Keratins as markers of differentiated taste cells of the rat

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Abstract. Cytokeratins in taste buds were immunocytochemically evaluated with monoclonal antibodies. In each of six different epithelial sites in the rat oral cavity, intragemmal cells of taste buds were immunoreactive for keratin polypeptides 8, 18, and 19, as well as for keratin 7, which has not been previously reported in taste buds. Keratin-18-like immunoreactivity was present in fewer than half of the intragemmal cells, whereas all intragemmal cells were immunopositive for keratins 7, 8, and 19. Apart from some salivary duct cells, no other cells in the tongue were immunoreactive for any of these four keratins. Morphological and immunocytochemical profiles indicate that taste buds are islets of simple epithelium embedded in an expanse of stratified squamous epithelium. These simple epithelial cells and their keratins are nerve-dependent, since denervation eliminated all four keratins and replaced elongated taste cells of the vallate papilla with stratified squamous epithelium. We conclude that antibodies against keratins 7, 8, or 19 are useful markers for intragemmal cells in studies of taste bud development, degeneration, regeneration, turnover and tissue culture.

Introduction

The diversity among cytokeratins is useful for monitoring the progression of epithelial cell differentiation [6, 12, 17, 19, 20, 29, 38]. At least 20 cytokeratin polypeptides are expressed in epithelial cells [3, 14, 29] and more than 100 keratin antibodies are available for studying epithelial cell differentiation [13].

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Each epithelial cell expresses a small subset of keratins. Typically these keratin subsets are present in coordinate basic/acidic pairs; two strands of a basic keratin (keratins 1–8) are associated with two strands of an acidic keratin (keratins 10–20) [3, 13, 42]. Exceptions to basic/acidic pairing include the lack of a standard basic partner for keratin 19, and in some simple epithelia the apparent affiliations of three keratins, like keratins 8/18/19 or 7/8/18 [3].

Epithelia can be classified as simple (one layer) or stratified (multiple layers). In the oral cavity, one finds stratified epithelia that are unkeratinized (keratins are present but there is no stratum corneum; e.g., buccal mucosa, epiglottis, and vallate trenches), parakeratinized (the stratum corneum has nuclei; e.g., the surface of fungiform and vallate papillae and the medial wall of the nasopalatine canal in rat [18]), or orthokeratinized (the stratum corneum lacks nuclei; e.g., hard palate, filiform papillae). Taste buds in the rat oral cavity appear in six sites: four that are essentially unkeratinized (foliate and vallate papillae of the tongue, epiglottis and the soft palate) and two that are parakeratinized (fungiform papillae and the nasopalatine canal).

Simple epithelia contain some combination of keratins 7, 8, 18, and 19 [14, 17, 19, 32]. Of these, keratins 8, 18, and 19 have been associated with taste buds [1, 12, 15, 22, 23, 26, 27, 33, 39, 40, 45]. Since the cells in taste buds form a single layer extending from the basal lamina to the taste pore at the surface, taste buds meet the formal morphological definition of simple epithelia. Based upon taste bud morphology and immunocytochemistry, we and others have suggested that taste buds are simple epithelia [27, 32, 40]. Since keratin 7 is found in many simple epithelia [14, 17, 30], we sought evidence for the presence of keratin 7 in taste cells. This report describes keratin 7-, 8-, 18- and 19-like immunoreactivity in salivary duct cells and the fusiform cells of taste buds (intragemmal cells), and the absence of immunoreactivity for these four keratins in perigemmal cells on the lateral margin of taste buds and in basal cells.

Table 1 Monoclonal antibodies tested on taste buds. Reactions ranged from negative (−) to strongly positive (++). Conc., concentration

Keratin specificity	Conc. range	Antibody	Source[a]	Rat taste bud reaction
1, 2, 10, 11	1:50–200	AE-2	Jansen	(−)
3	1:50–200	AE-5	ICN	(−)
4	1:200–400	6B10	Sigma	(−)
7	1:40–80	RCK105	Monosan	(++)
7	1:20–100	Q3CK7	Amersham	(+) (+/− human)
7	1:50–200	LDS68	Sigma	(+/−) (++ human)
8	1:200–400	LE41	Amersham	(++)
13	1:40–80	2D7	ICN	(−)
14	1:200–1000	CKB1	Sigma	(−)
13, 16	1:50–200	8.12	ICN	(−)
18	1:20–50	PKK3	Labsystems	(+)
18	1:20–100	LE65	Amersham	(++)
19	1:100–200	4.62	Sigma	(++)
19	1:200–400	170.2.14	BM	(++)
19	1:10–20	LP2K	Dr. E B Lane	(++)
20	1:10	20.5	ARP	(−) (++ human)
20	1:1 supern.	20.10	ARP	(++)

