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Quantitative study of taste buds in fungiform and circumvallate papillae of young and aged rats*

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INTRODUCTION

There are few studies on taste buds in aged animals and results are contradictory. Taste bud loss is reported to occur in circumvallate papillae of aged human and murine tongues (Arey, Tremaine & Monzingo, 1935; Conger & Wells, 1969; Mochizuki, 1937). Recently, however, Arvidson (1979) found no change in taste bud numbers in human fungiform papillae throughout life, from birth to old age.

It is important to determine whether receptor loss does occur in old mammals, since it is frequently suggested that reports of taste disturbances in elderly people logically relate to a decrease in taste bud number (Cohen & Gitman, 1959; Cooper, Bilash & Zubek, 1959; Schiffman, Hornack & Reilly, 1979). Therefore, a quantitative study of taste buds in fungiform and circumvallate papillae in young and old rats has been made.

MATERIAL AND METHODS

Animals

Tongues were obtained from 12 Wistar-derived rats aged 5–7 months (6 males, 6 females) and 12 rats aged 23–24 months (6 males, 6 females) from the Gerontology Research Center, National Institute on Aging, Baltimore. The animals in this colony were reared and maintained under barrier husbandry conditions to exclude the introduction of major infectious disease agents, and have a mean lifespan (50% mortality) of about 24 months. They were provided with National Institutes of Health-Purina laboratory chow and water *ad libitum*, and a 12/12 hour light–dark cycle. The mature, 5–7 months animals are referred to in this study as 'young' rats and the 23–24 months animals as 'old' rats.

Animals were anaesthetised with ether and killed by cardiac puncture. Tongues were removed and fixed in 10% neutral buffered formalin for several days. Subsequently, the single, circumvallate papilla was dissected from the posterior part of the tongue. In eight tongues from each age group, one lateral half of the anterior part of the tongue, extending from the tip to the edge of the intermolar eminence, was dissected also. Miller & Preslar (1974) demonstrated that fungiform papillae are equally distributed on the two lateral halves of the rat tongue and that only a few

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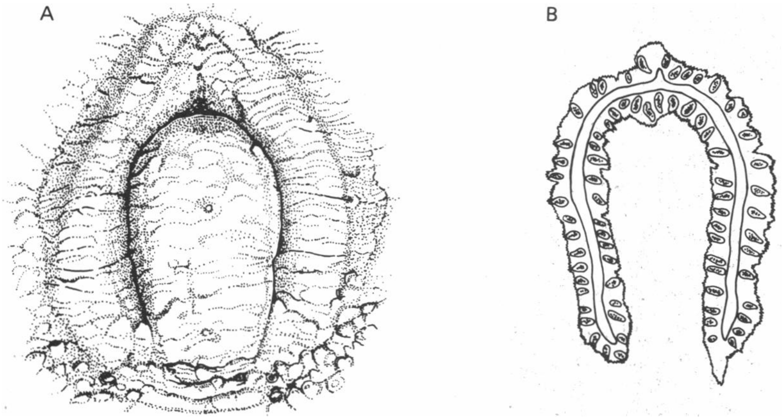

Fig. 1 (A–B). (A) Drawing of the single circumvallate papilla on the posterior area of the rat tongue. The anterior part of the tongue is oriented downwards in the drawing. The rat circumvallate papilla is distinctive because it is separated from surrounding tissue on the lateral and posterior boundaries only. (B) Drawing of taste buds in a horizontal, transverse section through the papilla. Taste buds in the papilla and trench walls were counted. Papilla width and length measurements were made of the papilla itself and did not include the trench wall.

papillae are located more posteriorly than the edge of the eminence. Therefore, tongue halves provided an adequate sample.

Circumvallate papillae and anterior tongue halves were dehydrated and embedded in paraffin. For the circumvallate papillae, serial sections 10 μm thick were cut in a plane parallel to the surface of the tongue, from the dorsal to the ventral surfaces. Anterior parts of the tongue were serially sectioned at a thickness of 10 μm in a coronal plane, perpendicular to the long axis of the tongue. All sections were stained with haematoxylin and eosin.

