Scanning Electron Microscopy

Volume 1986 | Number 4 | Article 16

9-29-1986

Basal Lamina at the Epithelial-Connective Tissue Junction in the Rat Forestomach, Esophagus, Tongue and Palate: Scanning Electron Microscopic Study

Merideth T. Hull
Indiana University School of Medicine

K. A. Warfel
Indiana University School of Medicine

SCANNING ELECTRON MICROSCOPY/1986/IV (Pages 1395-1401)
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 USA

0586-5581/86$1.00+05

BASAL LAMINA AT THE EPITHELIAL-CONNECTIVE TISSUE JUNCTION IN THE RAT FORESTOMACH, ESOPHAGUS, TONGUE AND PALATE: Scanning Electron Microscopic Study

Meredith T. Hull* and K. A. Warfel

Department of Pathology, Indiana University School of Medicine
Indianapolis, Indiana

(Received for publication January 16, 1986, and in revised form September 29, 1986)

Abstract

The squamous epithelium lining the rat foregut was removed by incubating fresh, unfixed specimens in 2N sodium bromide. The surface morphology of the exposed subepithelial basal lamina was examined by scanning electron microscopy. Areas examined included hard and soft palates, oropharynx, tongue, esophagus, and forestomach. The basal lamina was continuous over all surfaces. Basal lamina corrugations were not present at all sites. The saucer-like defects of lymphocyte migration that are present in the basal lamina beneath the squamous epithelium of the skin were not observed in rat foregut. The epithelial-connective tissue interface of the rat esophagus does not have the coiled and branched papillae seen in esophagi of adult humans. The three dimensional shapes of the connective tissue cores of the various lingual papillae are well-demonstrated by this technique and are distinct. The basal lamina of the hard and soft palates are also distinct.

Key Words: Basement membrane, basal lamina, squamous epithelium, epithelial-connective tissue junction, esophagus, tongue, palate, forestomach, scanning electron microscopy

*Address for correspondence:
Meredith T. Hull, Department of Pathology, Indiana University School of Medicine, 926 W. Michigan St., Indpls. In 46223
Phone:(317)274-2498

Introduction

The basal lamina underlying squamous epithelium has many functions, including facilitating epithelial-connective tissue adhesion, providing scaffolding for epithelial repair, modulating epithelial proliferation and differentiation, and regulating movement of some macromolecules (2,4,17). Also, in some settings characterized by abnormal cell proliferation, such as carcinoma-in-situ, the basal lamina provides a physical barrier to stromal invasion by malignant cells, at least until the neoplasm apparently develops the ability to exploit the thixotropic nature of the basement membrane or produces type IV collagenase (12,14).

The rat foregut, which is covered by stratified squamous epithelium, has a high degree of regional surface specialization. Understanding the topography of this interface in the normal rat esophagus and oral cavity would be useful to studies of epithelial-connective tissue interactions in experimental carcinogenesis, since, for example, the epithelial-connective tissue interface is altered in dysplasia and carcinoma adjacent to areas of invasion (8). Because of this potential use and because the basal lamina and superficial connective tissue underlying squamous epithelia are so intimately involved in many aspects of foregut morphology and function, this study was undertaken to characterize the topography of the basal lamina at the epithelial-connective tissue junction.

Materials and Methods

Esophagi and forestomachs were excised from 6 adult male Sprague-Dawley rats. Tongue and hard and soft palate including attached oropharynx were dissected from 18 adult rats. This population included 13 Sprague-Dawley and 5 Wistar rats. These included 8 females and 10 males. Following excision, the tissues were immersed in 2N sodium bromide at 60^{o}C. for 1 hour as described elsewhere (19). With the aid of a dissecting microscope, stratified squamous epithelium was gently elevated and removed from the underlying connective tissue and basal lamina. The latter tissue was fixed immediately in 3% glutaraldehyde in 0.1 M phosphate buffer, post fixed in osmium tetroxide, dehydrated in graded ethanol solutions, critical-point dried and sputter coated with gold-palladium.