[a] Source addresses:
ARP, American Research Products, Belmont, MA 02178, USA; Amersham, Amersham Corporation, Arlington Heights, IL 60005, USA; BM, Boehringer Mannheim Biochemicals, Indianapolis, IN 46250 USA; ICN, ImmunoBiologicals, Lisle, IL 60532, USA; Jansen, Accurate Chemical and Scientific Corp., Westbury, NY 11590, USA; Labsystems, Lab Products International Ltd., Raleigh, NC 27651, USA; Monosan, 5400 AM Uden, The Netherlands; Sigma, Sigma Chemical Company, St Louis, MO 63178, USA; Dr. E. B. Lane, University of Dundee, UK
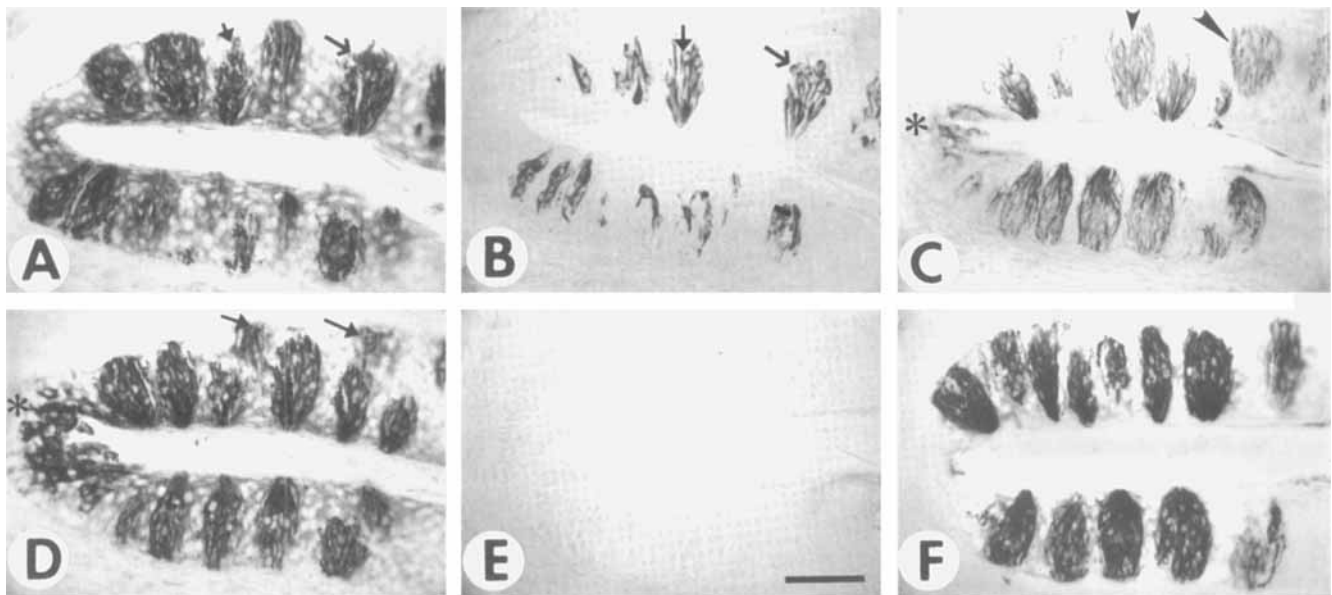

Fig. 1A–F Keratin-like immunoreactivity (IR) in rat vallate taste buds. A–D are serial sections; *arrows* and *arrowheads* indicate two taste buds that appear in all four sections. A Keratin-8-like IR, MAb *LE41*. B Keratin-18-like IR, MAb *LE65*. C Keratin-7- like IR, MAb *Q3CK7*. The *asterisk* in C and D indicates immunoreactive salivary duct cells. D Keratin-19-like IR, MAb *170.2.14*. E Control, no primary antibody. F Keratin-7-like IR, MAb *RCK105*. The *scale bar* in E is 60 μm for A–F

