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Three-dimensional architecture of the connective tissue core and surface structures of the lingual papillae in the rabbit

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Summary. Three-dimensional characteristics of the epithelial cell layer and connective tissue interface of the tongue were studied using scanning electron microscopy. In this study, the fragments of tongue were fixed in modified Karnovsky's fixative solution. Subsequently, the specimens were treated with 10% NaOH solution for 4-7 days at room temperature and postfixed in 1% OsO_4 in 0.1M phosphate buffer (pH 7.4) for 2 hours at 4 °C. They were dehydrated through a graded ethanol series, and critical-point dried with CO_2. The specimens were coated with gold and observed in a scanning electron microscope, JEOL JSM-6100. The results showed numerous papillae on the dorsal surface of the tongue divided into four groups (filiform, fungiform, foliate and vallate papillae). Filiform papillae are conically shaped; fungiform papillae have an irregular round surface; foliate papillae are oval in shape and have some parallel projections; and vallate papillae are located in the posterior part of the tongue and have a depression around the center. After the treatment with 10% NaOH solution, the original arrangements of connective papillae could be seen. This characteristic three-dimensional distribution of the collagen fiber bundles is typical for each superficial papillae depending on whether it is filiform, fungiform, foliate or vallate.

Key words: Scanning electron microscopy, Rabbit, lingual papilla, Connective tissue, Tongue

Introduction

The connective tissue has several functions such as nourishing the epithelial cells of the epithelium with blood and supplying the receptors with nerve fibers. On the other hand, the epithelium functions to protect the connective tissue.

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Tongues of several species of animals were studied not only for their relation to taste organs but also to classify the morphological characteristics of the lingual papillae according to the animal order. One reason for this morphological variety is the kind of food ingested and the degree of specialization of the mastigatory system.

The epithelium-connective tissue surface of the tongue of several animals has been investigated by Kulaa-Mikkonen and Sorvari (1985) in human, Iwasaki et al. (1987) in three mammalian species, Kobayashi et al. (1987) in the newborn dogs, Nagato et al. (1989) in the rat lingual filiform papillae, Kobayashi (1990) in the guinea pig, Morais et al. (1994) in the nine banded armadillo and Watanabe et al. (1997) in the *Calomys callosus*.

In the present study, three dimensional architecture of rabbit tongue was studied to clarify the relationship between the arrangements of collagen fibers of different kinds of papillae and their external appearance using the light and scanning electron microscope.

Materials and methods

In this study, tongues of eight adult rabbits, *Oryctolagus cuniculus*, of both sexes weighing between two and three kg were used. To collect the material, the animals were anesthetized with sodium pentobarbital and perfused with modified Karnovsky solution containing 2.5% glutaraldehyde, 2% paraformoldehyde in 0.1M sodium phosphate solution (pH 7.4). For electron microscopy, six tongues were fixed in the same solution for 24 h at 4 °C, then, rinsed with distillated water and three of them were treated with 10% NaOH solution for 4-7 days at room temperature about 25 °C as described by Ohtani (1992). After that, all the specimens were rinsed with distillated water and postfixed in 1% OsO_4 in 0.1M sodium phosphate buffer (pH 7.4) for 2 h at 4 °C. Then, they were dehydrated through graded ethanol series and critical-point dried. The specimens were mounted on metal stubs, coated with gold and observed by scanning electron microscopy (JEOL JSM-6100). For light microscopy, two tongues were fixed in

10% formalin or Bouin's fixative solution for 12 h and embedded routinely in paraffin. Then, specimens were cut, stained with hematoxylin-eosin, picro-sirius, azocarmin and observed in a Zeiss fotomicroscope.

Results

In light microscopic observations, the rabbit tongue exhibited a cornified epithelium, but the dorsal epithelium was thicker than the lateral and lower ones (Fig. 1).

In the rabbit tongue, a proeminence was observed in the middle part called torus lingual or intermolar proeminence and several types of papillae which could be divided in four main groups (filiform, fungiform, foliate and vallate papillae).