Representative 1.0 mm cubes of tissue from the epithelial-mesenchymal junction were removed from specimens prior to fixation for SEM and processed for transmission electron microscopy. These tissue cubes were fixed in 3% glutaraldehyde in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in Epon. Thin sections cut from Epon blocks were stained with uranyl acetate and lead citrate and examined on a Philips 300 transmission electron microscope. Scanning electron microscopic specimens were examined on a Philips 500 scanning electron microscope.

Results

No differences were found in the surface morphology based on sex or rat strain. As in previous studies, sodium bromide processing removed the squamous cell epithelium (Fig. 1). Transmission electron micrographs confirmed that the epithelial-connective tissue separation was between the membranes of the basal squamous epithelial cells and the basal lamina (7)(Fig. 2). Also, TEM showed that the basal lamina and sub-basal fibrous connective tissue were well-preserved and attached normally to each other. As such, the scanning electron micrographs presented here demonstrate the external surface of the basal lamina.

The basal lamina was continuous over all surfaces of the tissues studied. The fore-stomach was covered by prominent, transversely and obliquely oriented ridges with blade-like shapes (Fig. 3). The basal lamina was smooth to undulating and contained few corrugations. The esophagus was covered by folds which were longitudinally oriented and extended the entire length of the esophagus (Fig. 4). A system of smaller ridges was oriented parallel to and obliquely to the major folds. Corrugations which were 0.4-0.6 micrometers in diameter were present as a diffuse reticular network (Fig. 5). There were no basal lamina fenestrations or coiled and branching papillae.

In the tongue, findings reflected the fact that different types of papillae are located in specific regions. The anterior half of the dorsal surface was covered by peak-shaped connective tissue papillae that were the connective tissue cores of the simple conical filiform papillae (Fig. 6). The basal lamina on these was arranged in wavy, vertically oriented corrugations. Lateral bridging corrugations were uncommon. The corrugations at the papillary bases blended into the irregularly oriented ones covering the interpapillary spaces on the anterior tongue. Near the tip of the tongue the papillae were taller, more narrow, and less corrugated (Fig. 7). Most showed an oblong depression on the apical posterior surface.

Fungiform papillae were also present on the anterior half of the dorsal surface. Their connective tissue papillary cores were two to three times as high as the connective tissue cores of the simple conical filiform papillae (Fig. 8). The apical surface of each fungiform connective tissue core was characterized by a single deep crater (Fig. 9). The external

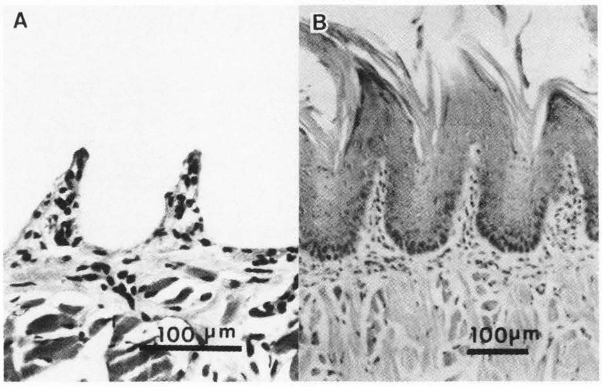

Figure 1. Light micrographs of rat tongue before (B) and after (A) removal of epithelium by sodium bromide processing.

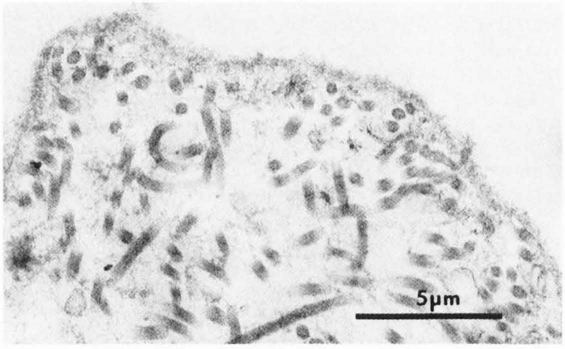

Figure 2. TEM of exposed basal lamina. The sub-basal connective tissue is well-preserved.