Methods

Tissue preparation. Sprague-Dawley albino rats (Harlan Sprague Dawley, Indianapolis, IN) were kept on a 12-h light:12-h dark cycle and housed with *ad libitum* food and water, as approved by the University of Michigan committee on animal care. Five rats were anesthetized with an i.m. injection of 170 mg ketamine and 7 mg xylazine per kg b.w. and the vallate papilla denervated by bilaterally avulsing the IXth nerve. All vallate taste buds degenerated and disappeared during the 15- to 21-day survival period. To collect normal and denervated tongues, all rats were deeply anesthetized with an i.p. injection of sodium pentobarbital (70 mg/kg b.w.). The anesthetized animals were perfused intracardially with either TRIS-buffered mammalian Ringer's solution [25] or 0.9%
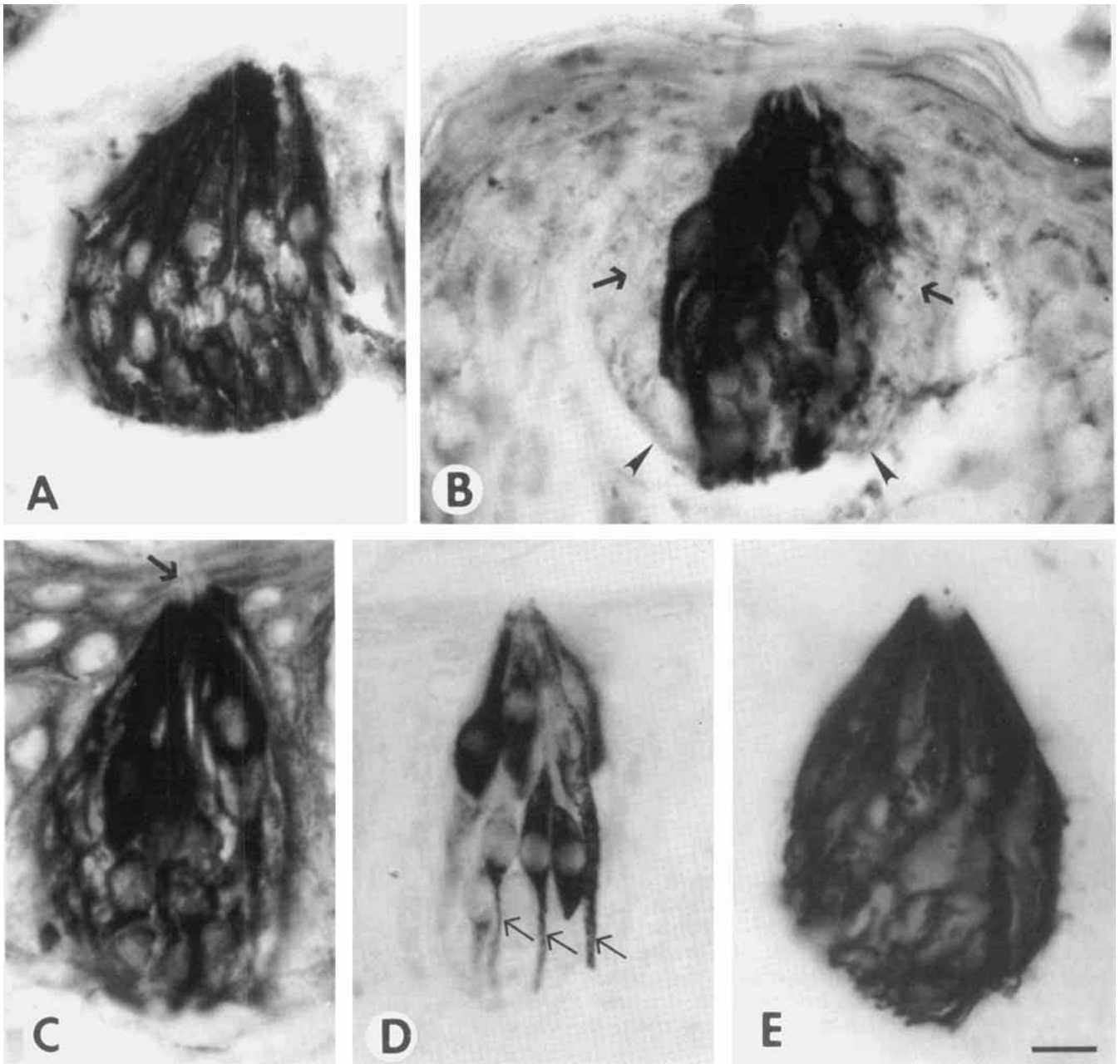
Fig. 2A–E Keratin-like IR in longitudinal sections of individual lingual taste buds. **A** Keratin-7-like IR in a vallate taste bud, MAb *RCK105*. **B** Keratin-7-like IR in a fungiform taste bud, MAb *Q3CK7*. *Arrows* indicate unreactive perigemmal cells. *Arrowheads* indicate unreactive putative basal cells. **C** Keratin-8-like IR in a foliate taste bud; MAb *LE41*. The *arrow* indicates the taste pore. High antibody concentrations were used to darken the surrounding tissue in order to demonstrate the location of perigemmal and basal cells in **B** (1:10 dilution) and to demonstrate the taste pit in **C** (1:50 dilution). **D** Keratin-18-like IR in a foliate taste bud, MAb *LE65*. *Arrows* indicate three of several fusiform intragemmal cells. **E** Keratin-19-like IR in a foliate taste bud, MAb *4.62*. The *scale bar* in **E** is 10 μm for **A** and **D** and 9 μm for **B, C,** and **E**

NaCl solution containing 0.02% sodium heparin and 0.5% procaine hydrochloride, followed by acid alcohol fixative (70% ethanol, 10% acetic acid).