In the scanning electron microscope, different types of papillae could be examined. Filiform papillae occupied the entire dorsal surface in the front of the terminal sulcus and continued to the lateral borders; their size and shape, however varied according to the location.

In the anterior part, filiform papillae were numerous, conical shaped with vertical orientation and a depression on the posterior part (Fig. 2); in front of the torus the filiform papillae were relatively tighter and taller than the papillae in the anterior part (Fig. 3); and at higher magnification, the limits of the epithelial cells and a network pattern of microridges were seen in this papillae (Fig. 4).

In the torus lingual, filiform papillae looked like a hand with a primary papillae and a secondary papillae growing from the tip of the primary papillae (Fig. 5). The secondary papillae differ in number ranging between three and five projections. Near the vallate papillae, lower conical filiform papilla with vertical orientation and disperse distribution were seen (Fig. 6).

Fungiform papillae were present in the anterior part of the tongue. They were elliptical or circular in shape and filiform papillae was higher than fungiform papillae (Fig. 7). It was possible to observe some scaling cells and at higher magnification the taste pores and the contourn of the polygonal cells could be seen (Fig. 8).

A pair of foliate papilla was found in the latero-posterior part of the tongue, each was oval in shape and had some parallel projections (ridges) separated by grooves (Fig. 9); in the lateral surface there were several taste pores.

In the posterior part of the tongue there was a pair of vallate papillae. Each papilla had a circular form with a depression around the center (Fig. 10); the upper surface the mucosa was irregular and in the lateral surface there were several taste pores.

After the removal of the epithelium using the

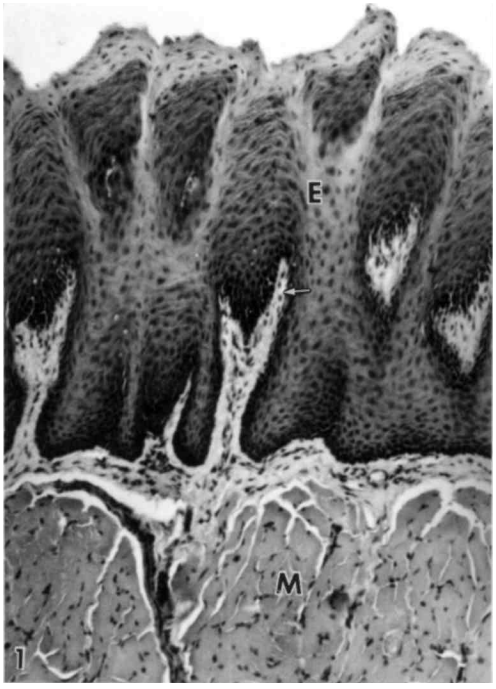


Fig. 1. Light micrograph of the dorsal epithelium of the rabbit tongue shows the epithelium (E), connective papilla (arrow) and muscular layer (M). x 113

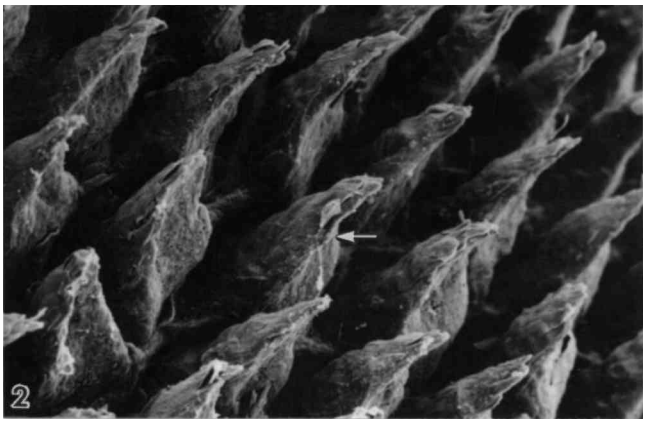


Fig. 2. SEM image of the anterior part of the rabbit tongue. Observe the filiform papillae with depression in the posterior part (arrow). x 174