Taste bud quantification and papilla size

Circumvallate papilla

Figure 1A illustrates the single circumvallate papilla on the rat tongue. As described almost forty years ago by Fish, Malone & Richter (1944), the rat circumvallate papilla is distinctive because the papilla proper is separated from surrounding structures by a trench on three sides only, lateral and posterior. Every profile of a taste bud in each serial section of the circumvallate papilla and of the opposite trench wall was counted (Fig. 1B). Since papillae were sectioned transversely, from dorsal to ventral aspect of the tongue, this obviated the problem of cutting taste buds in the curved wall of the papilla at several angles, as can occur in coronal or cross section. Once a total count of all taste bud profiles was made for one papilla, the average number of sections occupied by a single taste bud was determined. Ten individual buds were selected and traced to learn whether they occupied three, four or five sections, for example. The mean number of sections for these ten buds was then divided into the total number of taste bud profiles. Therefore the formula used by Arey, Tremaine & Monzingo (1935) and by Bradley, Cheal & Kim (1980) for quantifying taste buds was applied:

$$\frac{\text{total number of taste bud profiles in all sections}}{\text{average number of profiles for ten single taste buds}} = \text{total number of taste buds per circumvallate papilla.}$$

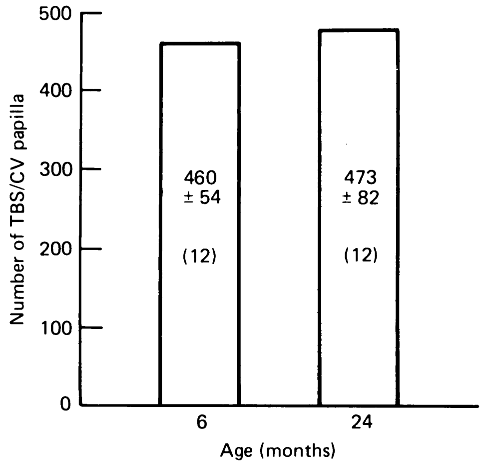

Fig. 2. Histograms of average number of taste buds (TBS) in the circumvallate (CV) papilla of young (6 months) and old (24 months) rats. The mean number of taste buds and standard deviation are noted within each bar, and the number of animals per age group is in parentheses. There is no difference in taste bud number between the two age groups.

Average taste bud size was calculated by multiplying the average number of sections occupied by one bud by 10 μm (the thickness of one section).

In an alternative method for quantifying taste buds, all taste *pores* in ten circumvallate papillae from each age group were counted. Using the two methods, the average difference in counts was 9·0 %; the pore count yielded smaller mean values for each group. No divergence in general conclusions resulted from the two different methods.

The length and width of the circumvallate papilla were measured from a projected image of the middle histological section of each papilla (Fig. 1 B). The depth was determined by counting the total number of sections through each papilla and multiplying by 10 μm.

Fungiform papillae

Every occurrence of a fungiform papilla profile on each sectioned tongue half was noted on a data sheet and the occurrence of a taste bud profile was marked beside the appropriate papilla section. Thus, all individual papillae were studied to determine whether each contained a taste bud. By summating the number of sections in which a papilla or taste bud appeared, papilla and taste bud size were obtained. These measurements were straightforward because, in general, few fungiform papillae appeared in any single tongue half section, and in the rat one papilla usually contained only one taste bud (Fish *et al.* 1944).

Data analysis

Measurements of taste bud number, taste bud size, and papilla size were compared between the two age groups using Student's *t* test to determine whether group means were similar.

RESULTS

Circumvallate papilla and taste buds

As illustrated in Figure 2, there was no difference in numbers of taste buds in circumvallate papillae from young and old Wistar-derived rats ($P > 0{\cdot}10$). Not only

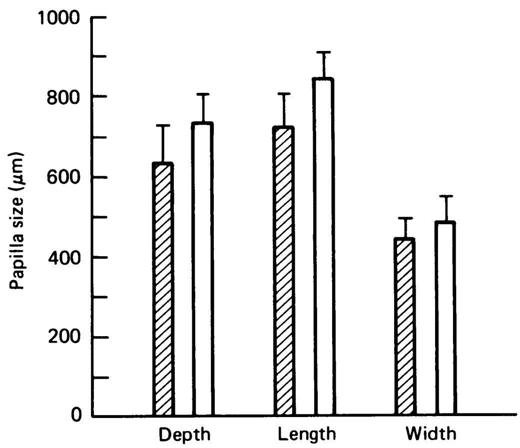

Fig. 3. Means and standard deviations for circumvallate papilla depth, length and width in young (hatched bars) and old (open bars) rats. Papillae in older rats were significantly deeper and longer, but not significantly wider.