Tongues and other tissues of interest were excised and immersed in acid alcohol fixative for at least 1 h, and then cryoprotected [2] and mounted for frozen sectioning. Ten-micrometer-thick cryosections were cut at −25° C, mounted on gelatin-coated slides, and stored at −20° C. Frozen human tongue vallate and fungiform tissue was supplied by Inglis J. Miller, Jr., Bowman Gray School of Medicine, Winston-Salem, NC, USA.

Immunohistochemistry. Primary monoclonal antibodies (MAbs), their keratin specificities, concentrations, and commercial sources are shown in Table 1. Three primary MAbs were specific for keratin 7: *Q3CK7* [14, 41], *RCK105* [30], and *LDS68*, which was reactive only with human tissues [35]. Three MAbs were specific for keratin 19: *LP2K* [37], *170.2.14* [14, 37], and *4.62* [7]. *MAb 4.62* cross-reacts with bovine desmosomes [7] but did not react with rat desmosomes. Additional MAbs reactive with taste buds included MAb *LE41* to keratin 8 [12]; MAbs *LE65* and *PKK3* to keratin 18 [12, 44], and MAbs *20.5* and *20.10* to keratin 20 [4,15]. Other pri-
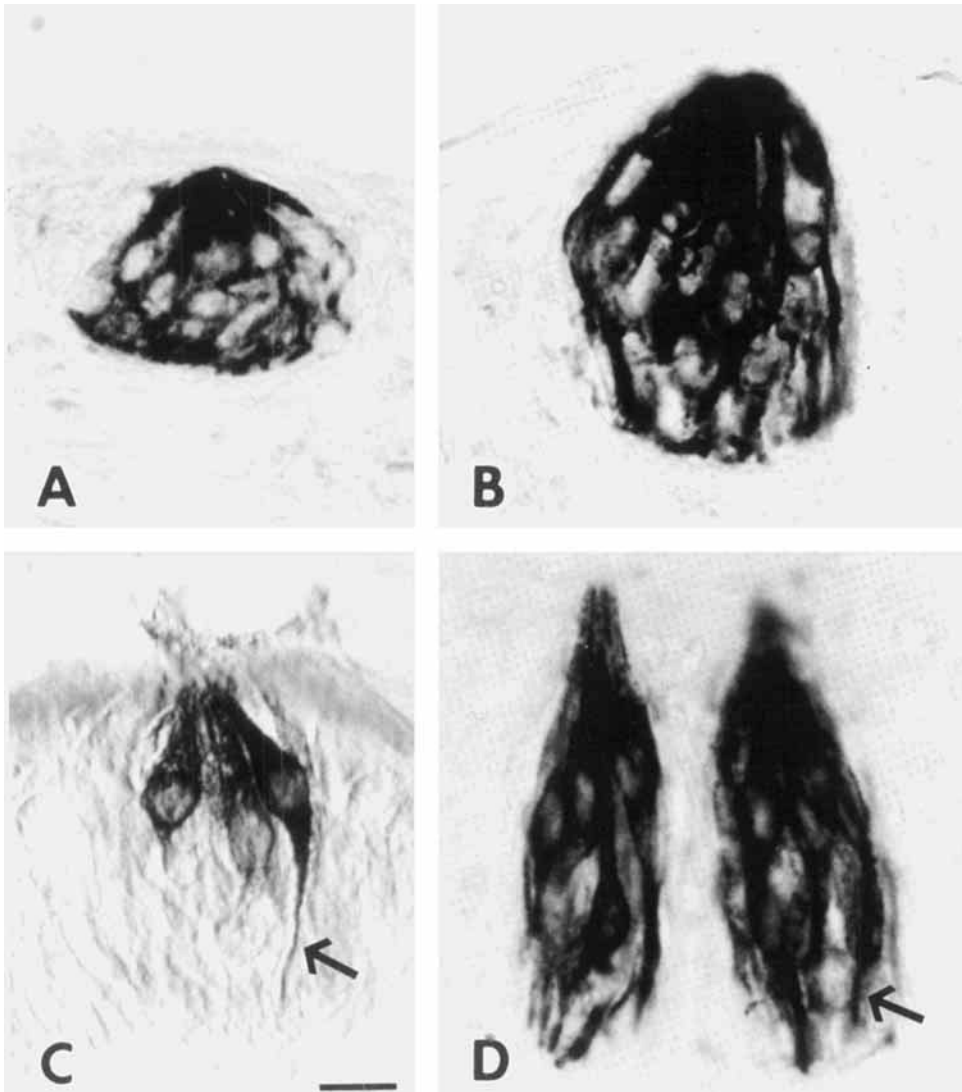
Fig. 3A–D Keratin-like IR in longitudinal sections of individual non-lingual taste buds. MAb *RCK105* reveals keratin-7-like IR in an epiglottal taste bud (**A**) and a taste bud of the nasopalatine papilla (**B**). **C** Keratin-18-like IR in a subset of cells in a taste bud of the nasopalatine papilla, MAb *LE65*. **D** Keratin-8-like IR in elongated cells of two palatal taste buds, MAb *LE41*. *Arrows* in **C** and **D** indicate the narrow basal processes of fusiform cells; note the absence of immunopositive basal cells. The *scale bar* in **C** is 10 μm for **A–D**