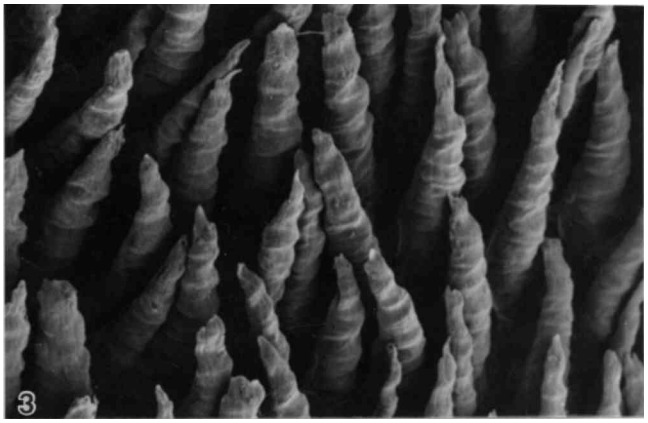


Fig. 3. SEM image of the filiform papillae in front of the torus of the rabbit tongue. Note the elongated filiform papillae and its distribution. x 166

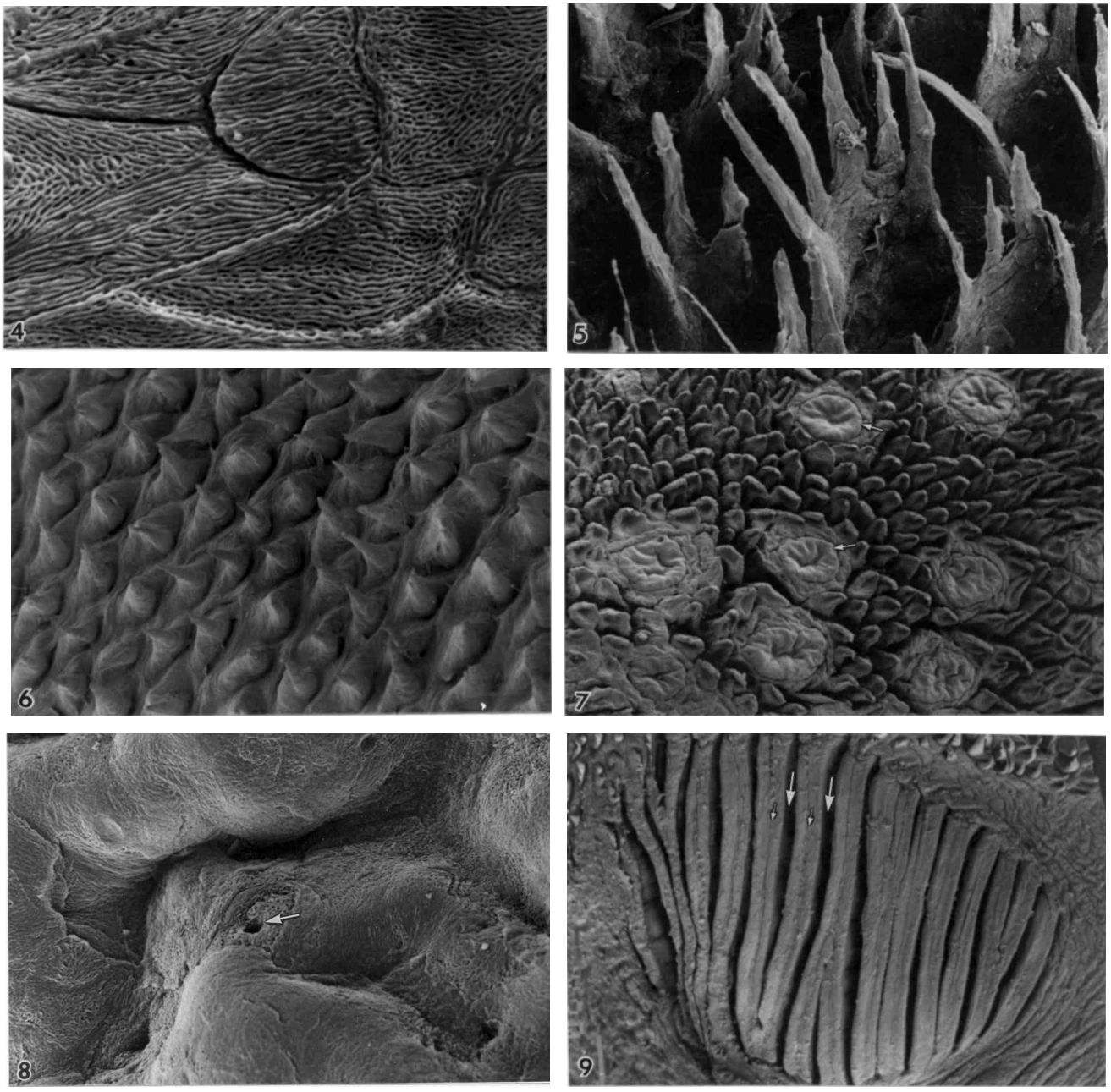


Fig. 4. SEM image of the filiform papillae in front of the torus of the rabbit tongue. At higher magnification, the polygonal cells and microridges can be observed. x 3,885

Fig. 5. SEM image of the lingual torus of the rabbit tongue, filiform papillae looks like a hand. x 201

Fig. 6. SEM image of the posterior part of the rabbit tongue. Note the form and distribution of the filiform papillae. x 69

Fig. 7. SEM image of the anterior part of the rabbit tongue. Observe the fungiform papillae (arrows) and the filiform papillae around. x 48

Fig. 8. SEM image of the fungiform papillae of rabbit tongue, observe the taste pore (arrow) and the scaling cells. x 1,143

Fig. 9. SEM image of the foliate papillae of rabbit tongue. Note the parallel ridges (small arrows) and the grooves (large arrows). x 23

