A Histomorphometric Study of the Age-related Changes of the Human Taste Buds in Circumvallate Papillae

Yoshinaka Shimizu
Department of Oral Pathology, Tohoku University School of Dentistry, Sendai, Japan


The objective of this research is to investigate the age-related changes of human taste buds in circumvallate papillae by a histomorphometric study. The materials were obtained from 241 Japanese cadavers, ranging in age from 0 to 97 years old. The taste bud numbers in the papilla wall, and the taste bud cell numbers per taste bud section were calculated. The transcripational activities of taste bud cells and lingual epithelial cells were investigated by the argyrophilic nucleolar organizer regions (AgNORs) technique. The taste bud density and the taste bud cell density in the older age groups was lower than that of the younger age groups. The AgNOR number of lingual epithelial cells decreased significantly in the older age groups, but the taste bud cells did not. Unlike the maintenance of transcripational activity of taste bud cells, the reduction in the taste bud density and the taste bud cell density may influence taste systems with aging.

Key words: aging, human taste buds, circumvallate papillae, histomorphometry, AgNORs

Yoshinaka Shimizu, Department of Oral Pathology, Tohoku University School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-77, Japan

Introduction

Taste thresholds have been reported to be physiologically higher in the elderly than in the young (1-3). The taste buds are the final sense organ of taste, and the sensation of gustation arises from impulses generated in taste bud cells. They are located mainly in the mouth, pharynx and larynx, and are primarily found on the tongue (4-6).

Histological reports have shown that the taste bud number on human tongue papillae diminish with age (7,8) and that taste perception could be related to taste bud density (9,10). On the other hand, recent histological studies have shown no relationship between taste bud number and age in human fungiform papillae(11,12).

Taste bud cells, first recognizable at 13-15 weeks in the human fetal tongue, continue to be differentiated from the surrounding epithelial cells (13). The life span of a taste bud cell is about 250±50 hours in rat lingual fungiform papillae (14). There are no age-related studies on the differences in the life spans of human lingual taste bud cells. Nucleolar organizer regions(NORs) are certain specific, acidic nonhistone portions of DNA which code for ribosomal RNA(rRNA), and ultimately direct protein synthesis (15). They are selectively stained black, and appear as dots in a light microscope under the silver colloid technique (16). The number of NORs in interphase nuclei may reflect the state of cellular proliferation (17), transcriptional activity (16) and the degree of malignancy of tumor cells (18). The age-related changes of cellular transcriptional activity in taste bud cells were unclear.

It has recently been suggested that human taste bud histology and taste physiology are substantially preserved in old age. To clarify histologically age-related changes to taste buds, the present comparative study of the taste bud cell density and the cellular activity is shown for different ages.

Fig. 1 A: The portion of circumvallate papilla for the histological section.
Fig. 1 B: The appearance of sliced surface.
Fig. 1 C: Three sites (I, II, III) for counting AgNOR numbers.
Materials and Methods

Experimental tissue

The materials for the present study were taken from 241 human cadavers. The ages ranged from 0 to 97 years old. Macroscopically, cases with pathological lesion were excluded. The materials were fixed in 10% formalin and cut into transverse slices at the posterior portion of the tongue containing circumvallate papillae (Fig. 1A, B). They were routinely processed for paraffin histology. Serial 3-μm vertical sections were stained with hematoxyline and eosin (H-E). Microscopically, specimens with pathological changes were excluded.

Fig. 2 A: Light microscopy of circumvallate papilla (59 years old male, H-E stain, ×20). Some taste buds (arrows) shown in circumvallate papilla wall.

Fig. 2 B: Taste buds (28 years old male, H-E stain, ×200).

Fig. 2 C: A taste bud (69 years old female, AgNOR stain, ×400).

Measurement of taste bud density

The length of the circumvallate papilla walls was measured using a computer supported image processing system TRI/P (Ratoc System Engineering, Tokyo) in the vertical section stained with H-E. The number of taste

![Graph](image1)

Fig. 3-1: Scatter diagram for age-related changes of taste bud density.

![Graph](image2)

Fig. 3-2A: Relationship between age group and total number of taste buds.

![Graph](image3)

Fig. 3-2B: Relationship between age group and length of circumvallate papilla wall.

![Graph](image4)

Fig. 3-2C: Relationship between age group and taste bud density.
buds measured on the circumvallate papilla walls was
determined. The taste bud density was calculated as the
number of taste buds divided by the length of the
circumvallate papilla walls (Fig.1C).