mary MAbs used include: MAb *AE–5*, specific to keratin 3 [34]; MAb *AE–2* to keratins 1, 2, 10, 11 [46]; MAb *6B10* to keratin 4 [43]; MAb *2D7* to keratin 13 [43]; MAb *Ks 8.12* to keratins 13,16 [9]; and MAb *CKB1* to keratin 14 [28].

Immunohistochemistry was carried out using either indirect immunofluorescence or an avidin-biotin peroxidase (ABC) method (Vector Labs). For the ABC-peroxidase method, mounted tissue sections were hydrated in four 5-min washes of 0.1 M phosphate-buffered saline (PBS), pH 7.4, containing 0.4% Triton-X 100 (TX-100, Sigma). The slides were then incubated for 30-min with 3% normal goat serum (Cappel, Organon Teknika) in PBS/TX-100, followed by a 1-h incubation with the primary antibody at room temperature. The slides were washed with PBS four times for 5-min each, followed by a 45-min application of the secondary antibody, biotin-conjugated goat anti-mouse IgG preadsorbed with rat serum proteins (B-8774, Sigma). Three additional washes in PBS preceded both the 30-min application of Vectastain avidin-biotin complex (PK-4000 kit, Vector Laboratories, Burlingame, CA) and the 5- to 10-min incubation with a PBS solution containing 0.5 mg/ml 3,3'-diaminobenzidine (Sigma), 0.01% hydrogen peroxide and 0.04% $NiCl_2$ to tint the reaction product blue.

For indirect immunofluorescence, either a fluorescein-conjugated secondary antibody (F-8771, Sigma) was used, or a strepta-vidin-Texas Red conjugate (95405A, Gibco BRL) was applied following incubation with the biotinylated secondary antibody.

Hoechst 33258 (100 μM in PBS, Sigma) was used as a fluorescent nuclear counterstain. Little or no anti-keratin staining was observed in rat tongue fixed with 4% paraformaldehyde or when the primary antibody was replaced with an inappropriate antibody, e.g., *AE–5* (anti-cytokeratin 3, ICN).

Results

Rat vallate taste buds were immunoreactive with monoclonal antibodies (MAbs) against keratins 7, 8, 18, and 19. Serial sections indicated a given taste bud was immunoreactive for all four keratins. There was no staining when the primary antibody was omitted (Fig. 1).

Immunoreactivity of taste buds was examined at six locations within the rat's oral cavity: the epiglottis, soft palate, nasopalatine papilla, and the vallate, fungiform, and foliate papillae. At each of these six sites the intragemmal cells of taste buds were selectively immunopositive for keratins 7, 8, 18, and 19. (Figs. 2 and 3).

In preliminary experiments we found that vallate taste buds in the rat were immunopositive for keratin 20

(MAb *20.10*), and we also confirmed the observation of Moll et al. [15] that human taste buds have keratin 20-like immunoreactivity (MAb *20.5;* data not shown).

The MAbs reactive with rat intragemmal cells included two for keratin 7, one for keratin 8, two for keratin 18, three for keratin 19, and one for keratin 20. Six antibodies of varied keratin specificity failed to stain taste cells. See Table 1.
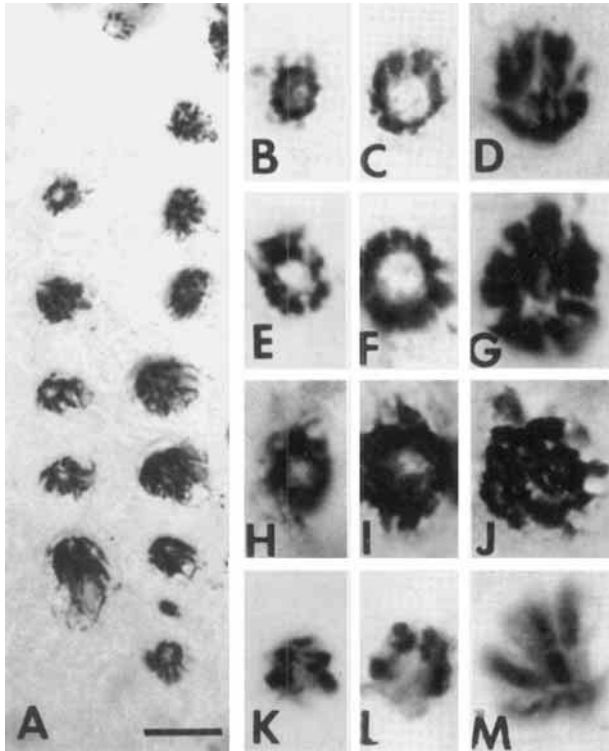