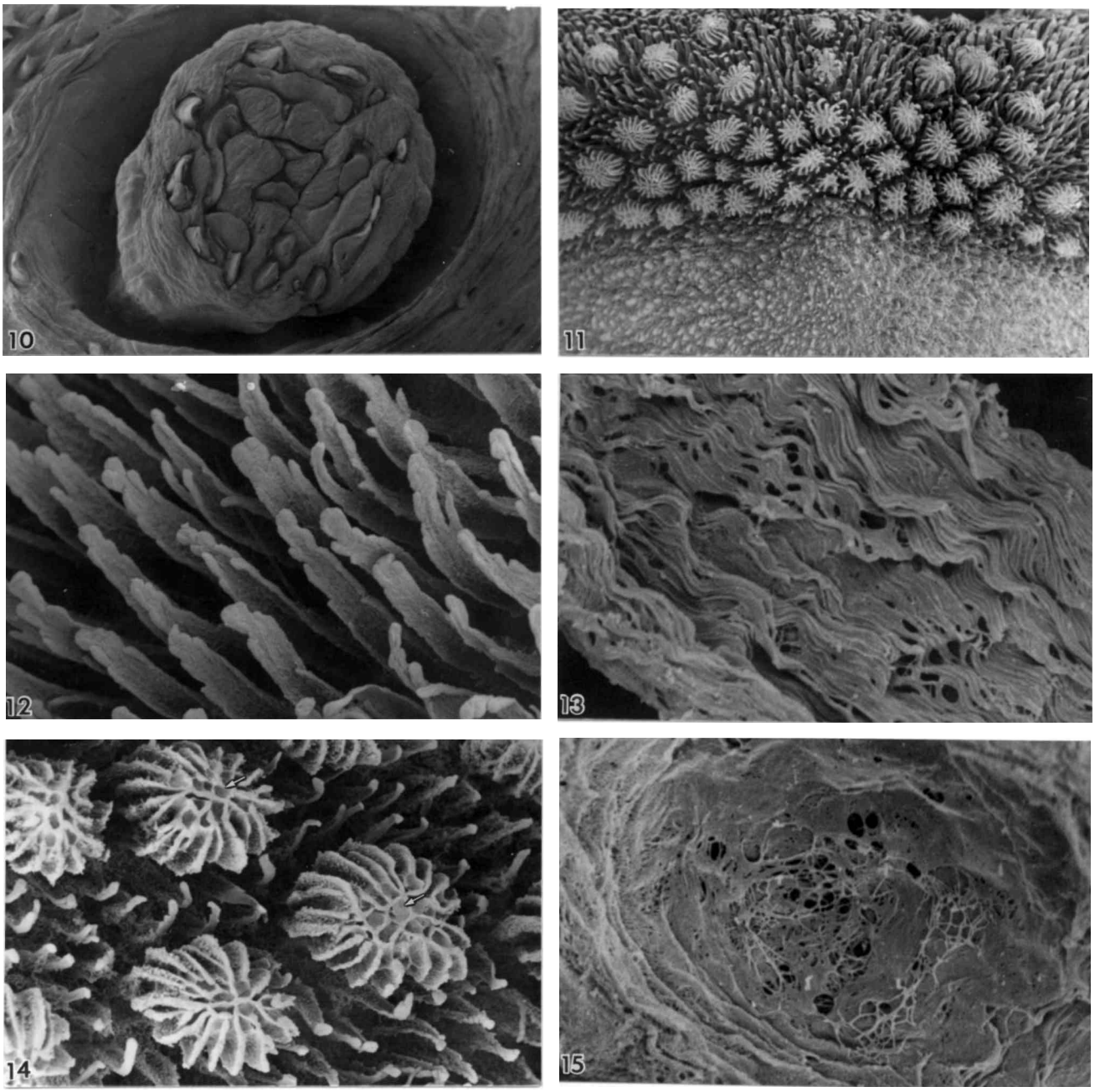


Fig. 10. SEM image of the posterior part of rabbit tongue. Vallate papillae with a depression around can be noted. x 69

Fig. 11. SEM image of the anterior part of rabbit tongue. Samples treated with NaOH solution, showing the three-dimensional characteristics of the connective papillae. x 23

Fig. 12. SEM image of the filiform papillae of the anterior part of the rabbit tongue. Note in the apex several small projections. x 165

Fig. 13. SEM image of the filiform papillae of the anterior part of the rabbit tongue. Observe the collagen fibers bundles. x 2,286

Fig. 14. SEM image of the fungiform papillae. Samples treated with NaOH solution, showing the columnar appearance with depressions on the top (arrows) and filiform papillae. x 83

Fig. 15. SEM image of the fungiform connective papillae. Observe details of the round depression. x 2,286

treatment with 10% NaOH solution, different forms of connective papillae could be seen (Fig. 11). The connective tissue core (CTC) of the filiform papillae of the anterior region had in the apex several small projections (Fig. 12) and at higher magnification the collagen fibers could be seen (Fig. 13). The connective papillae of the filiform papillae of the other regions were similar to the superficial shape.

The CTC of the fungiform papillae had a vertical orientation and were higher than the CTC of the filiform papillae. The connective papillae had a columnar shape and in the apex there were some depressions (Fig. 14). At higher magnification the collagen fiber arrangement could be seen (Fig. 15). These depressions were filled with taste buds as could be observed in the light microscope (Fig. 16).

The connective tissue of the foliate papilla was parallel laminar in shape. Each projection or ridge had three laminar sheets of connective tissue as could be observed in light micrographs (Fig. 17) and they were classified according to the localization, if they were beside the groove or not. The sheets near the grooves were called groove folds and the other sheet was called septal fold (Fig. 18). It was possible to observe some round depressions in the groove folds corresponding to the basal part of the taste buds (Fig. 19).

The arrangement of the connective tissue of the vallate papilla was irregular with several central and lateral prolongations. The central prolongations were like thorns and the lateral ones were laminar (Fig. 20). At higher magnification the arrangement of the collagen fibers could be seen (Fig. 21). In light micrographs, it was possible to observe the presence of several taste buds in the walls of the depression around the center and glands in the posterior part (Fig. 22).