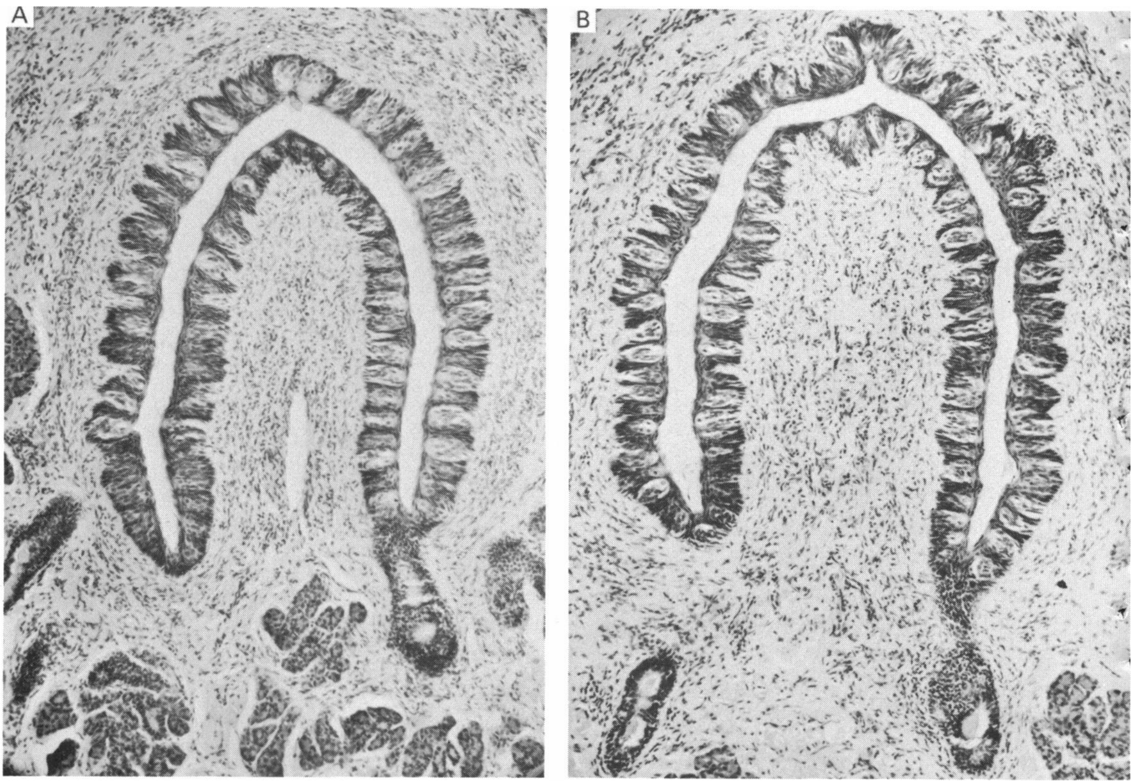

Fig. 4 (A–B). Photomicrographs of a transverse section through the circumvallate papilla of a young (A) and old (B) rat. General papilla morphology is similar in the two age groups, but old papillae are larger than young. Haematoxylin and eosin. × 100.

