial cells of the lizard, *Takydromus tachydromoides*. The specimens were prepared by a method involving osmium postfixation and acid treatment to remove extracellular attached material.

Bump-shaped protuberances were arranged symmetrically on both sides of the median line of the palate. Epithelial surfaces besides the bump-shaped protuberances were widely covered with fine folds. At higher magnification, a network pattern of microridges was closely distributed over the entire surface of the palate, and each cell marginal thickening was clear. The protuberances were also observed in the latero-posterior region of the floor of the mouth. A number of nicks were arranged symmetrically on both sides along the median line of the fore-region of the floor. Taste buds were scattered in the epithelial surfaces between nicks. At higher magnification, microvilli as well as microridges were observed on the epithelial cell surfaces of the floor. There were no papillar structures on the ventral tongue surface. Fine pits were densely distributed over the entire epithelial cell surface. The cell margin was clearly distinguishable as a depressed line. The epithelial surface of the laryngeal part of the pharynx was entirely covered with ciliated cells. Taste buds were also scattered on the inner gingival epithelial surfaces of the upper and lower jaws along the dental arches. Relatively indistinct microridges were widely distributed on the gingival epithelial cell surface of the upper jaw. Scrollwork and network patterns of microridges were clearly observed on the gingival epithelial surface of the lower jaw.

Introduction

The oral epithelial cell surfaces of mammals have been examined by a number of investigators using scanning electron microscopy (Cleaton-Jones and Fleisch, 1973¹⁾; McMillan, 1974²⁾, 1979³⁾; Cleaton-Jones, 1975⁴⁾; Andrews, 1976⁵⁾; Appleton and Heaney, 1977⁶⁾). It has been suggested that the different features of oral epithelial cells may be sufficiently distinctive to be correlated with the degree of surface keratinization. On the other hand, it is also important from the viewpoint of comparative anatomy to clarify

the surface features of the oral epithelium in animals other than mammals, since there has been a notable lack of such studies to date. In the present study, the Japanese lizard, *Takydromus tachydromoides*, a representative of the reptiles, was used to examine the surface features of the oral epithelium by scanning electron microscopy. For this purpose, the mucus covering the oral epithelial surface was removed by acid hydrolysis employing the same method as in previous studies involving lingual surface observations (Iwasaki *et al.*, 1984 a⁷⁾; Iwasaki and Kobayashi, 1984 b⁸⁾; Iwasaki and Miyata, 1985⁹⁾).

Materials and Methods

Specimens of the palate, floor, ventral tongue surface, laryngeal part of the pharynx and gingivae of the upper and lower jaws from three male and three female adult Japanese lizards, *Takydromus tachydromoides*, were used. The animals were perfused from the heart with Karnovsky fixative (Karnovsky, 1965¹⁰) containing glutaraldehyde and paraformaldehyde under ether anaesthesia. The tongues were then removed and refixed with the same fixative. After rinsing in 0.1 M cacodylate buffer, the materials were post-fixed in phosphate-buffered 1% osmium tetroxide solution (Millonig, 1962¹¹) at 37°C for 2 hours, and treated with 8 N hydrochloric acid at 60°C for 30 minutes to remove extracellular attached material by acid hydrolysis. This was followed by dehydration, critical point drying and gold-ion sputtering in that order. Finally, the specimens were observed by scanning electron microscopy.

Results

The major part of the epithelial surface of the palate was almost flat at the lower magnification of scanning electron microscopy and many bump-shaped protuberances were seen, arranged symmetrically on both sides of the median line forming antero-posterior rows. The diameters of these protuberances were 30–60 μm (Figs. 1 and 2). The surface of the flat epithelium adjacent to the bump-shaped protuberances was widely covered with fine folds expanded antero-posteriorly (Fig. 2). At higher magnification, a network pattern of microridges was observed closely distributed over the entire surface of the palate containing the bump-shaped protuberances. Cell marginal thickening was clearly recognized (Fig. 3). Bump-shaped protuberances were also observed in the latero-anterior region of the floor of the mouth. A number of nicks, elongated transversely, were arranged symmetrically on both sides along the median line of the fore-region of the floor (Fig. 4). Taste buds bearing cilia on their surfaces were surrounded by cells with a