Measurement of taste bud cell density

The circular taste bud profiles in a given section
were traced and the size was calculated microscopically
(×200) using TRIP. Taste buds extending parallel from
the basal lamina to the epithelial surface were selected.
The total number of taste bud cell nuclei was counted.
The taste bud cell density was calculated as the number
of nuclei divided by the size of a taste bud in a section.

AgNORs staining

Paraffin sections were dewaxed in xylene and
hydrated through graded ethanol to deionized water.
They were dried at 68°C for 2 hours and underwent the
AgNOR stain procedure at room temperature for 30
minutes. The reaction mixture comprised 2% gelatin
and 1% aqueous formic acid. This was mixed in a
proportion of 1:2 volumes with 50% aqueous silver
nitrate under darkened room conditions (15). The
sections were washed in running deionized water for 30
minutes, dehydrated to xylene, mounted and the
AgNOR number was counted immediately.

The number of AgNORs was analyzed by using taste
bud cells on the circumvallate papillae wall (Fig.1C 1 ),
the surface epithelium of the circumvallate papillae
(Fig. 1C 2 ) and the epithelium around the circumvallate
papillae on the tongue (Fig. 1C 3 ). The average
AgNOR number per cell was calculated in each region.

Statistical analysis

The materials were divided into five arbitrary age
groups, group 1: 0-15 years (23 males, 25 females ),
group 2: 16-35 years (24 males, 12 females), group 3: 36-
55 years (37 males, 19 females), group 4: 56-75 years (39
males, 29 females) and group 5: 76- years (22 males, 11
females). A statistical analysis of the data by the
Wilcoxon rank-sum test was done with a microcom-
puter PC-9801RA (NEC,Tokyo) and the statistical
analysis program Doctor Chameleon (Sankaido, Tokyo).

Results

Age-related changes in taste bud density

The taste buds had pear- and barrel-shaped structures embedded in the epithelium on the circumvallate
papillae wall (Figs. 2A, 2B). They were much lighter
than the surrounding cells of the epithelial layer. Some
taste buds had a taste pore or basement membrane.
1. The age changes in taste bud density

![Fig. 4-1: Scatter diagram for age-related changes of taste bud cell density.](image)

![Fig. 4-2A: Relationship between age group and taste bud size.](image)

![Fig. 4-2B: Relationship between age group and taste bud cell number.](image)

![Fig. 4-2C: Relationship between age group and taste bud cell density.](image)
A scatter diagram (Fig.3-1) showed an estimate of taste bud number per circumvallate papilla. The regression line was for all cases, its slope showed the diminution of taste bud density with age and was not statistically significant.

2. The age group changes of taste bud numbers (Table 1)
   A. The mean number of taste buds: 8.51 ± 8.42 (means ± S.D.). The decrease in the number of taste buds in group 5 was statistically significant (p<0.01) (Fig.3-2A).
   B. The mean length of the circumvallate papilla wall: 1.02 ± 0.52 (means ± S.D.) mm. The length in group 2 significantly increased, but afterwards there was no significant change (Fig.3-2B).
   C. The density of taste buds: It decreased significantly, to reflect the diminution of the total number of taste buds. (Fig.3-2C)

**Age-related changes in taste bud cell density**

Under a light microscope (×200) two types of taste bud cells were observed, light-staining cells and dark-staining cells. Their nuclei showed round shapes or ellipse shapes. The proportion of the two types of taste bud cell varied in each taste bud.

1. The age changes of taste bud cell density

A scatter diagram (Fig.4-1) showed the age-related changes of taste bud cell density. The regression line drawn for all taste buds showed a decrease in cell density with aging. The slope was statistically significant (p<0.01).

2. The age group changes of taste bud cell density (Table 2)
   A. Size: It showed a statistically significant increase with aging. (Fig.4-2A).
   B. Number: It decreased in the 16-35 age group and showed no significant change afterwards (Fig.4-2B).
   C. Density: It decreased significantly with aging (Fig.4-2C).

**Age-related change of the AgNOR number of taste bud cells**

The AgNORs staining response was shown in Fig.2C. Well-defined, black, silver-stained dots were observed in all nuclei. The number of dots per nucleus was counted.