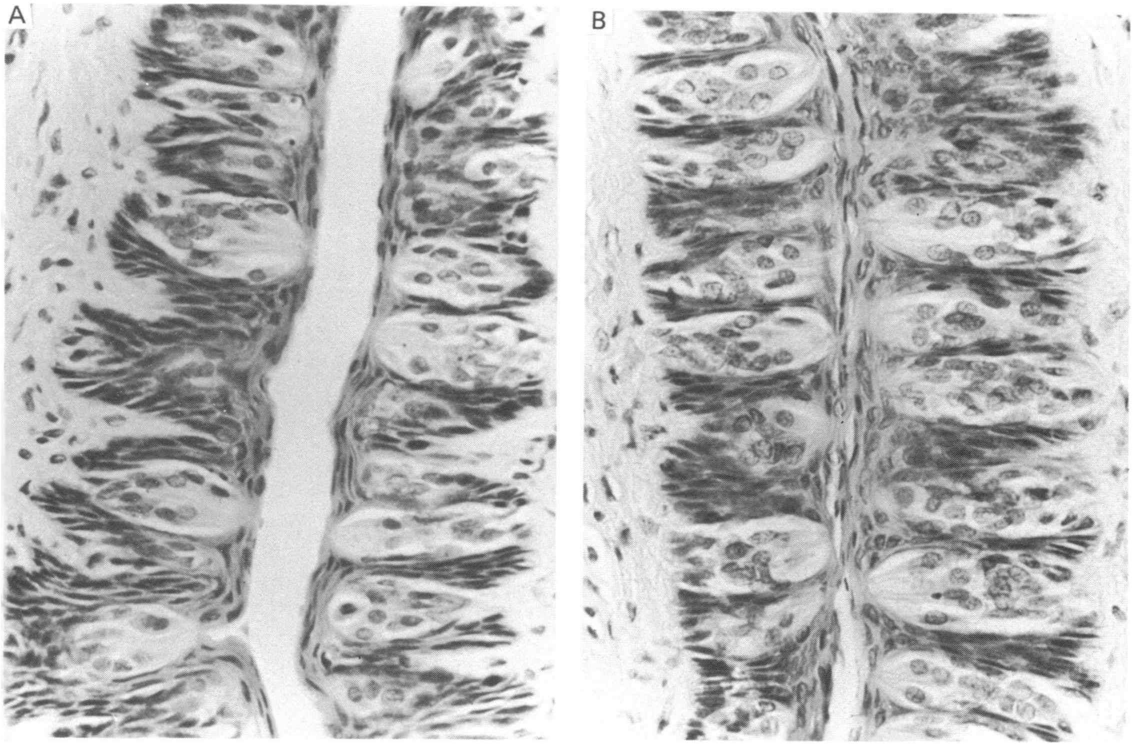

Fig. 5 (A–B). Photomicrographs of taste buds in the circumvallate papilla of a young (A) and old (B) rat. As seen with the light microscope, the taste buds are morphologically similar. × 350.

were average numbers of taste buds similar, but also the ranges overlapped (385–572 in young rats, 345–610 in aged rats). When taste buds were quantified by counting every taste pore, average values of 394 taste buds for young rats and 450 taste buds for old rats were obtained. There was no difference between the two age groups ($P > 0{\cdot}10$). Furthermore, within either group of animals, there was no sex difference in the number of taste buds per papilla ($P > 0{\cdot}10$).

The size of taste buds in both young and old rats averaged 46 μm. There was, however, a significant difference in the size of the circumvallate papilla. In old rats, papillae were deeper ($t = 3{\cdot}01$, D.F. $= 22$, $P < 0{\cdot}01$) and were longer ($t = 4{\cdot}12$, D.F. $= 22$, $P < 0{\cdot}001$) than in young rats (Fig. 3). Old papillae were not significantly different in width, however ($P > 0{\cdot}10$).

Therefore, although the number and size of taste buds in the circumvallate papilla did not differ with age, the papilla itself was larger in older than in younger rats. General papilla morphology was similar in young and old rats (Fig. 4). As seen with the light microscope, taste buds at the two ages were morphologically similar also (Fig. 5).