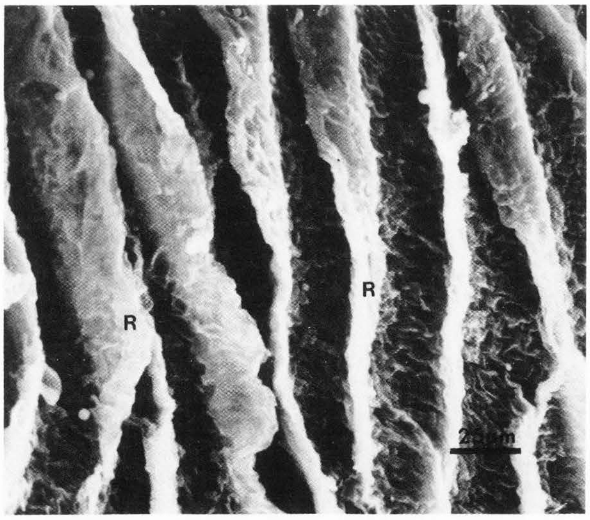

Figure 3. SEM of basal lamina of forestomach showing transversely and obliquely oriented ridges (R). Long axis of the forestomach is left to right in figure. Basal lamina shows undulations.

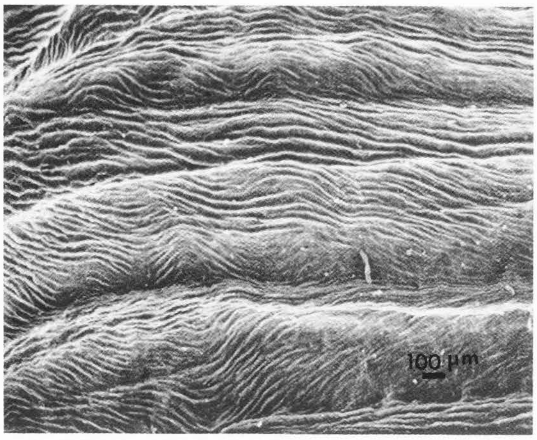

Figure 4. SEM of basal lamina of distal esophagus. Longitudinal axis is left to right in figure. Longitudinally oriented large folds and smaller ridges are evident.

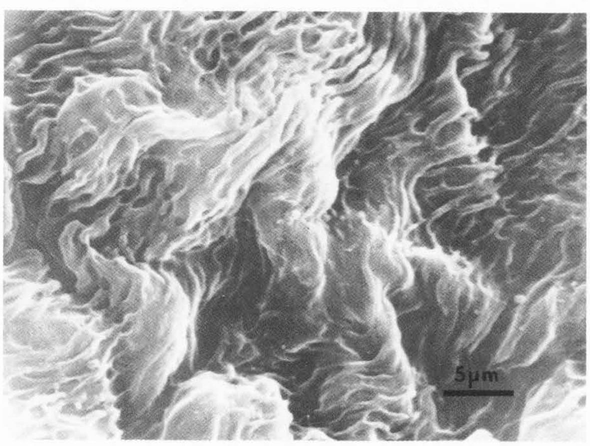

Figure 5. SEM of smaller ridges showing basal lamina corrugations.

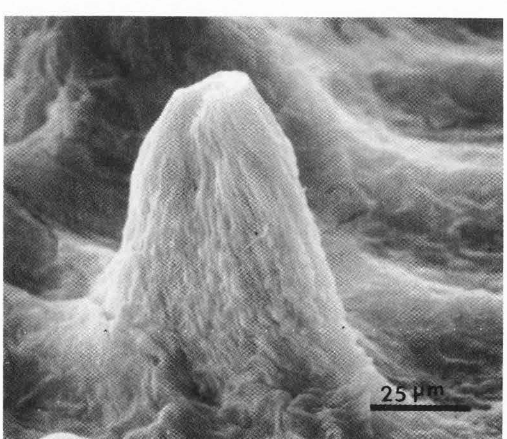

Figure 6. SEM of simple conical filiform papilla with vertical basal lamina corrugations.