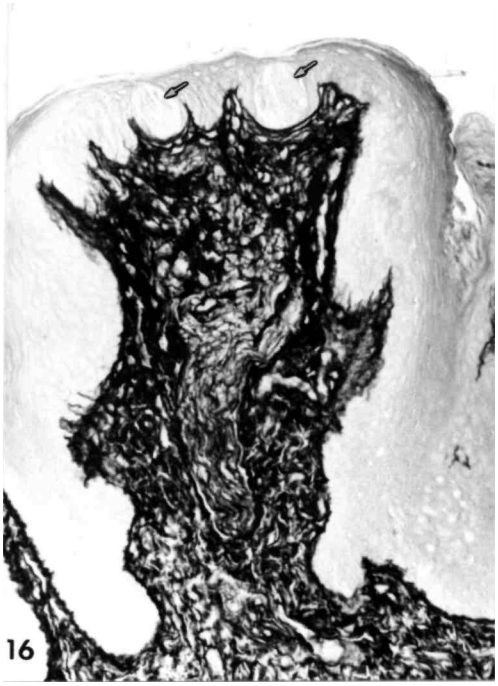


Fig. 16. Light micrograph of the fungiform papillae. Observe details of the connective tissue and the taste buds (arrows). x 186

Discussion

Our observations with light and scanning electron microscope revealed the characteristics of the lingual mucosa of rabbits. The distribution of filiform papillae was almost the same as in other mammals and reptiles reported by several authors.

In mammals, the smaller papillae were in the anterior and lateral border while the taller ones were situated in the torus lingual; this arrangement could help the complex mastigation pressing food against the palate. These aspects were related by Fish et al. (1944), Nagato et al. (1989) and Iwasaki et al. (1997) in rats, Iwasaki et al. (1996) in the mouse and Watanabe et al. (1997) in the *Calomys callosus*. However, these authors describe giant conical papillae in this place while we

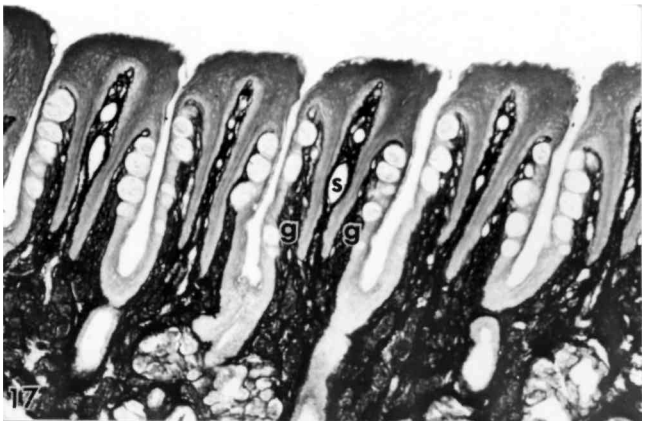


Fig. 17. Light micrograph of the foliate papillae. Note the connective tissue with septal fold (s) and groove folds (g) and the taste buds (arrows). x 63

Fig. 18. SEM image of the connective tissue of foliate papillae. The parallel folds can be observed. x 23

found filiform papillae like a hand.

In the posterior part, the radix of the tongue, near the faringe, the papillae were absent showing only mucosae prega. These aspects were related by Iwasaki et al. (1987) in rats and Iwasaki et al. (1988) in squirrel monkeys.

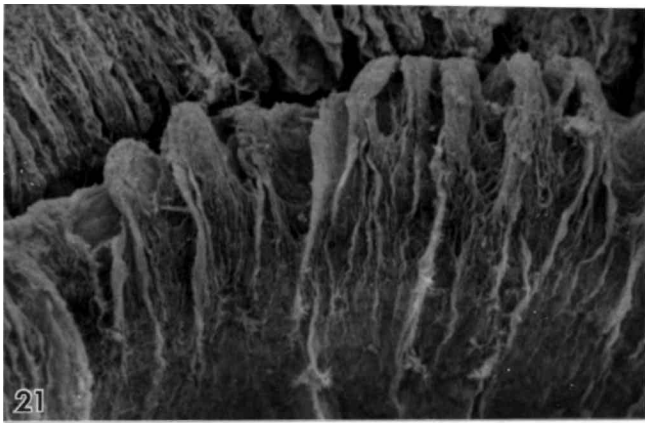
Fungiform papillae were localized in the anterior part of the tongue. They were elliptical or circular in shape and filiform papillae was higher than fungiform papillae. These characteristics were reported in rats (Miller and Preslar, 1975), in guinea pigs (Kobayashi, 1990), in the *Tupaia gli*s (Kobayashi and Wanichanon, 1992), in the *Tragulus javanicus* (Agungpriyono et al., 1995) and in rabbits (Ojima et al., 1997). The presence of taste pores in the surface were described by Chamorro et al. (1986) in cows and horses and by Agungpriyono et al. (1995), in the *Tragulus javanicus*.