Fungiform papillae and taste buds

In tongues of young rats, an average of 99 % of all fungiform papillae contained taste buds, and in old tongues, 98 % contained taste buds (Fig. 6). A mean number of 116 papillae on young tongues and 113 on old tongues was examined, with an overall range of 101 to 127 papillae. There was no difference in the numbers of fungiform papillae without taste buds in young compared to old rat tongues

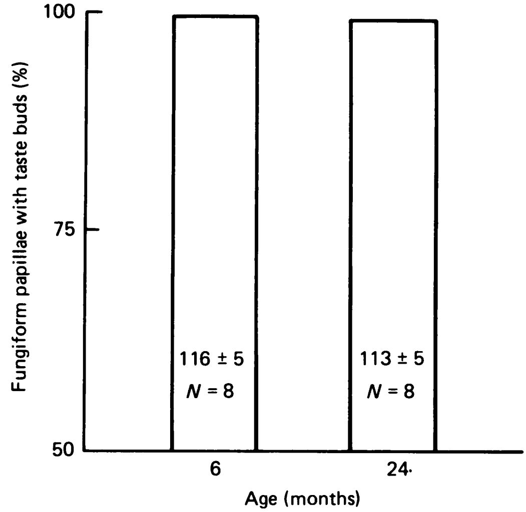

Fig. 6. Percentage of fungiform papillae that contain taste buds in young (6 months) and old (24 months) rats. The means and standard deviations for actual number of papillae examined are within each bar and the number of animals in each age group is also noted. There is no difference in the number of fungiform papillae that contain taste buds between the two age groups.

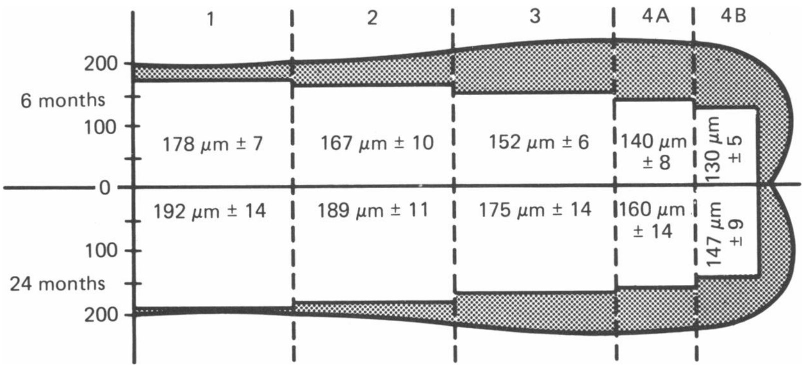

Fig. 7. Fungiform papilla size for four tongue quarters in young (6 months) and old (24 months) rats. Means and standard deviations are noted in the tongue diagram. For every quarter, papillae are significantly larger in old rats. Quarter 1: $t=2{\cdot}47$, $P<0{\cdot}05$; 2: $t=3{\cdot}95$, $P<0{\cdot}01$; 3: $t=3{\cdot}97$, $P<0{\cdot}01$; 4A: $t=3{\cdot}29$, $P<0{\cdot}01$; 4B: $t=4{\cdot}34$, $P<0{\cdot}001$; for all quarters: D.F. = 14.

($P>0{\cdot}10$), nor were large numbers of taste buds deficient in an individual tongue in either group. Three young and three old tongues had a taste bud in every papilla. Taste bud sizes were similar, also, averaging 42 μm in young and 44 μm in old animals.

Anterior parts of the tongue were longer in old ($13{\cdot}4 \pm 1{\cdot}4$ mm) than in young animals ($12{\cdot}4 \pm 0{\cdot}6$ mm) ($t=1{\cdot}82$, D.F. = 14, $P<0{\cdot}10$). Fungiform papillae were larger, also, in rats aged 24 months. To quantify papilla size, each tongue was divided into successive quarters 1 to 4 from the intermolar eminence to the tip (Fig. 7). Division into quarters was necessary because in an individual rat, papilla size increased from anterior to posterior. In addition, the most anterior quarter was divided into halves (4A and 4B), because papilla density was greatest in this quarter