network pattern of microridges on their surfaces (Fig. 5). Neither protuberances nor nicks were observed in the middle and posterior regions of the floor. At higher magnification, microvilli as well as microridges were widely seen on the epithelial cell surfaces of the anterior part of the floor. Cell marginal thickening was clear (Fig. 6). A scrollwork pattern of microridges was widely distributed on the epithelial surfaces of the lateral and posterior areas of the floor. Cell marginal thickening was also significant (Fig. 7). The ventral surface of the tongue appeared almost smooth at lower magnification, but the polygonal outline of each cell was relatively distinct (Fig. 8). At higher magnification, many fine pits appeared compactly distributed on the ventral epithelial cell surface of the tongue. The boundary lines between adjacent cells appeared as narrowly distinct hollows (Fig. 9). The epithelial surface of the laryngeal part of the pharynx appeared smooth at lower magnification (Fig. 10). However, at higher magnification, an almost complete covering of ciliated cells became apparent (Fig. 11). Papillar protuberances, bearing round taste buds at the apex, were arranged sporadically on the inner gingival epithelial surface of the upper jaw along the dental arches (Fig. 12). Relatively obscure microridges were widely distributed on the epithelial cell surface. Marginal thickening of these cells was recognizable (Fig. 13). Taste buds were also scattered on the inner gingival epithelial surface of the lower jaw along the dental arches. However, no distinct papillar protuberances were recognized around the taste buds and the shape of the taste buds was nearly oval (Fig. 14). At higher magnification, some of the taste buds appeared to be surrounded by a small protuberance. Scrollwork and network patterns of microridges were clearly observed on the epithelial surface of this region. Cell marginal thickening was clearly seen (Fig. 15).

Discussion

Hydrolysis of extracellular connective tissue components with hydrochloric acid has often been used in combination with enzymic diges-

tion to enable visualization of the basal aspect of tissues and cells (Evan *et al.*, 1976¹²⁾; Uehara and Suyama, 1978¹³⁾; Nagato *et al.*, 1980¹⁴⁾). Desaki and Uehara (1981)¹⁵⁾ indicated that hydrochloric acid treatment without collagenase digestion could hydrolyze both connective tissue and basal lamina. Recently, it has become possible in mammals to remove mucous substance from the lingual dorsal surface without damage to the epithelial cells themselves by employing osmium postfixation and acid hydrolysis (Iwasaki *et al.*, 1984 a⁷⁾; Iwasaki and Kobayashi, 1984b⁸⁾; Iwasaki and Miyata, 1985⁹⁾) according to a method almost identical to that developed by Desaki and Uehara (1981)¹⁵⁾ for removing connective tissue. In the present study, this acid hydrolysis method was applied to the oral epithelia of the lizard, and the results clearly showed that fixed mucus was removed almost completely from the epithelial cell surfaces, while there was no distinct damage to the cell surface itself. It is concluded therefore that the acid hydrolysis method is effective not only in the mammalian tongue but also the oral epithelia of lizards for obtaining distinct scanning electron microscopical images.

It has recently been reported that microridges are widely distributed on the oral epithelial surface in relation to keratinization (Cleaton-Jones and Fleisch, 1973¹⁾; McMillan, 1974²⁾, 1979³⁾; Cleaton-Jones, 1975⁴⁾; Andrews, 1976⁵⁾; Appleton and Heaney, 1977⁶⁾). However, there have been few reports on the fine structure of the reptile oral epithelial surface as revealed by scanning electron mi-

croscopy. The results of the present study indicated that microridges were well developed on the surface of the palate, the floor and the gingivae of the upper and lower jaws. Fahrenbach and Knutson (1975)¹⁶⁾ proposed that microridges represented an adaptational structure of the epithelial cells to friction, while Sperry and Wassersug (1976)¹⁷⁾ reported that microridges might play a role in holding mucus and appeared to facilitate the spread of mucus. The present results also suggest that the microridges may be adaptive in holding mucus on the oral epithelial surface of the lizard. The histological features of such epithelial cells with microridges must be studied in further detail.

Mori (1984)¹⁸⁾ reported that a small number of taste buds was located on the lingual dorsal and ventral epithelia and a large number of taste pores were observed on the epithelium of the nicks of the floor in *Takydromus tachydromoides*. The results of the present study indicated that taste pores were also present in the inner gingival epithelia of the upper and lower jaws along the dental arches. Further detailed studies will be needed to ascertain whether or not the fine structures of these taste buds located in the different areas are the same.

