The Surface Structures of the Laryngopharyngeal Epithelium of the Mouse with Special Reference to the Degree of Keratinization

By

Takashi NAKANO and Hiroshi MUTO

Department of Anatomy, Aichi Medical University, Nagakute, Aichi 480-11, Japan

—Received for Publication, July, 5, 1986—

Key Words: Keratinization, Epithelium, Taste bud, Laryngopharynx, Mouse

Summary: In the central zone of the ventral wall of the mouse laryngopharynx, a fairly large number of taste bud papillae were observed. The epithelial surface of the radix region of the papilla showed microridges of which outlines were not so distinct. As the surface was traced from the radix towards the top, microridges became more indistinct, and in the top region the surface showed honeycomb or relatively smooth appearance. In the interpapillary area of the central zone and in the lateral zone, the surface was covered with distinct microridges. It is suggested that the epithelium in the taste bud papilla represents more advanced keratinization than in the interpapillary area and the lateral zone, and that the keratinization is rather strong in the top region compared with the radix region in the papilla.

It has been pointed out in the oral epithelium that there exists the topographical difference in the surface appearance concerning the degree of keratinization (Osmanski and Meyer, 1967; Yukino, 1967; Farbman, 1970; Hayward et al., 1973; McMillan, 1979; Iwasaki et al., 1983; Iwasaki and Miyata, 1985). Klein and Schroeder (1979) described that the functional relationships between the taste buds and the surface appearance of the surrounding epithelium could not be ruled out. On the other hand, Fisker et al. (1982) reported that the surface appearance and the degree of keratinization were among the factors governing preferential colonization and initial intraepithelial penetration of oral microorganisms.

As described above, it is of interest to study the topographical difference in the surface appearance of the epithelium in the oral cavity as well as in the neighboring regions, with special reference to the degree of keratinization. This study reports the topographical difference in the surface appearance between the taste bud papilla and the surrounding epithelium in the mouse laryngopharynx, and discusses the cause of the difference in the degree of keratinization.

Materials and Methods

The materials were the pharyngeal regions removed from 25 SMA mice (10 male and 15 female) aged from 6 to 12 months. Light microscopic specimens were fixed in Zenker’s fluid containing 5% acetic acid, decalcified in 6% nitric acid, dehydrated in a graded ethanol series, and embedded in paraffin. They were serially cut into frontal
or sagittal sections at a thickness of 5–7 µm and were applied with hematoxylin and eosin stain.

Scanning electron microscopic samples were fixed with 2.5% glutaraldehyde in phosphate buffer, postfixed with 2% osmium tetroxide, immersed in 2% tannic acid, and stained with 2% osmium tetroxide. They were dehydrated in graded ethanol. After replacement with isoamyl acetate and drying at critical point with liquid CO₂, they were coated with gold and observed under a Jeol-U3 scanning electron microscope.

Results

Light microscopic findings

In the ventral wall of the laryngopharynx which appears to be keratinized throughout, two epithelial zones are clearly differentiated; the central zone and the lateral one (Fig. 1). The central zone shows a varying number of epithelial papillae, which are distributed from the caudal border of the interarytenoid notch to the rostral fourth of the plate of the cricoid cartilage. The papilla usually contains one taste bud (Fig. 1, 2), while in sections traversing the papillary center, up to three taste buds are encountered. Depending on the plane of sections, these taste buds are observed to rest on the basal membrane, and to possess a taste pore at the epithelial surface (Fig. 2). The epithelium in the taste bud papilla shows a well-defined cornified layer composed of several closely approximated and overlapping cornified cells with a smooth outer surface (Fig. 1–3). Keratohyalin granules are poorly defined in the taste bud papilla (Fig. 3).

In the interpapillary area, the cornified layer consists of loosely arranged and flaky cells, presenting a very uneven surface appearance (Fig. 1, 3). The granular layer in the interpapillary area contains obvious large keratohyalin granules not only in the cytoplasm but in the nucleoplasm (Fig. 3).