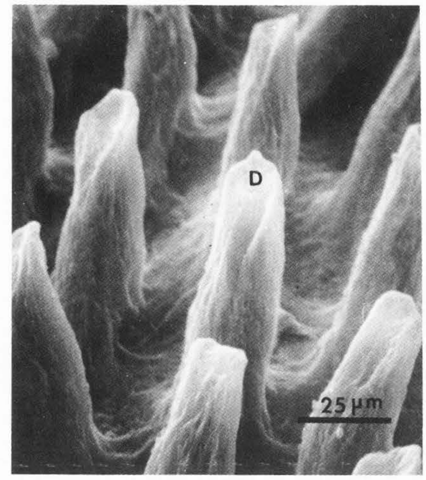

Figure 7. SEM of basal lamina of simple conical filiform papilla near the tip of the tongue. Note the apical posterior depression (D).

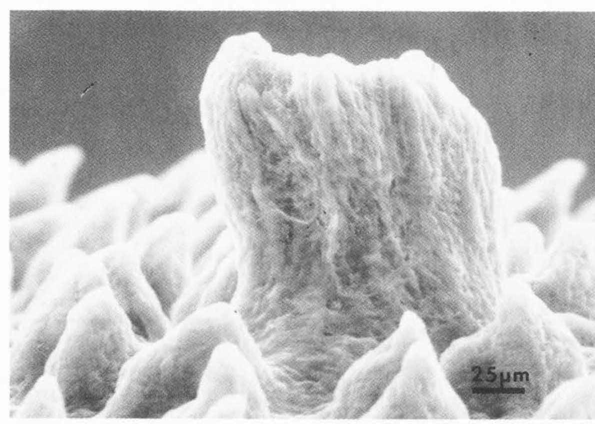

Figure 8. SEM of fungiform papilla showing lateral basal lamina and size relationship to adjacent simple conical filiform papillae.

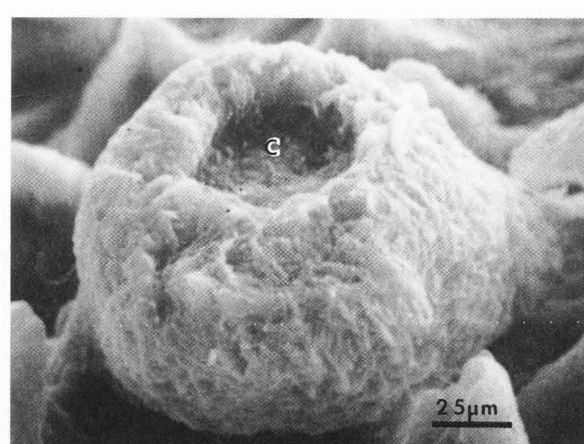

Figure 9. SEM of apical crater (C) of fungiform papilla.

lateral walls of the fungiform cores had vertically oriented corrugations that were not quite as distinct as those on simple conical filiform cores. The basal lamina on the walls of the apical craters were undulating to flat without organized corrugations.

Giant conical filiform papillae occupied an area posterior to the fungiform and simple conical papillae. The connective tissue papillae were taller and had narrower apices than the connective tissue papillae of the simple conical forms. Many had notched apical tips. Deep grooves were present on both anterior and posterior surfaces. Vertical coarse trabeculations on the basal lamina were present on these cores (Fig. 10). In the region posterior to the giant conical papillae the papillary cores were those of true filiform papillae. The connective tissue cores of true filiform papillae arose as a single base with anteroposterior flattening, but the apical one-half to two-thirds were composed of three to five secondary finger-like divisions (Fig. 11). Their basal lamina corrugations were poorly developed and most of the surface was smooth.

The connective tissue core of the solitary circumvallate papillae on each tongue showed a broad apex and vertical walls covered by slightly undulating basal lamina with no organized corrugations. Connective tissue cores of foliate papillae were present at the posterolateral aspects of the tongue and were characterized by long rows arranged in an anteroposterior direction (Fig. 12). Secondary papillae occupied the apical one-half of these connective tissue rows. The basal lamina on foliate papillae was smooth to undulating and without corrugations.

The lateroventral surface of the tongue was undulating and free of papillae; organized corrugations were absent (Fig. 13). Parallel ridges were present on the ventral surface adjacent to the reflection of the basal lamina onto the floor of the mouth and anterior pharyngeal columns. Their long axes were oriented in an anteroposterior direction. Orifices for von Ebner glands that were present in the posterolateral aspects of the tongue were oval and surrounded by prominent basal lamina ridges (Fig. 12). The basal lamina of the duct orifices was continuous with the basal lamina covering the remainder of the tongue.