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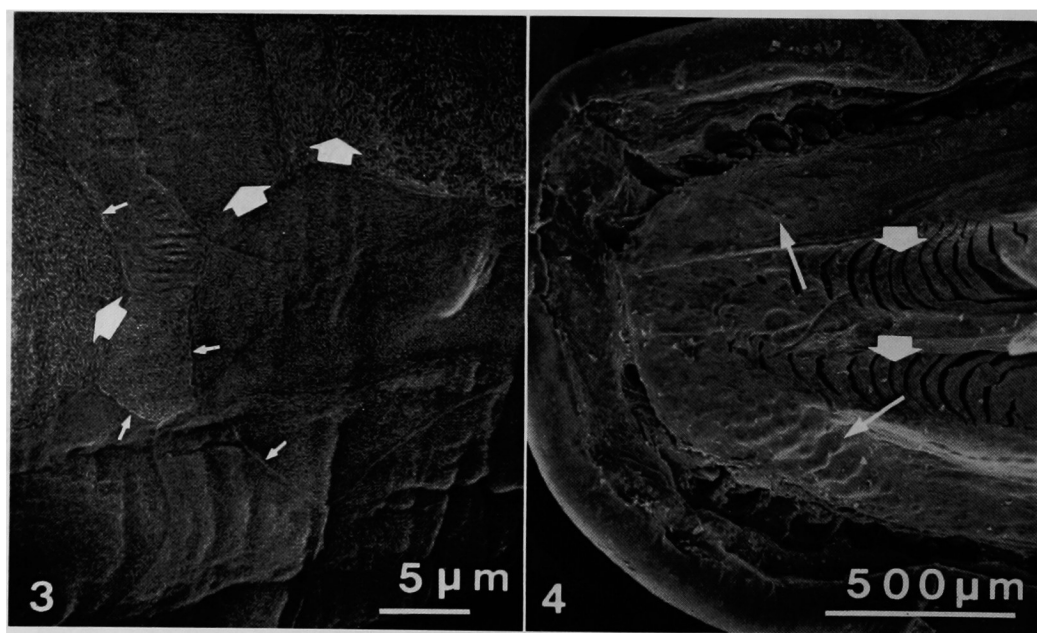
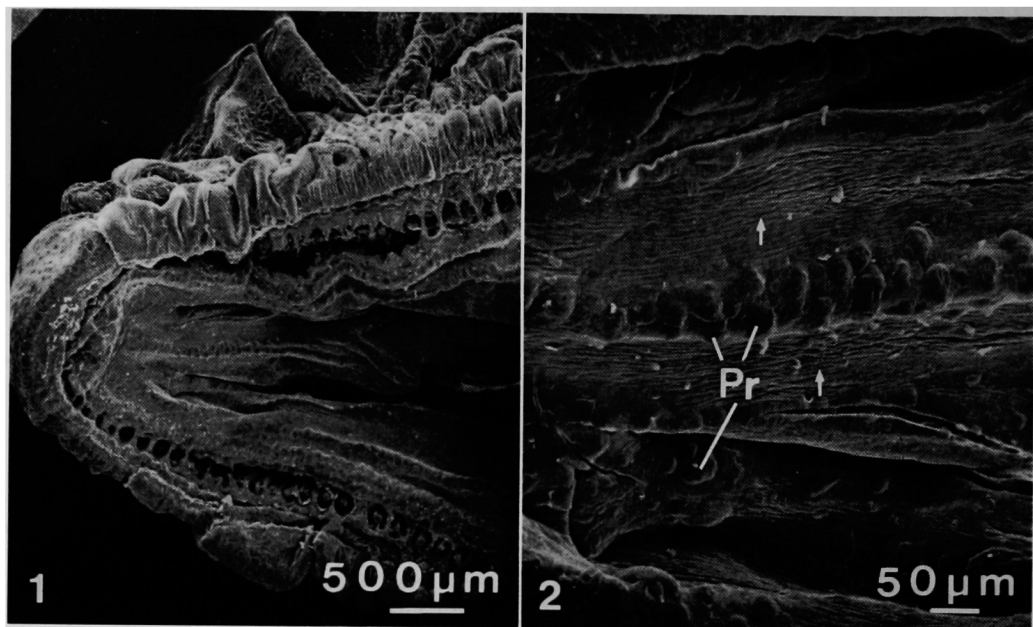
抄録：爬虫類有尾目のカナヘビについて、舌背以外の口腔内上皮、すなわち、口蓋、口腔底、舌下面、咽頭喉頭部および上下顎歯肉の上皮表面の微細構造を、酸消化によって粘性付着物を除去した試料において、走査電子顕微鏡によって観察した。