Our observations confirmed the characteristics of the foliate papillae reported by Watanabe et al. (1988) and Kobayashi (1992), which presented some parallel projections (ridges) separated by grooves and three laminar sheets of connective tissue called groove folds and septal fold.

Our results demonstrated that vallate papillae of rabbits were similar in shape to cows and horses (Chamorro et al., 1986), in the squirrel monkey (Iwasaki et al., 1988) and in the *Macaca Fuscata* (Iwasaki et al., 1992). In relation to the number and disposition several animals like rats and mouses have only one central papilla while the man has several papillae disposed in an inverted V.

In our study was used the NaOH-method reported by Ohtani (1987) which removes the epithelium layer evidencing the collagen fibers of lamina propria in original dispositions. On the other hand, Kobayashi (1992) and Kobayashi and Wanichanon (1992) employed HCl solution at low temperature in orther to remove the epithelial layer.

The observation showed full details about the connective tissue like interdigitations which have as



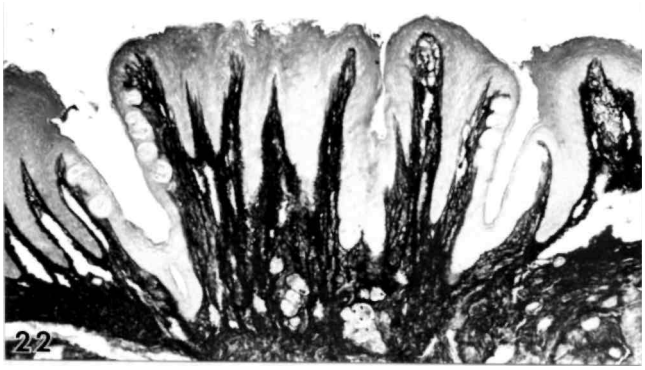


Fig. 19. SEM image of the foliate papillae. Observe the basal aspects of the taste buds (arrow). x 94

Fig. 20. SEM image of the vallate papillae after the treatment with NaOH solution. Observe the irregular projections in the center and laminar in the margins. x 40

Fig. 21. SEM image of the vallate papillae, the arrangement of the collagen fibers coud be seeen. x 422

Fig. 22. Light micrograph of the vallate papilla. Observe details of the connective tissue and the taste buds. x 63

function to increase the mechanical connection and the blood and nerve supply. It was also possible to note spaces between the bundles of collagen fibers, these spaces are the original localization of nerves fibers and vessels.

After the removal of the epithelium, the CTC of the rabbit tongue was observed and, in general, the CTC of the filiform papillae was similar to the shape of the lingual papillae. This result was also related by Nagato et al. (1989).

The CTC of the fungiform papillae had a columnar shape and were higher than the CTC of the filiform papillae. This was also observed in rats (Miller and Preslar, 1975), in guinea pigs (Kobayashi, 1990), in the *Tupaia glis* (Kobayashi and Wanichanon, 1992), in the *Tragulus javanicus* (Agungpriyono et al., 1995) and in *Calomys callosus* (Watanabe et al., 1997).

Our data showed that the arrangement of the connective tissue of the vallate papilla was slightly irregular with several central and lateral prolongations, corresponding to the characteristics described in the *Tupaia glis* by Kobayashi and Wanichanon (1992).

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