In the palate, the basal lamina covering the hard palate was different from that on the soft palate. The hard palate contained rows of short, blade-like nodules arranged in modified chevrons with the apices directed anteriorly (Fig. 14). The last row usually was straight rather than chevron-shaped. Frequently the last row was a continuous ridge rather than discrete nodules. The nodules were continuous with small ridges that extended posteriorly (Fig. 15). Basal lamina on the nodules had a pattern of undulations and poorly organized corrugations. The parallel ridges of the hard palate changed abruptly to an irregular pattern of interconnecting ridges on the soft palate (Fig. 16). Orifices for soft palate glands were present in the posterior aspect of the soft palate, and the basal lamina of the glands was continuous with that of the soft palate. The basal lamina

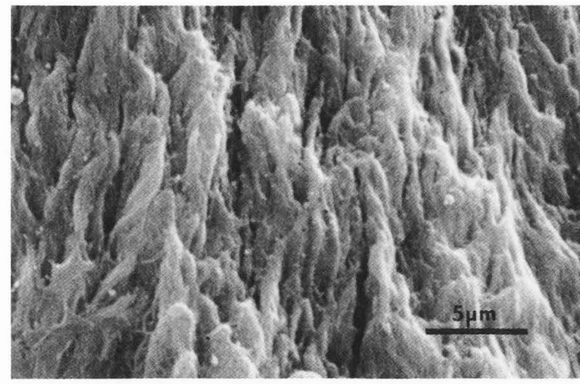

Figure 10. SEM of basal lamina wall of giant conical papilla. Note the coarse, vertically oriented trabeculations.

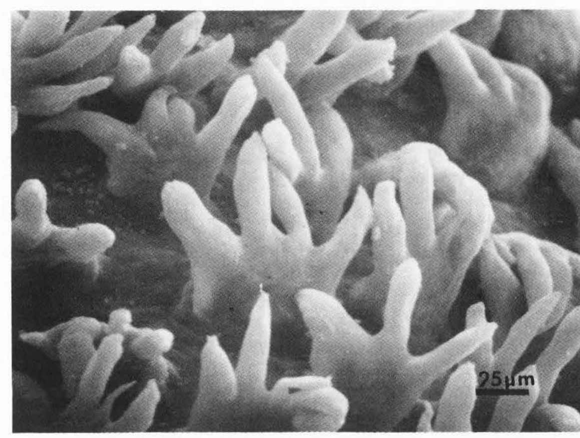

Figure 11. SEM of basal lamina of true filiform papillae. The top of the figure is directed toward the anterior aspect of the tongue.

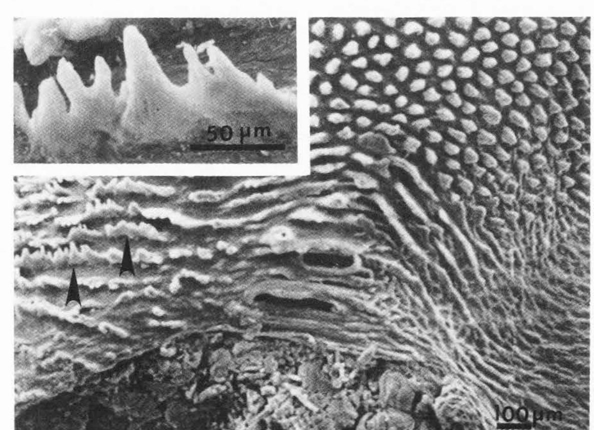

Figure 12. SEM of basal lamina of the posterolateral aspect of the tongue. Figure right is anterior. Note foliate papillae (arrows). Insert: Detail of foliate papillae.