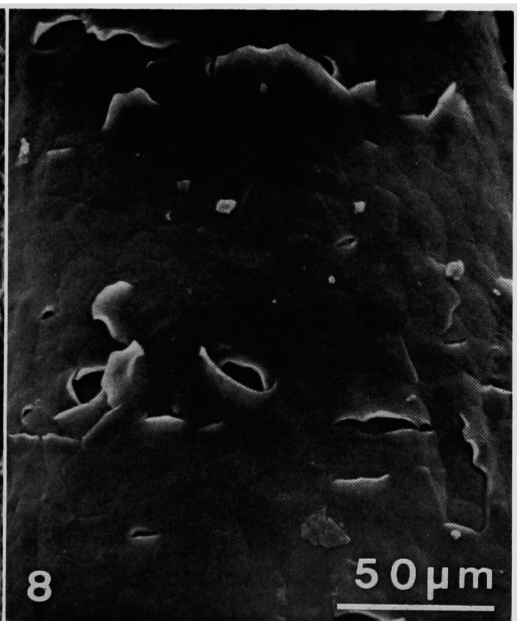
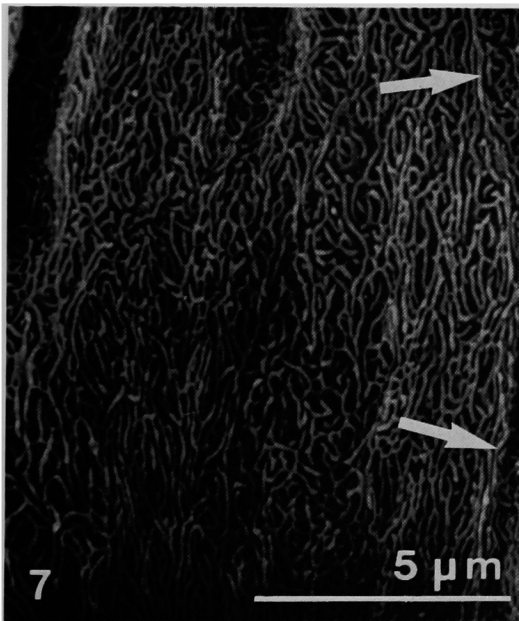
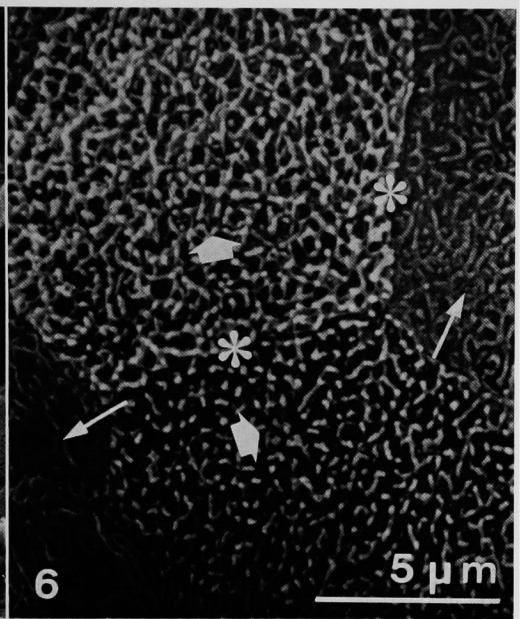
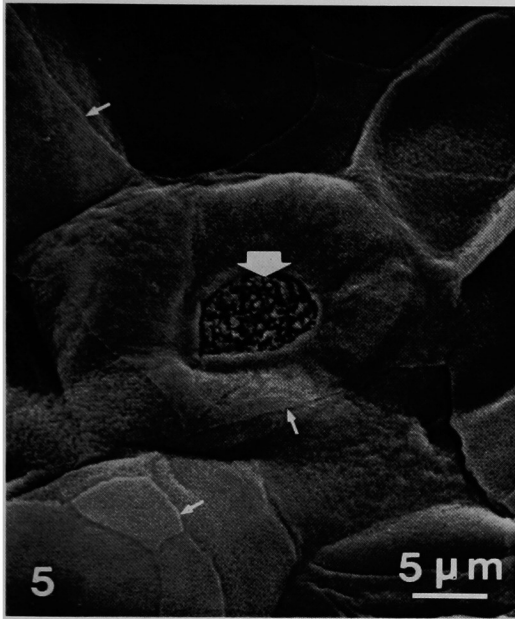
その結果、口蓋表面をやや拡大して観察すると、正中線からわずかに外側よりの左右ほぼ対称的な線に沿って、コブ状の隆起が前後に多数連なって配列し、それ以外の上皮表面は、前後に伸びる多数の細かいヒダによって覆われている。この上皮表面を強拡大で観察すると、いずれの部位でも、網目状の微小堤が密集し、各細胞辺縁の肥厚も比較的明瞭である。口腔底でも、前方外側にはコブ状の隆起が観察され、正中線の左右に、横に長い上皮の切れ込みが前後に連なって15条前後配列している。上皮表面を強拡大で観察すると、微絨毛様の構造および網目状あるいはウズ巻状の輪郭の明瞭な微小堤が観察される。細胞辺縁の肥厚も明瞭である。舌下面の表面は乳頭様構造はみられず、弱拡大ではほぼ平滑な表面を呈している。ここでは、各細胞の多角形の輪郭が比較的明瞭に認められる。さらに強拡大で観察すると、各細胞の表面には、細かい小孔が

