Scanning Electron Microscopic Observations on the Taste Pores and Taste Hairs in Rabbit Gustatory Papillae

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Summary. The taste pores and taste hairs of the papillae foliatae and circumvallatae in the rabbit’s tongue were observed under the scanning electron microscope. The animals were perfused with buffered glutaraldehyde through the A. carotis communis and the papillae were post-fixed with buffered osmium tetroxide.

1. The taste pores were distributed more frequently on the top of the undulating epithelial surface lining the papilla groove, and opened in the form of circular crater with a relatively uniform diameter.

2. Each pore was surrounded by 3 to 5 cell bodies arranged side by side to form a ring.

3. Two types of taste hairs were found: the one occupied the major part of the pore and corresponded to the common taste hairs or microvilli in its size and shape and its location in the pore, while the other was a club-like projection, only one to three in number, and it extended out of the free margin of the pore an unusually long and thick stem with its top generally swollen.

4. Development of the hairs in papillae circumvallatae was not so conspicuous as in papillae foliatae and the occurrence of the club-like projection was correspondingly very seldom.

5. The club-like projection is considered to correspond to the ending of the dark cell or type III cell which Murray and Murray et al. have reported with sectional views by transmission electron microscopy.

Scanning electron microscopy (SEM) in biological studies has recently accumulated a great amount of literature coming from many branches of science. Although some points still remain to be solved in its application to biological subjects, especially with respect to the preparation of soft tissue samples, SEM has proved its advantages over the conventional, transmission electron microscopy (TEM) in elucidating the three-dimensional structures of either the surfaces or sections of cells and tissues, and it is creating ever increasing interest in its future promise.

The use of SEM in the study of taste organs is already reported by some authors such as Arenberg et al. (1969) with the guinea pig and human material, GraziaDei (1969) with the fish, frog and rat, and Beidler (1969a; b; 1970) with the frog, rat, rabbit and puppy as material. An additional report has been written by the present authors (Shimamura and Tokunaga, 1970) on the microstructure of the surface of fungiform papillae in the frog. However, experiences in the application of SEM to the studies of taste organs of vertebrates including man are still very limited, and even the technique of preparing adequate samples from such materials has not been established. One important problem is that the structural details of cells and tissues as prepared by conventional techniques and seemingly intact and well preserved when viewed under lower magnification, frequently turned out to show a doubtful
picture when viewed under higher magnification, thus indicating poor preservation of the structural details.

The present study was undertaken to work out some improvement in the conventional methods of sample preparation from the taste organ, and based on the improved method, to make observations on the taste pore of mammals which is the tiny and sole communication of the taste bud with the oral cavity. Opinions as to how the taste pore opens in the superficial cells of the papillae groove and how the taste hairs grow in the taste pore were also topics of the present study, and the findings were discussed in relation to the current information obtained with TEM.

**Material and Method**

The tongues of rabbits weighing about 3 kg were perfused with a 2.5% glutaraldehyde solution in cacodylate buffer (pH 7.4) through the A. carotis communis. The foliate and circumvallate papillae were excised and transferred into physiologic saline to cleanse the surface from mucinous substances. They were next transferred to another change of saline solution and subjected to the action of an ultrasonic vibrator. The material was immersed in the same fixing solution for more than 6 hrs, during which period the grooves of the papillae were exposed by the aid of an anatomical microscope. Postfixation was then made for 60–90 min in a 1% osmium tetroxide solution in the same buffer. After being washed by fresh solution of the same buffer, the sample was soaked in a mixture of isoamyl acetate and ethanol (5:1) until it came to remain in the bottom of the solution. The samples were then transferred into a pure solution of isoamyl acetate for about 20 min, changing the solution every 5 min. The sample was then taken out onto a piece of filter paper and then rapidly transferred into the vacuum evaporator and dried.

They were subjected to double coating with carbon and gold and observed by a JSM-2 type SEM (JEOL).

**Result**

**Epithelial lining of papillae and taste pores**

The entire epithelial surface lining the papilla grooves extended with a rather undulant appearance. The undulation occurred more conspicuously from the middle depth to the bottom of the groove. The paving pattern of individual flat epithelial cells covering the groove was readily recognizable owing to their well-defined cell borders.

Although the contour of individual cells was subject to extreme diversity, cells of irregular polygonal contours were the most common. The size of these cells was also highly variable. The epithelium of this region contained as a rule a small number of cells in the desquamative stage as shown in Figure 3. In the groove surface near the dorsal portion of the papillae, there were also seen small rises under which lay nuclei of degenerated cells (Fig. 7).