Fig. 4A–M Cross-sections of the apical tips of vallate taste buds immunostained for keratins 7, 19, 8, and 18. **A** Two rows of vallate taste buds immunoreactive for keratin 7, MAb *RCK105*. **B–M** show representative sections of 12 taste buds sectioned at a progressively deeper depths from left to right panels. **B–D** Keratin 7, MAb *RCK105*. **E–G** Keratin 19, MAb *4.62*. **H–J** Keratin 8, MAb *LE41*. **K–M** Keratin 18, MAb *LE65*. The *scale bar* in **A** is 23 μm for **A** and 8 μm for **B–M**

Based upon their staining intensity and specificity, the most effective antibodies for rat intragemmal cells were: *RCK105* for keratin 7, *LE41* for keratin 8, *LE65* for keratin 18, and *4.62* and *LP2K* for keratin 19. Staining was restricted to salivary duct cells (described below) and taste buds.

RCK105 was the most effective in identifying keratin 7-like immunoreactivity in rat taste buds. It was more effective than *Q3CK7* in staining foliate and vallate taste buds (Fig. 1C vs 1F). Keratin-7-like immunoreactivity was observed in human fungiform and vallate taste buds with MAb *LDS68*, which is selective for keratin 7 (data not shown).

In longitudinal sections, all intragemmal cells appeared to be immunoreactive for keratins 7, 8, and 19 (Figs. 2 and 3). In contrast, keratin-18-like immunoreactivity was present only in a subset of intragemmal cells (Figs. 2D and 3C). Higher concentrations of *LE65* did not increase the proportion of keratin 18 immunoreactive taste cells. Unlike MAb *LE65*, MAb *PKK3* was not exclusively reactive with salivary duct cells and a subset of intragemmal cells, for it also had some affinity for cells of the general lingual epithelium.

We utilized cross-sections of taste buds to examine intragemmal cells that extended to the taste pit. Vallate taste buds that lay in rows were evenly spaced (Fig. 4A). Taste buds appeared as immunostained dark rings surrounding the unstained taste pit, in cross-sections of the apex of the taste bud. It was characteristic of keratins 7, 8, and 19 that these rings of immunopositive cells were complete and unbroken, implying these three keratins were ubiquitous in the apical process of intragemmal cells (Fig. 4B–J). In contrast, with MAbs for keratin 18, there were partial rings of stained cells (Fig. 4L). When the plane of the cross-section was below the taste pit, the ring was replaced by a stained disk, solid and circular for keratins 7, 8, and 19, but irregular and non-circular for keratin 18 (Fig. 4D, G, J, M). In cross-sections of taste buds the keratin-18-positive cells occupied no consistent position within the taste bud (Fig. 4K–M). In contrast to the ubiquitous staining for keratins 7, 8, and 19, fewer than half of the cells of the intragemmal cells in longitudinal or cross-sections were immunopositive for keratin 18.