1. The age changes of the AgNOR number of taste buds cells (Fig.5-1)

A scatter diagram (Fig.5-1) showed the age-related changes of the AgNOR number of taste bud cells. In the analysis of the linear regression of 100 cases, no statistically significant pattern was seen in the AgNOR...
Table 1: Changes of taste bud density in age groups

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number (M/F)</th>
<th>Bud number (mean ± S.D.)</th>
<th>Wall length (mean μm ± S.D.)</th>
<th>Taste bud density (mean /μm ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 &amp; below</td>
<td>48 (23/25)</td>
<td>7.85±6.40</td>
<td>0.74±0.52</td>
<td>10.75±8.39</td>
</tr>
<tr>
<td>16–35</td>
<td>36 (24/12)</td>
<td>7.22±7.99</td>
<td>1.07±0.38</td>
<td>6.09±6.65</td>
</tr>
<tr>
<td>36–55</td>
<td>56 (37/19)</td>
<td>9.42±8.98</td>
<td>1.13±0.47</td>
<td>7.44±6.75</td>
</tr>
<tr>
<td>56–75</td>
<td>68 (39/29)</td>
<td>9.69±10.2</td>
<td>1.10±0.50</td>
<td>6.29±5.69</td>
</tr>
<tr>
<td>76 &amp; above</td>
<td>33 (22/11)</td>
<td>6.78±5.85</td>
<td>1.15±0.55</td>
<td>4.42±3.49</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>241 (154/87)</strong></td>
<td><strong>8.51±8.42</strong></td>
<td><strong>1.02±0.52</strong></td>
<td><strong>7.14±6.72</strong></td>
</tr>
</tbody>
</table>

(statistically different: *p<0.05, **p<0.01, ***p<0.001)

Table 2: Changes of taste bud cell density in age groups

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number (M/F)</th>
<th>Size (mean×10^2 μm^2 ± S.D.)</th>
<th>Cell number (mean ± S.D.)</th>
<th>Density (mean×10^7/μm^2 ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 &amp; below</td>
<td>48 (23/25)</td>
<td>15.16±7.56</td>
<td>13.05±5.05</td>
<td>9.15±3.65</td>
</tr>
<tr>
<td>16–35</td>
<td>36 (24/12)</td>
<td>15.52±6.08</td>
<td>11.87±4.31</td>
<td>8.11±2.72</td>
</tr>
<tr>
<td>36–55</td>
<td>56 (37/19)</td>
<td>15.06±7.14</td>
<td>12.07±4.56</td>
<td>8.37±3.23</td>
</tr>
<tr>
<td>56–75</td>
<td>68 (39/29)</td>
<td>17.58±6.38</td>
<td>12.26±4.10</td>
<td>7.39±2.35</td>
</tr>
<tr>
<td>76 &amp; above</td>
<td>33 (22/11)</td>
<td>17.02±7.22</td>
<td>12.24±4.88</td>
<td>7.36±2.37</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>241 (154/87)</strong></td>
<td><strong>16.28±6.94</strong></td>
<td><strong>12.29±4.56</strong></td>
<td><strong>8.01±2.93</strong></td>
</tr>
</tbody>
</table>

(statistically different: *p<0.05, **p<0.01, ***p<0.001)

Table 3: Changes of AgNOR number of taste bud cells in age groups

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number (M/F)</th>
<th>AgNOR number (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>15 &amp; below</td>
<td>20(12/8)</td>
<td>2.48±0.45</td>
</tr>
<tr>
<td>16–35</td>
<td>20 (11/9)</td>
<td>2.31±0.29</td>
</tr>
<tr>
<td>36–55</td>
<td>20 (14/6)</td>
<td>2.29±0.23</td>
</tr>
<tr>
<td>56–75</td>
<td>20 (13/7)</td>
<td>2.42±0.30</td>
</tr>
<tr>
<td>76 &amp; above</td>
<td>20 (13/7)</td>
<td>2.35±0.35</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>100 (63/37)</strong></td>
<td><strong>2.37±0.32</strong></td>
</tr>
</tbody>
</table>

(statistically different: *p<0.05, **p<0.01, ***p<0.001)
number of taste bud cells.
2. The age group changes of the AgNOR number of taste bud cells (Table 3)

The average number of AgNORs varied between different age group populations for each site. The number of AgNORs was very high in infants. It decreased as age advanced and was the lowest in the old age groups.

A. Site I: The mean number was 2.37 ± 0.32. A slight decrease of the mean number of AgNORs was recognized in the 76- age group as compared to the younger groups, but no significant difference was shown in the five age groups (Fig.5-2A).

B. Site II: The mean number was 1.79 ± 31 (Fig.5-2B). It was significantly less in the 76- age group than in the 16-35 (p<0.01) and 36-55 age groups (p<0.01).