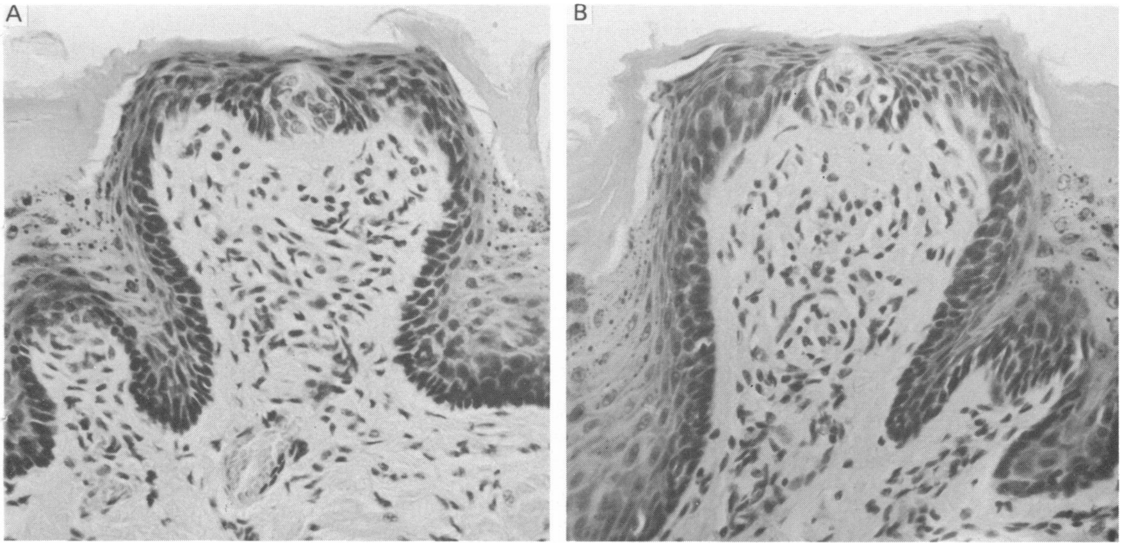

Fig. 8 (A–B). Photomicrographs of a fungiform papilla ana taste bud from a young (A) and old (B) rat tongue. As seen with the light microscope, the papillae and taste buds are morphologically similar. × 200.

(Miller & Preslar, 1974) and papillae nearest the curved tongue tip were often sectioned at various angles, making measurements difficult.

Average fungiform papilla size within each tongue quarter was larger in old compared with young rats (Fig. 7). Other than change in size, no differences in papilla or taste bud morphology were apparent with the light microscope (Fig. 8).

DISCUSSION

In Wistar-derived rats aged 6 and 24 months, there are no differences in numbers of taste buds in the circumvallate papilla on the posterior part of the tongue or in fungiform papillae on the anterior part of the tongue. However, both fungiform and circumvallate papillae are larger in older rats. The average taste bud number in circumvallate papillae in this study compares closely with data from Guth (1957) who reported an average of 375 taste buds per papilla in adult Osborne–Mendel rats. Numbers of fungiform papillae are similar to published data also (Fish *et al.* 1944; Miller & Preslar, 1974). Fungiform papillae do not decrease in number with age, as seen either in the present study or in that of Fish *et al.* (1944) who evaluated tongues from 103 rats aged 8 hours to 466 days.

The observation that the number of taste buds in the circumvallate papilla is not decreased in old rats contrasts with some previous studies of taste bud number in relation to age in other species. Three major factors are probably related to the discrepant results: the extent of tissue that is examined; the degree of pathological change in aged specimens; and whether statistics are applied in data analysis.

In the only other published study of taste bud number in aged rodents (Conger & Wells, 1969), a 30% decrease in total taste buds in the circumvallate papilla was reported in mice over the age range 6 to 27 months. Stained taste pores were counted in alternate sections of the 15 to 24 middle histological sections from papillae that had been cut coronally (cross section). Thus, a relatively small number of sections was actually used for analysis. The authors, multiplying the two quotients buds/section and sections/papilla, stated that "since the papilla size changed but little with dose or age, the simple measurement of buds per section is substantially proportional to total buds per papilla" (Conger & Wells, 1969, p. 33). However no statistical analysis was applied and an examination of data in their Figure 2 indicates that papillae are actually larger in older animals. If taste buds were less densely distributed in larger papillae in older animals, Conger & Wells would have counted fewer taste buds in the alternate, middle sections. Counts of all taste buds in serial sections should be made for accurate quantification and since inter-animal variation can be high, results must be analysed statistically.