Fig. 5A–C A This fungiform papilla was stained with Hoechst nuclear dye. *Arrows* indicate immunonegative perigemmal cells. The *horizontal dashes* locate the apex and base of the taste bud visible in **B**. **B** Bright field illumination of the section in **A** reveals intragemmal cells immunopositive for keratin 8, MAb *LE41*. **C** In this section of a different fungiform papilla, the cytoplasm of basal cells and neighboring suprabasal cells is immunopositive for keratin 14, MAb *CKB1*, while intragemmal cells are unstained. The *scale bar* is 25 μm for **A, B** and 22 μm for **C**
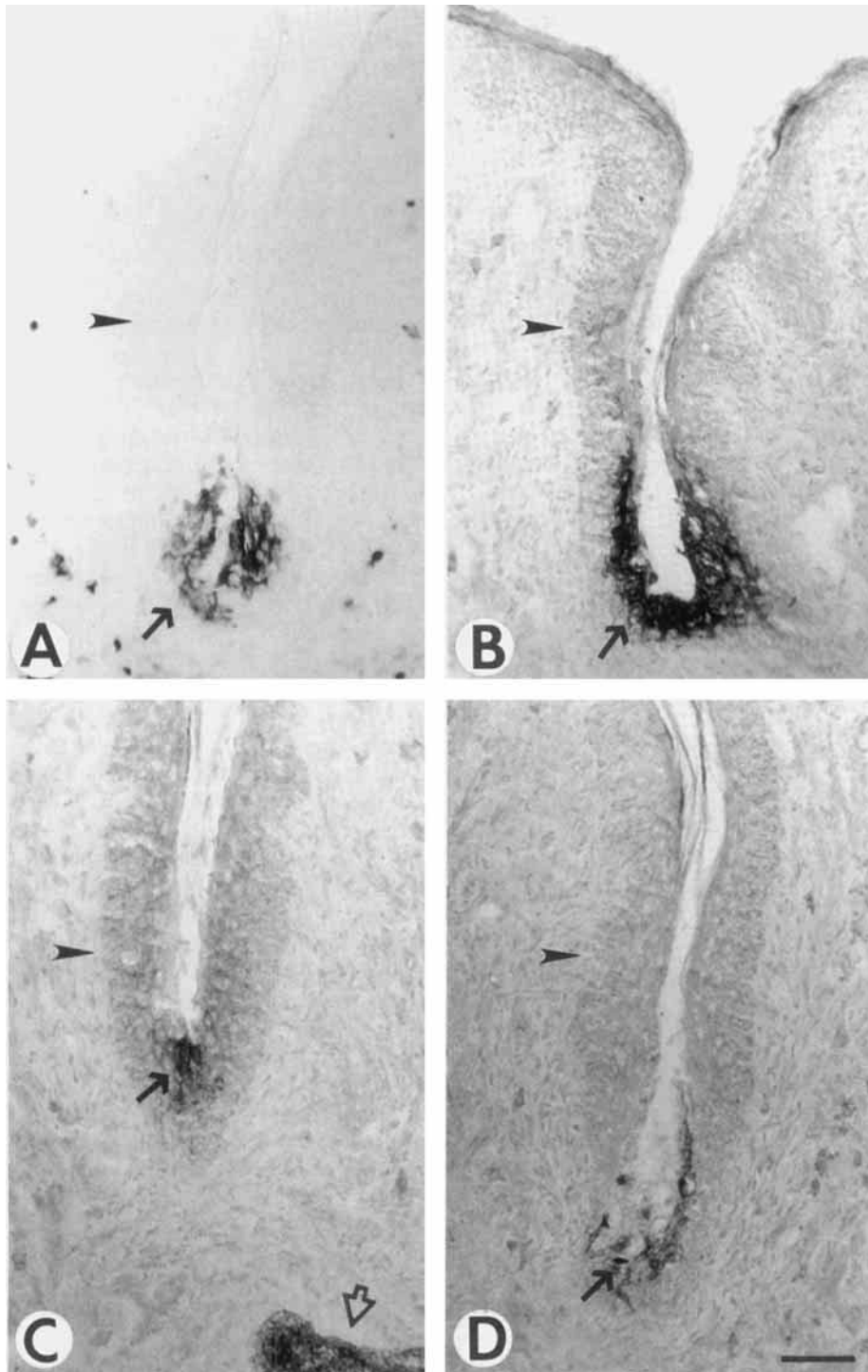
Fig. 6A–D Sections of denervated vallate trenches stained for keratin immunoreactivity. The gustatory epithelium has no immunoreactive taste cells. *Arrowheads*, gustatory epithelium; *arrows*, salivary cells. **A** Keratin 19, MAb *4.62*. **B** Keratin 7, MAb *Q3CK7*. **C** Keratin 8, MAb *LE41*. *Open arrow*, portion of a salivary duct wall. **D** Keratin 18, MAb *LE65*. The primary antibody concentrations were 1:200 for **A** and 1:100 for **B–D.** The *scale bar* in **D** is 50 μm for **A–D.** The *sections* in **B–D** were overstained to reveal the morphology of the trench and the boundary of the gustatory epithelium