In the lateral zone the epithelium shows no papillae and is similar in appearance to that in the interpapillary area of the central zone (Fig. 1, 2). That is, the outer surface shows a loose arrangement along wavy line, and the granular layer contains obvious large keratohyalin granules.

The submucosa in the central zone contains well-developed fatty tissue, while in the lateral zone it is poorly developed (Fig. 1).

Scanning electron microscopic findings

In the ventral wall of the laryngopharynx, 13–22 taste bud papillae are observed. Although no definite pattern of arrangements is noticed among the papillae, they are localized in the central zone and are absent in the lateral zone (Fig. 4). The taste bud papilla, about 80–130 µm in diameter, is dome-like in form and is sharply delineated by a circumferential depression, slightly protruding from the general level of the surface in the interpapillary epithelium. It contains one, sometimes two and many more taste pores near the top (Fig. 5).

At low magnifications, the epithelial surface of the taste bud papilla appears to be relatively flat throughout the papilla (Fig. 4, 5). However, close examinations reveal that the surface of the papilla is somewhat different according to the region of location (Fig. 6). In the radix region of the papilla, the surface is covered with the microridges, which are approximately 0.3–0.4 µm wide with space of 0.1–0.4 µm between them. The microridges show some tendency to form anastomoses and, therefore, the outlines are not so distinct (Fig. 6, 7). As the surface is traced from the radix towards the top, microridges become more indistinct. In the top region of the papilla, the surface shows honeycomb appearance of interconnections of microridges surrounding
depressions, or relatively smooth appearance (Fig. 6, 8). The lateral boundaries of the surface cells are sharply defined in the top region (Fig. 6).

The surface in the interpapillary area of the central zone is divided into small patches by deep furrows which run transversely across the long axis of the laryngopharynx and, therefore, it appears to be very uneven and corrugated (Fig. 4, 5). At higher magnifications, the surface is covered with microridges, consist of fine surface ledge of about 0.3-0.4 μm in width, as the same size as those in the papilla (Fig. 9). The length of the microridges varies considerably. Although occasional individual cells have a parallel arrangement of the microridges, there is no specific pattern of arrangements but rather they run in all directions. Typical honeycomb appearance is not observed in the interpapillary area. The cell boundaries are less pronounced (Fig. 9).

The epithelial surface in the lateral zone is similar in appearance to that in the interpapillary area of the central zone. It is divided into small patches by deep furrows (Fig. 4). Close examination reveals that the surface is covered by various pattern of microridges of 0.3-0.4 μm in width. Linear ridges corresponding to lateral cell boundaries are not so distinct (Fig. 10).

Discussion

It is difficult to conclude whether an epithelium is keratinized or not. At least, there is no doubt about the presence of keratinization in normal epidermis. Brody (1959, 1960) observed the “keratin pattern” with less opaque filaments embedded in an opaque interfilamentous substance in the cornified layer of human and guinea pig epidermis. In a mucosal epithelium the issue is more confused. In some areas of the oral mucosa, the keratin pattern was observed (Yukino, 1967; Farbman, 1970; Hayward et al., 1973; McMillan, 1979). However, in other areas of the oral mucosa, the keratin pattern was not observed and the cells in the cornified layer had their opaque filaments embedded in a less opaque interfilamentous substance (Kurahashi and Takuma, 1962; Listgarten, 1964; Albright and Listgarten, 1962; McMillan, 1979). This appearance, known as “abnormal keratin pattern”, was observed in the esophagus of various mammals including man (Oehmke, 1964; Parakkal, 1967; Ito and Ishii, 1970). According to McMillan (1979), examination in light microscope of sections stained with Mallory’s triple connective-tissue stain revealed that rat hard palate which exhibited the keratin pattern in transmission electron microscope showed complete keratinization, while rat cheek which exhibited the abnormal keratin pattern showed incomplete keratinization. That is, the mucosal keratinization is included within a wide continuous spectrum between complete and incomplete keratinizations.