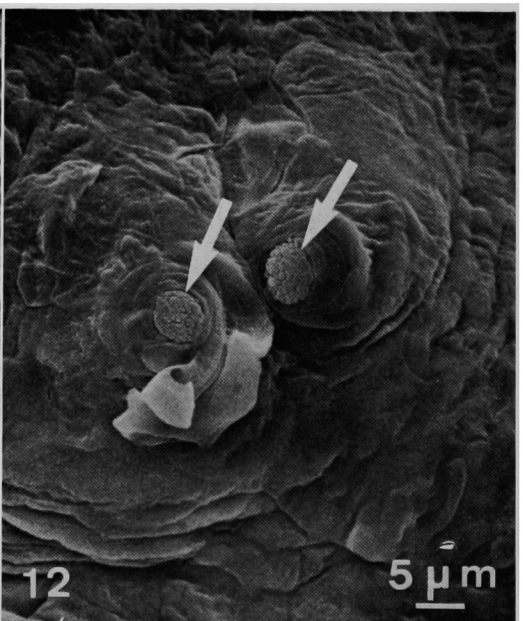
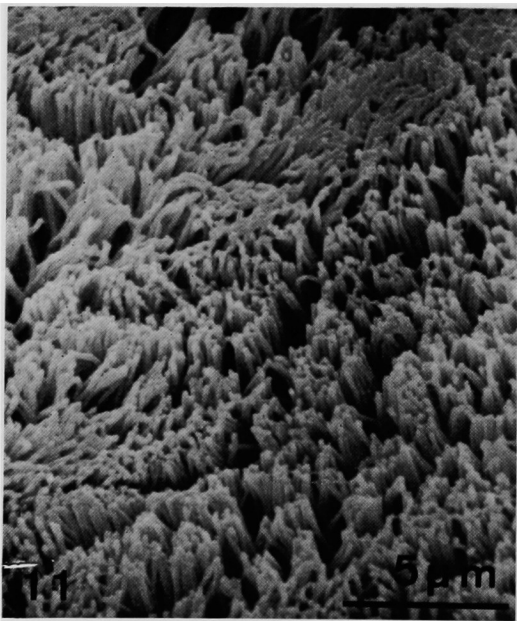
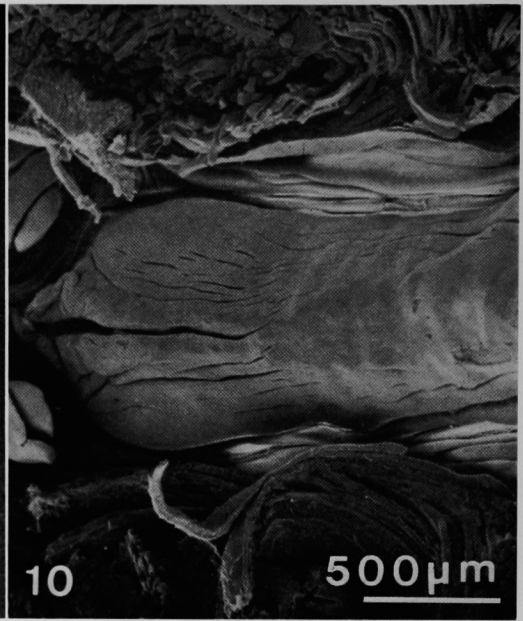
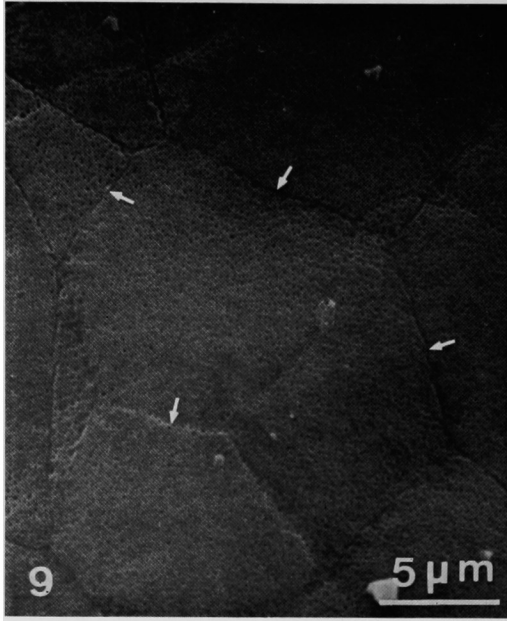
密に、ほぼ均一に分布しており、各細胞の境界部はくぼみによって、はっきりと境されている。咽喉頭部の上皮は、弱拡大では、あまり凹凸のない平らな面として観察されるが、強拡大では、ほぼ全面に繊毛が密生しているのが認められる。上顎内側歯肉には、乳頭様の膨らみが歯列に沿って散在しており、その頂上には、味蕾が分布している。歯肉上皮細胞表面は、輪郭のやや不明瞭な微小堤が広く分布している。下顎内側歯肉にもやはり歯列に沿って味蕾が分布しているが、その周囲には、ごくわずかの膨らみを有するものもあるが、明瞭な乳頭様構造はみられない。上皮細胞表面には、明瞭なウズ巻状あるいは網目状の微小堤が観察できる。