The taste pores were distributed more frequently over the top of the undulating surface, and opened in the form of a circular crater relatively uniform in diameter. The maximum diameter was shown to reach 4 μ. These pores were seen scattered from the middle depth to the bottom of the groove, one pore being separated from its
neighbors by 20–30μ in the middle depth and by much smaller distances in the bottom portion of the groove. No definite pattern of arrangement was noticed among these pores.

The pore was generally surrounded by 3 or 4 cell bodies arranged side by side to form a ring, but some pores were surrounded by 5 cells. The portions of these cells forming the free margin appeared slightly bent down into the pore (Fig. 3–6).

**Appearance of taste hairs**

In the foliate papillae, dense growth of taste hairs could be observed by SEM within the taste pore. There could be discriminated two varieties of hairs, of which the first type occupied the major part of the taste pore and corresponded in size and shape to the common taste hairs or microvilli. The top of these hairs did not reach as high as the free margin of the pore as it was usually seen by TEM, but appeared somewhat shrunk downward. In all probability this may be a result of shrinkage by drying in the preparation of the sample. The height of the individual hairs within the pore was irregular and all the tips were slightly tapered.

The taste hairs of the second variety, as more than three in number, extended out of the free margin of the pore with an unusually long and thick stem as shown in Figure 3 taken at lower magnification. The stem of the hair was slightly bent with its tip generally swollen in a club-like shape as shown in Figures 4 and 5. There were, however, some hair-like projections of irregular shape without a swollen tip (Fig. 6). These taste hairs of the 2nd variety are hereafter referred to as club-like projections by the present authors. The club-like projections showed a maximum diameter of about 0.3μ at the base of the stem and about 0.5μ at the swollen tip, although there were those of many intermediate sizes. A projection or projections of this type occurred in every taste pore examined throughout the epithelial surface...
Fig. 3. Epithelial surface extending from the middle region to the groove bottom (right side) of foliate papilla, showing distribution of taste pores. In each of the pores can been seen the club-like projections. Note various patterns of arrangement of superficial cells and the appearance of the cells arranged to form the pore inlet. Arrow 1: red cell, A, B: ×1,000
of the groove.

The taste pores in papillae circumvallatae resembled those in papillae foliatae, except that they occurred not only in the middle and lower parts of the groove but also near the dorsal surface. Development of taste hairs was not so conspicuous as in foliate papillae and the number of club-like projections was likewise very few (Fig. 2).

Fig. 4. A close-up view of the part indicated by arrow 2 of Figure 3. A club-like projection is extending from within the pore, and a large number of hairs of common type are also seen growing within the pore. ×31,000

Discussion

Detailed studies on the small processes or microvilli on the pore portion of taste bud cells have been done by FARBMAN (1965a, b) in the fungiform papillae of the rat, by SCALZI (1967) in rabbit's foliate papillae, by MURRAY, and MURRAY et al. (1967, 1969, 1970) in rabbit's foliate papillae and by UGA (1969) in rat's circumvallate papillae. A review of these reports suggested that the fine structure of the apical termination in the taste bud cells extending from the inner part of the taste pore
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(otherwise termed as "proximal or inner taste pore" or "taste pit" or "taste chamber") to the outer part ("distal or outer taste pore") was subject to variation among individual species of animal and among individual papillae studied. Such differences appeared to exist more or less even in the foliate papillae of the same animal at the microstructure level. Of the various descriptions as reported by these authors with regard to the apical termination of taste bud cells, we were particularly interested in "bulbous termination in dark cell or type III" by MURRAY (1969) (see Fig. 5 of his paper), and MURRAY et al. (1969) (see Fig. 2 of their paper) and "rod-shaped processes without any villous structure in dark cell" by UGA (1969) (see Fig. 5 of his paper). Of these forms described, the apical bulbous structure in the former authors's report seems to correspond to what we termed in the present study as a club-like projection. With regard to "apical dilatation" of taste bud cells, we have an early report of GRAY and WATKINS (1965) on the circumvallate papillae in rabbits. According to these authors the maximum thickness of the terminal dilatation was 2μ and this was explained to be protruding from within the "outer taste pore" to the external environment as much as 3μ or more (see Fig. 1 in their article). MURRAY and MURRAY (1967) have commented on this unusual terminal dilatation of microvilli as follows. "We usually do not see bulges, although they do occur. In such cases, the bulging ends seen in Figure 1 of GRAY and WATKINS' report are very pale in contrast to the

Fig. 5. A higher magnification of the part indicated by arrow 4 of Figure 3. A part of the free margin of the pore shows a fluffy appearance. ×10,000
Fig. 6. A higher magnification of the part indicated by arrow 3 of Figure 3. A variant shape of the club-like projection, having a flat irregular stem without a swollen tip. ×10,000

Fig. 7. View of the superficial cells near the dorsal portion of the foliate papilla showing a few small rises which contain nuclei of degenerating cells. A, B: ×10,000
rest of the villus, suggesting a degree of artificial swelling. This apparent difference may then be a result of fixation artifact."