It is of interest to discuss the topographical difference in the surface appearance of the mucosal epithelium concerning the degree of keratinization. In this study, microridges were found to be widely distributed on the epithelial surfaces in the radix region of the taste bud papilla, the interpapillary area and the lateral zone. However, as the epithelial surface of the papilla was traced from the radix towards the top, microridges became indistinct, and in the top region the surface showed honeycomb or relatively smooth appearance instead of microridges. Microridges are remains of usual intercellular cytoplasmic processes which were once necessary to maintain intercellular organization (Cleaton-Jones and Fleish, 1973; Cleaton-Jones, 1975; Klein et al., 1979; Nakano, 1986).

There is a tendency to believe that honey-
Honeycomb appearance is a characteristic on the exposed surface of keratinized epithelium, while nonkeratinized epithelium has the surface covered with microridges (Cleaton-Jones and Fleisch, 1973; Cleaton-Jones, 1975; Takagi, 1977; Klein and Schroeder, 1979; Nair and Rossinsky, 1984). However, this is not always the case because the anterior surface of the lingual filiform papilla of rat and musk shrew, which is keratinized, has partly microridges instead of honeycomb appearance (Yukino, 1967; Iwasaki et al., 1983). Nakano (1986), one of the present authors, reported at first that the width of intercellular processes and microridges in the cornified layer of the keratinized epithelium were about doubled compared to that in the underlying layers and in the nonkeratinized epithelium, and suggested that the intercellular processes in the keratinized epithelium increased in size, as a result of cell membrane distortion associated with keratinization, to form the tightly applied intercellular interdigitations as the cells moved towards the cornified layer. That is, the difference in width of microridges between keratinized and nonkeratinized epithelia was regarded as an important marker of the start of keratinization. The width of the microridges observed in this study was about the same size as that in keratinized epithelia in other anatomical sites (Nakano, 1986). This finding suggests that the epithelium in the radix region of the taste bud papilla, the interpapillary area and the lateral zone represents earlier stage in the differentiation of the epithelium towards keratinization.

Honeycomb appearance is formed, or at least contributed to, by interconnections of microridges and then it is transformed into relatively smooth appearance with the advance of keratinization (Matsumoto et al., 1976; Takagi, 1977; Iwasaki et al., 1983; Iwasaki and Miyata, 1985). These findings suggest that the epithelium in the taste bud papilla represents more advanced keratinization than that in the interpapillary area and the lateral zone. Furthermore, it appears that the keratinization is rather strong in the top region compared with the radix region in the taste bud papilla.

In the taste bud papilla which represented more advanced keratinization the keratohyalin granules were poorly defined while in the less keratinized interpapillary area and the lateral zone the epithelium contained obvious large keratohyalin granules. These findings support to an earlier description that in areas of the epithelium which showed complete keratinization the keratohyalin granules were poorly defined, while the incompletely keratinized epithelium contained obvious large keratohyalin granules (McMillan, 1979).

In the top region of the taste bud papilla the lateral boundaries of the surface cells were sharply defined, while in the interpapillary area and the lateral zone they were less pronounced. Furthermore, at the light microscopic level, the epithelium in the taste bud papilla showed a well-defined cornified layer composed of closely approximated cells, while in the interpapillary area and the lateral zone the cornified layer consisted of loosely arranged and flaky cells. These findings suggest that the cells in the taste bud papilla, which represent more advanced keratinization, have greater resistance to the mechanical stress than those in the interpapillary area and the lateral zone.

The light and the low power scanning electron micrographs revealed that the epithelial surface in the interpapillary area and the lateral zone was very uneven and corrugated. It is assumed that the appearance is a mechanism allowing for stretching of the epithelium during the swallowing. On the other hand, the epithelial surface in the taste bud papilla was relatively flat and appeared
to be less mobile. These findings are in agreement with the earlier description that microridges are associated with more flexible epithelial surfaces, while honeycomb appearance is associated with those subjected to less stretching (McMillan, 1979; Philipsen et al., 1982).