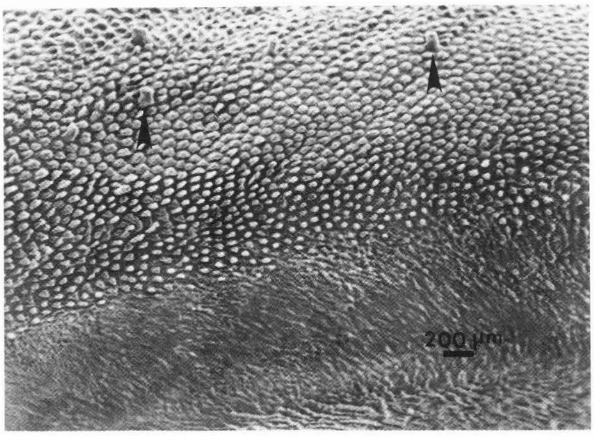

Figure 13. SEM of basal lamina of the antero-lateral tongue. Figure right is anterior. Note scattered fungiform papillae (arrows).

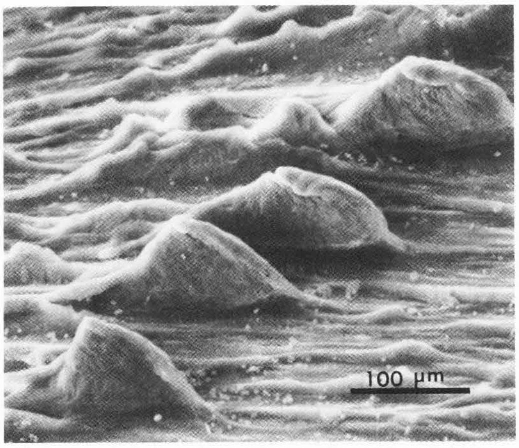

Figure 15. SEM of blade-like nodules (arrows in Figure 12). Note they are continuous with longitudinal ridges.

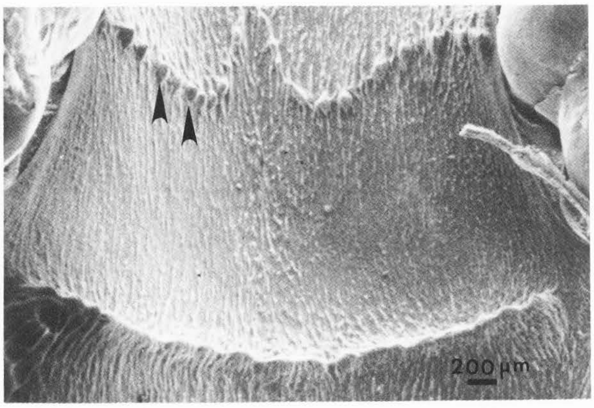

Figure 14. SEM of basal lamina of the hard palate. Figure top is anterior. Note the row of blade-like nodules (arrows).

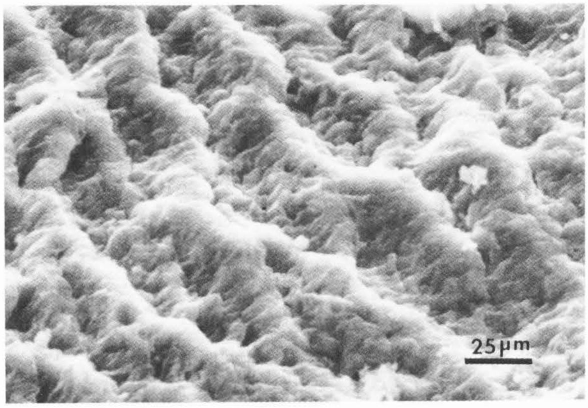

Figure 16. SEM of basal lamina of soft palate. It is characterized by a complex pattern of irregularly oriented interconnecting ridges.

of the oropharynx showed parallel, obliquely oriented ridges which converged medially and posteriorly. A well-developed pattern of corrugations was not observed.

Discussion

This study utilized a sodium bromide technique for separating the epithelial-connective tissue interface of stratified squamous epithelium (5,15). It is one we have used to study both skin and esophagus and one that provides an intact basal lamina on the connective tissue underlying the stratified squamous epithelium. We are not aware of studies showing the mechanism of action of the sodium bromide.