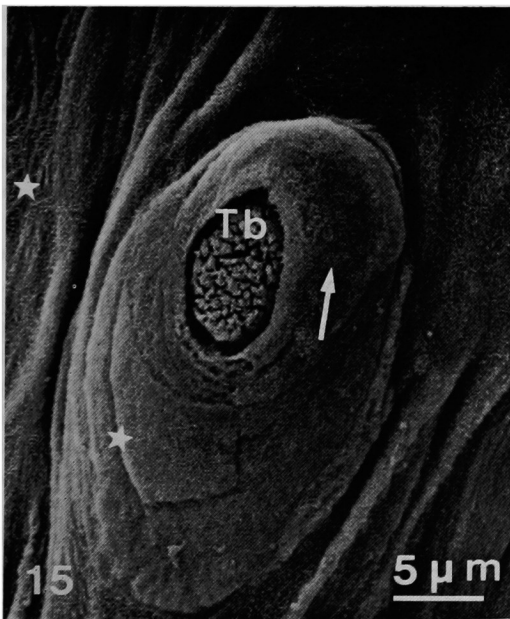
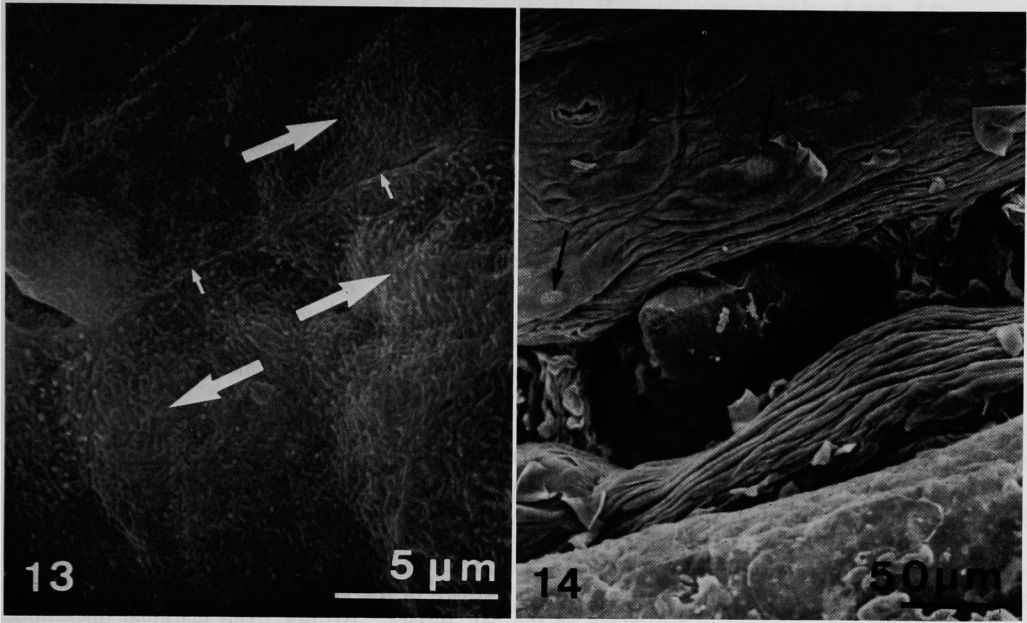
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Explanation of Figures