Uga (1969) observed similar pictures and remarked that these should be regarded as an artifact that could be produced by single fixation with 1% osmium tetroxide or with 0.6% KMnO₄ in Ringer solution, buffered at pH 7.4 with veronal acetate. The terminal dilatation observed by Gray and Watkins may then be nothing more than an artifact incidental to an inadequate fixation procedure. However, as Murray et al. (1969) have shown, the fact seems firmly established that even with double fixation by glutaraldehyde and osmium tetroxide, bulbous termination of microvilli and their protrusion from the outer taste pores can occur in certain type of cells of the taste bud.

The rod-shaped processes of the dark cell protruding from the outer taste pore which Uga (1969) described in rat's circumvallate papillae, had already been observed by Kitamura (personal communication, 1966) with the same papillae in the rat by the use of double fixation procedures involving both 2.5% glutaraldehyde perfusion from the heart and 1% osmium tetroxide fixation. The shape of the protruding end as described by these authors was quite different from that described by Murray, and Murray et al. with rabbit's foliate papillae. Such a difference, however, may well be explained on the basis of differences in species and taste papillae studied.

The club-like projection which we first described by the use of SEM is considered to represent the three-dimensional picture of the bulbous termination of the dark cell or of the type III cell which Murray, and Murray et al. have reported in their sectional view by TEM. They reported, however, that these type III cells were as much as 5 to 10% of the total number of the taste bud cells that ranged in number from 30 to 80, while our club-like projection occurred only 1 to 3 for every taste pore. Thus, this discrepancy remains to be explained.

Although the taste pores of mammalian and lower animals have been studied by several investigators by the use of SEM, we are the first to demonstrate clear pictures of the taste hairs as they occur in the taste pores. Similar, but less definite findings have been reported by Beidler (see Fig. 2 of 1969a and Fig. 6 of 1969b) in rat's fungiform papillae. A somewhat similar structure may be the "large ball-like structure" reported by Graziaedi (1969) in the taste pores in the lip scales of the guppy. Graziaedi emphasized that the structure was a real outgrowth of the taste bud cell and presented pictures taken by TEM in support of his observation by SEM.

Although the bulbous termination of taste hairs has been to date observed only in limited species of animals, careful study would reveal the occurrence of similar structures in other species even if in more variable appearance.

It is unknown whether the terminal dilatation occurring in a small number of taste bud cells is designed so that the contact area of the organ might be increased for any nutrient fluid to come.

One of the critical problems in applying SEM to studies of highly hydrated soft tissues of animals may be how to dry the samples without causing damage. Most of the scanning microscopists have usually adopted the method of Barber and Boyde (1968) which essentially consists of rapid drying after dehydration of samples in graded concentrations of acetone. Although some investigators preferred the use of the freeze drying method, this often resulted in damage of tissues by ice crystal
formation and consequently proved not suitable for good preservation of the tissue fine structure. Dehydration of samples by isoamyl acetate followed by drying in vacuo, which we have first employed in the present study, proved to bring out a dried sample with a more natural appearance, structural details of cells and tissues being well preserved as shown in the photographs presented.

Recently ARENSBERG et al. (1970) using tongue papillae have made an extensive study on as many as 15 kinds of sample preparation methods, involving fixation, dehydration and various drying procedures such as simple air drying, air drying in vacuo, controlled freeze-drying at low temperature and simple freeze-drying. Among the 15 methods studied, the method designated No. 9 has been proposed by the authors as the most excellent for conservation of intact tissues. This involved fixation by 4% glutaraldehyde, dehydration by a mixture of ethanol and ether at the temperature of −40°C and drying in an evacuated dessiccatior and by Linde Molecular Sieve (Type 13X). We failed, however, to recognize the proposed excellence of method No. 9 on examining the actual photographs presented.

On the other hand, we are confident of the merit of the method described above with which we could reproduce a clear view of the taste hairs in rabbit’s foliate papillae.

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