The rat esophageal epithelial-connective tissue interface is morphologically very different from that of the human. Rat basal lamina had major and minor longitudinal ridges and interconnecting corrugations with no stromal papillae. In contrast, scanning electron microscopy of human esophageal basal lamina showed a complex array of papillae and blade-like stromal folds (18). The papillae were simple or branched and had helical contours caused by subepithelial spiraling capillaries. Few corrugations were seen in human esophageal basal lamina, while they were relatively well-developed in rat esophagus. Differences may be related to the position of the esophagus during deglutition and the nature of the swallowed food. The vertical position of human esophagus and the larger size of swallowed substances may act to pull against the esophageal stroma and facilitate the formation of papillae.

The surface of the tongue has striking structural specialization, not all of which is not functionally explicable (1,3,6,9,11,13). In the rat's tongue, the anterior half of the dorsal surface is covered mostly by simple conical papillae while the posterior half of the dorsal surface is covered by filiform papillae. Between these two regions is an oval patch of giant conical papillae. Fungiform papillae are distributed in the paracentral areas of the dorsal anterior half of the tongue and are admixed with the more numerous simple conical papillae. There is a single posterior dorsal midline circumvallate papillae. Variable numbers of foliate papillae are present along the posterolateral aspect of the tongue. The lateroventral surface is smooth. Taste buds are present individually on the apices of fungiform papillae and in groups along the sides of circumvallate papillae.

Previous scanning electron microscopic studies of the rat oral cavity have been performed on mucosal surfaces still covered by intact squamous epithelium (16,20). Patterns of connective tissue cores of papillae in the rat have been studied by light microscopy of serially sectioned tongues (11). Connective tissue cores of simple conical papillae have been described as truncated cones. Cores of giant conical papillae have been estimated to be roughly truncated cones with slight compression in the anteroposterior direction. "En face" serial sections of true filiform papillae have shown three to five cores and accurately predicted the finger-like appearance of the secondary papillae seen on scanning electron micrographs. Studies on serially sectioned tissues are quite tedious to perform and do not always result in complete visualization of three dimensional surface. Such studies of rat tongue have demonstrated the gross shapes of papillae. The technique used in the study reported here has revealed other levels of basal lamina organization such as coarse trabeculations on giant conical papillae and intricate patterns of basal lamina corrugations on the anterior papillae.

Well-developed patterns of basal lamina corrugations were restricted to fungiform and simple conical papillae. True filiform papillae had corrugations that were poorly developed. Basal lamina on circumvallate and foliate papillae and on the ventral surfaces of the tongue was relatively smooth with no distinct corrugation pattern. The oropharynx, soft palate, and hard palate also show distinctive epithelial-connective tissue interfaces. An intermediately developed design of corrugations was present in these sites. These patterns of corrugations were somewhat different from those seen in previous studies of skin (7,10,19). Under the squamous epithelium of human skin the basal lamina is arranged in vertical corrugations on dermal papillae. The corrugations in children and non-senescent adults are taller and have more laterally oriented bridging. In old age, the corrugation pattern is lost, resulting in basal lamina with an undulating to relatively smooth surface.

The relationships between the surface patterns of basal lamina and the different functions attributed to it in conjunction with the underlying connective tissue are not known. Hypothetically, corrugations afford an increase in surface area for epithelial-mesenchymal interaction. Their loss in aged skin corresponds well with the clinically observable decreased epidermal-dermal adhesion of senescent skin (7). In the tongue, corrugations were most prominent on the anterodorsal surface, an area exposed to coarse food and presumably greatest shearing pressures. They were also present in the esophagus. The increased surface area afforded by the corrugations in these regions may reflect a greater local requirement for strength of adhesion.

Another interesting finding in similar studies of the basal lamina of human skin has been the presence of numerous sites of basal lamina deformation associated with lymphocyte passage (19). These saucer-like defects from lymphocyte migration were not identified in any portion of the rat foregut.

This technique for visualizing extensive surfaces of basal lamina underlying squamous epithelium of the tongue could provide a useful model for evaluating structural changes in the epithelial-connective tissue junction associated with differentiation, epithelial repair and neoplastic transformation with subsequent stromal invasion.