- Fig. 1 Scanning electron microscopy of the oral epithelial surface of the upper jaw containing the palate and gingiva. A major proportion of most of the teeth has been dissolved by the acid treatment. $\times 20$.
- Fig. 2 Scanning electron microscopy of the palate. Many bump-shaped protuberances (Pr) are arranged symmetrically on both sides of the median line. Folds (arrows) expanded antero-posteriorly are widely distributed on the epithelial surface. $\times 110$.
- Fig. 3 Higher magnification view of the epithelial cell surface of the palate. Network pattern of microridges (thick arrows) is closely distributed over the entire cell surface. Cell marginal thickening (thin arrows) can be clearly recognized. $\times 2300$.
- Fig. 4 The anterior part of the inner surface of the lower jaw. A major proportion of most of the teeth has also been dissolved by the acid treatment. Bump-shaped protuberances (thin arrows) are observed in the latero-anterior region of the floor. A number of nicks (thick arrows), elongated transversely, are arranged symmetrically on both sides along the median line of the fore-region. $\times 46$.
- Fig. 5 Higher magnification view of the epithelial surface between nicks. A taste bud (thick arrow) is located in the epithelium. Network pattern of microridges is widely distributed on the surface of the epithelial cells. Cell marginal thickening (thin arrows) is clear. $\times 2200$.
- Fig. 6 Higher magnification view of the epithelial cell surface of the anterior floor. Both microvilli (thick arrows) and microridges (thin arrows) are seen on the cell surface. Cell marginal thickening (asterisks) can be observed. $\times 6900$.
- Fig. 7 Higher magnification view of the epithelial surface of the lateral floor. A scrollwork pattern of microridges is widely distributed on the cell surface. Arrows: cell marginal thickening. $\times 6800$.
- Fig. 8 The ventral surface of the tongue. The polygonal outline of each cell is relatively distinct. $\times 430$.
- Fig. 9 Higher magnification view of the ventral epithelial cell surface of the tongue. Many pits are compactly distributed on the cell surface. The boundary lines (arrows) between adjacent cells appear as narrowly distinct hollows. $\times 3300$.
- Fig. 10 Lower magnification of the laryngial part of the pharynx. The left side of this photo is the direction of the lingual apex. $\times 36$.
- Fig. 11 Higher magnification view of the epithelial surface of the pharynx. It is covered with ciliated cells. $\times 4300$.
- Fig. 12 Scanning electron micrograph of the inner gingival surface of the upper jaw. Papillar protuberances (arrows), at the apex of each of which is a round taste bud, are arranged in the epithelium. $\times 1100$.
- Fig. 13 Higher magnification view of the gingival epithelial cell surface. Microridges (thick arrows) are relatively obscure. Cell marginal thickening (thin arrows) is recognizable. $\times 4300$.
- Fig. 14 Lower magnification view of the gingival surface of the lower jaw. A major proportion of most of the teeth has been dissolved by acid treatment. Taste buds (arrows) are also scattered on the inner gingival epithelial surface along the dental arches. $\times 270$.
- Fig. 15 Higher magnification view of a taste bud (Tb) on the inner gingival epithelial surface of the lower jaw. The taste bud in this micrograph is accompanied by a small surrounding protuberance. Scrollwork (thick arrows) and network (thin arrows) patterns of microridges can be clearly observed on the epithelial cell surface. Cell marginal thickening (asterisks) is clear. $\times 2300$.