C. Site III: The mean number was 1.91 ± 0.30 (Fig.5-2C).

It was significantly less in the 76- age group than the 0-15 age group (p<0.05).

Discussion

Age-related changes in taste bud numbers have been studied in various animals. Rat taste bud numbers showed no decrease with age in the circumvallate papillae (19,20). The taste bud numbers in chicken oral cavities increased during the embryo stage, and remained relatively constant thereafter (21). Cat (22) and sheep (23) epiglottal taste buds increased in number during development, and continued to increase until the epiglottis reached its adult size. In Rhesus monkey there was no significant difference with age in the average taste bud number in fungiform, circumvallate, and foliate papillae (10,24). Arey (7) and Mochizuki (8) demonstrated that taste bud numbers decreased in human lingual circumvallate papillae and foliate papillae (25,26) with age. Arvidson (11) and Miller (12) recently examined the taste bud number in human fungiform papillae taken from autopsy subjects. Recent anatomical evidence has been presented for no age-related decrease in the taste bud density of human lingual fungiform papillae (12,27).

Taste bud numbers have been counted in the fungiform, circumvallate and foliate papillae of the tongue. Those in the fungiform papillae were a low percentage of the total lingual taste buds in mammals (24,28,29), and were almost always found in the circumvallate papillae and the foliate papillae (24). Since there is a traumatic loss of fungiform papillae and foliate papillae in the anterior portion and the lateral edge of the human tongue (24), the circumvallate papillae in the middle portion of the tongue were examined in the present study.

Arey et al. (7) concluded that all combinations existed between high and low taste bud numbers in the circumvallate papillae and on their associated wall length. In the present study the wall length of the circumvallate papillae increased until adulthood and showed no significant difference thereafter, but the taste bud density decreased with age. Mochizuki (8) reported that the diminution in the number of taste buds in old age was due to their atrophy and their consequent disappearance. This study suggested that the diminution of taste bud numbers in the human circumvallate papillae with age might occur because of their atrophy.

The taste buds in rat vallate papillae have been quantitatively investigated by light microscopy (30,31). Hosley et al. (30) measured the postnatal increases in taste bud size and cell number. Mistretta et al. (31) suggested that the taste bud diameter did not differ with age, and that the general anatomical characteristics of taste buds were similar in young and old rats. The present study showed an increase in the taste bud size in human circumvallate papillae. Their cell density significantly decreased with age although there was no significant difference in taste bud cell numbers during adulthood. The parenchymal cells not only decreased in number, but also often increased in volume with age (32). The results of the present study suggested the hypertrophy of taste bud cells with age (33) to compensate for the taste physiological depression of old age (34).

Beider (14) demonstrated with a radioactive isotope (H-thymidine) that the turnover time of taste bud cells was shorter than that of epithelial cells. In the present study the mean AgNOR number of epithelial cells of the circumvallate papillae did not differ from that of the epithelial cells around the circumvallate papillae. The mean AgNOR number of taste bud cells was more than that of their lingual epithelial cells. It was suggested that the transcriptional activity of the taste bud cells was higher than that of the epithelial cells, which differentiated taste bud cells.

Denton et al. (35) reported that the NOR number in cultured lymphocyte showed a decrease with age. Age-dependent changes in cell proliferation and renewal rates were studied with H-thymidine in rapid and slow renewing cell populations of adult mice (36). In the mice lingual epithelium the rate of cell birth in the basal layer was more rapid in younger mice, which therefore led to a more rapid epithelial renewal time. The transcriptional activity of taste bud cells, which is expressed as the AgNOR number, didn't significantly change and was preserved with age in the present study. Taste bud cells maintained the transcriptional activity during aging, unlike lingual epithelial cells.

Taste bud cells could be closely related to the peripheral nerve. If the peripheral nerve is cut, taste bud cells degenerate and eventually disappear (37). Since taste bud cells are known to develop from the surrounding epithelial cells in response to specific gustatory nerve contact (38), it is conceivable that the differentiation of taste bud cells from surrounding epithelial cells is induced by neurotrophic messengers. The activity of
taste buds innervated by glossopharyngeal nerves may be maintained (39). The atrophy and depression of the peripheral nerves penetrating the lingual papillae might result in the atrophy of taste buds. Age-related changes in human taste buds, especially both hypertrophy and the transcriptional activity of taste bud cells, might serve to compensate for a depression of the taste system with aging. It was concluded that aging was an important factor in the consideration of the histomorphometric changes in taste buds.

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