References

1. Boshell JL, Wilborn WH, Singh BB. (1980). A correlative light microscopic, transmission and scanning electron microscopic study of the dorsum of human tongue. Scanning Electron Microsc. 1980; II:505-510.
2. Briggaman RA. (1982). Biochemical composition of the epidermal-dermal junction and other basement membrane. J. Invest. Dermatol., 78, 1-8.
3. Davies RO, Kare MR, Cagan Rh. (1979). Distribution of taste buds on fungiform and circumvallate papillae of bovine tongue. Anat. Rec., 195, 443-446.
4. Ekblom P, Alitalo K, Vehevi A, Timple R, Saxen L. (1980). Induction of a basement membrane glycoprotein in embryonic kidney: Possible role of laminin in morphogenesis. Proc. Natl. Acad. Sci. USA., 77, 485-489.
5. Felsher Z. (1947). Studies on the adherence of the epidermis to the corium. J. Invest. Dermatol., 8, 35-47.
6. Fish HS, Malone PD, Richter CP. (1944). The anatomy of the tongue of the domestic Norway rat. I. The skin of the tongue; the various papillae; their number and distribution. Anat. Rec., 89, 429-440.
7. Hull MT, Warfel KA. (1983). Age-related changes in the cutaneous basal lamina: Scanning electron microscopic study. J. Invest. Dermatol., 81, 378-380.
8. Hull MT, Warfel KA, Bloch T, Madura J, Eble JN. (1985). The epithelial-mesenchymal junction in colonic adenocarcinoma: Scanning electron microscopic study of colonic epithelial basal lamina. Lab. Invest., 52, 30A.
9. Hume WJ, Potten CS. (1976). The ordered columnar structure of mouse filiform papillae. J. Cell Sci., 22, 149-160.
10. Kawabe TT, MacCallum DK, Lillie JH. (1985). Variations in basement membrane topography in human thick skin. Anat. Rec., 211, 142-148.
11. Kutuzov H, Sicher H. (1951). The filiform and the conical papillae of the tongue in the white rat. Anat. Rec., 110, 275-288.

12. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. (1980). Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature., 284, 67-68.
13. Miller IJJr., Preslar AJ. (1975). Spatial distribution of rat fungiform papillae. Anat. Rec., 181, 679-684.
14. Simpson LO. (1980). Basement membranes and biological thixotropy: A new hypothesis. Pathology., 12, 377-389.
15. Staricco RJ, Pinkus H. (1957). Quantitative and qualitative data on the pigment cells of adult human epidermis. J. Invest. Dermatol., 28, 33-45.
16. Svejda J, Skach M. (1971). Die Zunge der Ratte im Raster-Elektron-enmikroskop (Stereoscan). Z. mikrosk.-anat. Forsch., 84, 101-116.
17. Vracko R. (1974). Basal lamina scaffold: Anatomy and significance for maintenance of orderly tissue structure. Am. J. Pathol., 77, 313-346.
18. Warfel KA, Hull MT. (1984). Scanning electron microscopic study of the epithelial-mesenchymal junction of the esophagus. Scanning Electron Microsc. 1984;II:697-701.
19. Warfel KA, Hull MT. (1984). Migration of lymphocytes through the cutaneous basal lamina in normal skin: An ultrastructural study. Anat. Rec., 208, 349-355.
20. Yoshioka I, Muto H. (1976). Surface structures of the tongue, palate and buccal mucosa of the rat: Scanning electron microscopic studies on the oral mucosa. Okajimas Fol. Anat. jap., 52, 297-312.

Discussion with Reviewers

J.L. Boshell: Will you hypothesize on the relationship between morphology of the basal lamina and keratinization pattern of the epithelium in areas that were studied?

Authors: The one area where there was a relationship between the topography of the basal lamina and the pattern of keratinization of the underlying epithelium was in the simple conical filiform papillae. Keratinization in the epithelium covering the anterior aspect of the papillae has been described as "soft keratin and the cells show a weak reaction for sulphydryl groups. The posterior aspects of these papillae are covered by cells which develop "hard" keratinization and contain cells which are sulphydryl-rich. [Farbman AI.(1970). The dual pattern of keratinization in filiform papillae on rat tongue. J. Anat., 106, 233-242] The cells lying in the posterior depressions (D) of the papillae (Fig. 7) are believed to give rise to the posterior population of cells which develop "hard" keratin.