The cause of the difference in the degree of keratinization has been the matter of discussion. Many investigators e.g. Goetsch (1910) and Parakkal (1967) described in the esophagus that the degree of keratinization was correlated with mechanical stress by diet; i.e. in animals which eat predominantly hard diet the epithelium undergoes more advanced keratinization than that in animals living on soft diet. Furthermore, Hicks (1968) reported that the keratinization in the urinary bladder epithelium was observed clinically to be associated with irritation by calculi. As a result of this study, it is reasonably assumed that the taste but papilla, which protrudes from the general level of the surrounding epithelial surface, is subjected to more considerable mechanical stress by diet and is, therefore, more highly keratinized than the inter-papillary area and the lateral zone. In other words, the difference in the degree of keratinization between the "less stretching" taste bud papilla and the "more flexible" inter-papillary area is interpreted as the difference in the respective adaptations to mechanical stress. However, there is no gainsaying the possibility that the topographical difference in the degree of keratinization is involved in genetic factors.

It is of interest to notice the presence of the submucosal fatty tissue in the central zone, and the absence of the tissue in the lateral zone. The submucosal fatty tissue seems to raise the taste pores above the surrounding epithelial surface to improve contact with food.

Acknowledgements

The authors should like to express thanks to Prof. Dr. I. Yoshioka for revising and advising, and also to Mrs. M. Iida and Mr. T. Miyake for their technical assistance in operating the scanning electron microscope.

References

11) Ito, T. and Ishii, H.: Esophageal epithelium. II. Electron microscopic observations. 5. Stratum corneum; Morphology of tracheal and esophageal mucosae by use of light and


Surface structures in the laryngopharyngeal epithelium
Explanation of Figures

Plate I

Fig 1. Frontal section of the laryngopharynx. The central zone shows taste bud papillae (arrows), and contains submucosal fatty tissue (F). The outer surface in the lateral zone (arrowheads) is very uneven. X 50.

Fig. 2. Higher magnification of figure 1. The epithelium in the taste bud papilla (arrows) shows a cornified layer composed of closely approximated cells with a smooth outer surface. In the lateral zone (arrowheads) the cornified layer consists of loosely arranged cells, presenting a very uneven surface appearance. F = submucosal fatty tissue. X 160.

Fig. 3. Sagittal section of the central zone. In the taste bud papilla (arrows), keratohyalin granules are poorly defined. The interpapillary area (arrowheads) presents very uneven surface appearance, and contains obvious large keratohyalin granules in the cytoplasm and in the nucleoplasm. F = submucosal fatty tissue. X 160.
Plate II

Fig. 4. Scanning electron micrograph showing the ventral wall of the laryngopharynx. Many taste bud papillae (arrows) are localized in the central zone (C), and are absent in the lateral zone (L). The epithelial surface in the taste bud papillae (arrows) appears to be relatively flat, while that in the interpapillary area (arrowheads) and in the lateral zone (L) is divided into small patches by deep furrows and appears to be very uneven and corrugated. X 100.

Fig. 5. Higher magnification of figure 4. arrows = taste pores. X 300.
Plate III

Fig. 6. Close examination of a taste bud papilla. In the radix region, the surface is covered with microridges of which the outlines are not so distinct (A). In the top region, the surface shows honeycomb appearance (B) or relatively smooth appearance (C). The lateral cell boundaries are sharply defined (arrowheads). arrow = taste pore. X 3,000.

Fig. 7. Radix region of the taste bud papilla. Microridges show some tendency to form anastomoses (arrows). X 3,000.

Fig. 8. Honeycomb appearance in the top region of the taste bud papilla. X 10,000.
Plate IV

Fig. 9. Close examination of the interpapillary area. Microridges consist of fine surface ledge. The cell boundaries (arrowheads) are less pronounced. X 3,000.

Fig. 10. Close examination of the lateral zone. The epithelial surface is similar in appearance to that in the interpapillary area. Arrowheads = cell boundaries. X 3,000.