In aged *human* circumvallate papillae it has been reported that taste bud number decreases by about 50 % after 74 years (Arey *et al.* 1935) or after about 60 years of age (Mochizuki, 1937). Although appropriate methods for counting all taste buds in serial sections were applied in both these studies, there was no statistical analysis. Furthermore, autopsy material from individuals with various diseases was used, although Arey *et al.* (1935) carefully excluded tongues and papillae that appeared 'highly pathological' from their study. It is interesting that the human circumvallate papillae least likely to exhibit age-related changes are those most centrally located on the tongue (Arey *et al.* 1935). The rat tongue contains only one circumvallate papilla which is centrally located.

In human foliate papillae, Mochizuki (1939) finds average numbers of 108 taste buds per papilla in juveniles (birth to 20 years), 136 in mature individuals (21–60 years), and 109 in elderly cases (61–90 years). He therefore reports a decrease in taste buds in old age. Clearly, there is no difference in taste bud number between the juvenile and elderly specimens, and if the difference between mature and elderly is statistically significant, it is very small in actual number of taste buds.

Arvidson (1979) has studied fungiform papillae in human tongues from individuals aged 2 days to 90 years, obtained at autopsy 5–12 hours after death caused by road traffic accident or sudden cardiac infarction. No significant differences are found in mean numbers of taste buds per papilla as a function of age, complementing the present results on taste buds in rat fungiform papillae. Although human fungiform papillae contain 0–27 taste buds each and rat papillae usually contain one, in neither species are taste buds reduced in old age.

Even in those studies reporting decreases, large numbers of buds remain in papillae in old age (Arey *et al.* 1935; Conger & Wells, 1969; Mochizuki, 1937). Since a major loss of taste buds (up to 85 %) from anterior and posterior areas of the rat tongue does not alter taste preference behaviour radically (Pfaffmann, 1952), it is unlikely that human taste behaviour is disturbed substantially due to the reported extent of taste bud loss in old age.

Since taste bud cells are replaced every 10 days on average in adult rats (Beidler & Smallman, 1965), an age-related loss of taste buds should not be predicted necessarily. However, olfactory neurons also turn over in adult rodents (Graziadei & Graziadei, 1978) and there is a reported decrease in these receptors in aged rats (Hinds & McNelly, 1981). Other sensory receptors, including inner and outer cochlear hair cells, photoreceptor cells, and Meissner touch corpuscles, have been reported to decrease in old age in various species (Han & Coons, 1979).

The present results may indicate a specificity in sensory receptor alterations during ageing. Alternatively, it might be suggested that results from additional age groups could indicate a more complex situation. For example, studies of rats even older than 24 months might reveal some taste bud loss, although it is emphasised that 24 months is the mean life span for the colony of rats investigated and, by this age, significant changes occur in various characteristics of the submandibular gland (Kuyatt & Baum, 1981).

Although taste buds are apparently not reduced in rats aged 24 months, membrane changes in the receptors might occur which could alter peripheral gustatory responses. During early postnatal development in rats, at an age when taste bud numbers are not changing (14–30 days), neurophysiological responses from peripheral taste nerves alter substantially (Ferrell, Mistretta & Bradley, 1981; Hill, Mistretta & Bradley, 1982). Presumably these changes relate to maturation of receptor membranes.

In conclusion, for Wistar-derived rats aged 6–24 months, roughly comparable to a human age range of 25–70 years, there is no loss of taste buds from anterior or posterior areas of the tongue. Such data support an emerging body of evidence that suggests only minimal age-related changes in the sense of taste in man (Baum, 1981; Dye & Koziatek, 1981; Grzegorczyk, Jones & Mistretta, 1979; Moore, Nielsen & Mistretta, 1982; Murphy, 1979; Weiffenbach, Baum & Burghauser, 1982).

SUMMARY

To ascertain whether an age-related decrease in number of taste buds occurs in the tongue of aged rats, taste buds were counted in fungiform and circumvallate papillae of Wistar-derived rats aged 5–7 months and 23–24 months. There was no difference in number or size of taste buds in papillae in anterior and posterior areas of the tongue from the two age groups. However, both fungiform and circumvallate papillae were larger in old rats. These results complement a recent study demonstrating no difference in numbers of taste buds in human fungiform papillae from birth to old age (Arvidson, 1979). Both anatomical investigations and human taste threshold studies indicate that age-related differences in the gustatory system are not as substantial as investigators have suggested in the past.

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