The intragemmal cells were elongated, extending from the apex to the base of the taste bud. That the immunoreactive intragemmal cells always had a fusiform shape was most obvious with keratin 18 (Figs. 2D and 3C), but was also evident for keratins 7, 8 and 19 after use of low concentrations of primary antibodies (e.g., Fig. 3D) or fluorescently tagged second antibodies. In addition to intragemmal cells, we surveyed the basal and perigemmal cells near taste buds. Basal cells were defined as polygonal cells that abutted the basal lamina; they were keratin-14-positive (MAb *CKB1;* Fig. 5C). No polygonal cells were immunopositive for keratins 7, 8, 18, or 19. Perigemmal cells were located along the lateral margin of the taste bud. They were too short to reach both the taste bud's apex and base. Perigemmal cells of fungiform taste buds were unreactive for keratins 7, 8, 18, and 19 (Fig. 2B). Hoechst stained serial sections of fungiform papillae revealed cell nuclei, except where nu-
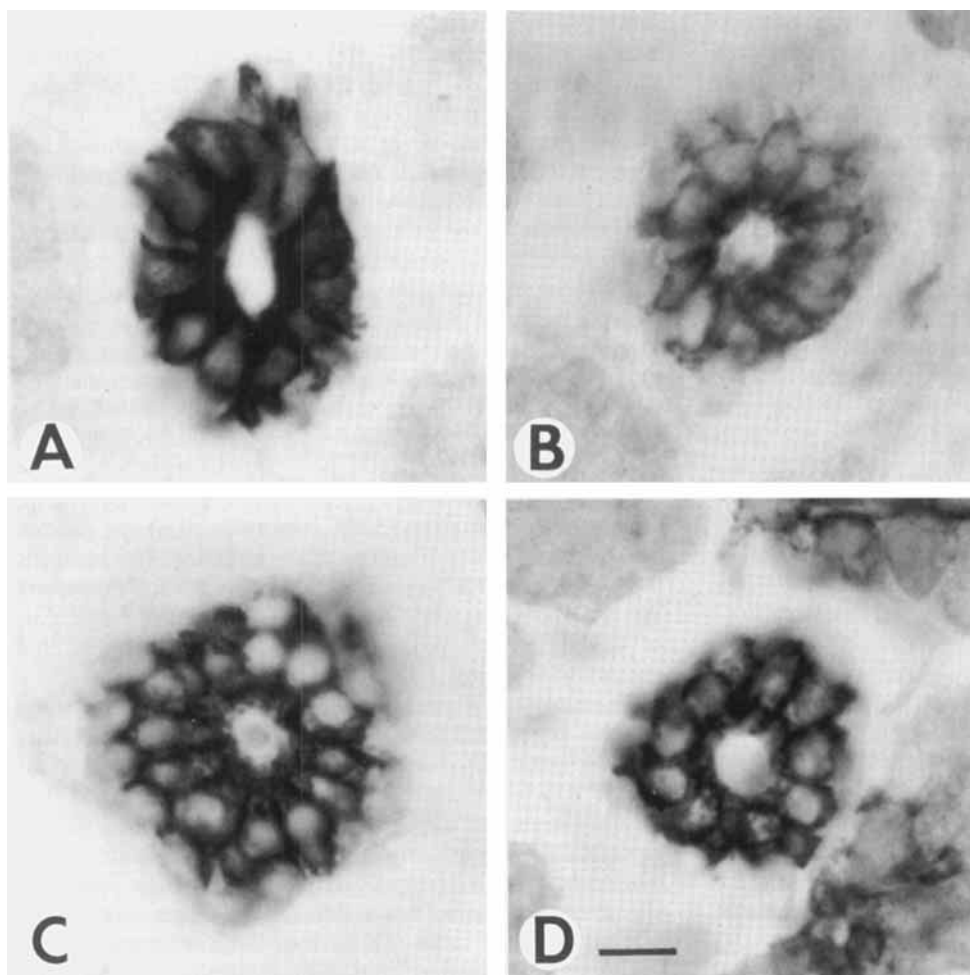
Fig. 7A–D Immunoreactivity of salivary ducts to: A Keratin 19, MAb *4.62*, B Keratin 7, MAb *Q3CK7*. C Keratin 8, MAb *LE41*. D Keratin 18, MAb *LE65*. The *scale bar* in D is 6 μm for A–D

clei of the intragemmal cells were obscured by the dense anti-keratin peroxidase product. This product did not quench the perigemmal cell nuclear fluorescence because these cells were keratin 7, 8, 18, and 19 immunonegative (Fig 5A, B). Basal cells did not react with antibodies against keratins 7, 8, 18, and 19. Compare the keratin-14-positive polygonal cells in Fig. 5C with the absence of keratin 8 or 18 immunoreactive basal cells in Figs. 2D, 3C, and 5B.

Denervated vallate papillae provided additional evidence that basal cells were immunonegative for keratins 7, 8, 18, and 19. All taste buds and all keratin 7, 8, 18, and 19 specific staining of taste cells were eliminated by denervation, while basal cells remained (see Discussion). The residual keratin 7, 8, 18, and 19 staining was limited to salivary duct cells, like those cells at the confluence of the salivary ducts and the vallate trench (arrows Fig. 6A–D).

Cross-sections of salivary ducts beneath both normal and denervated foliate and vallate papillae were immunopositive for keratins 7, 8, 18, and 19 (Fig. 7). Luminal cells were the most reactive. The staining patterns were generally similar for the four keratins, although in non-luminal cells keratin-8-like immunoreactivity was the most robust. All salivary glands were immunonega-

tive for keratins 7, 18, and 19. Serous glands were immunopositive for keratin 8.

Discussion

The histological appearence of taste buds meets the definition of a simple epithelium because taste buds consist of a one-cell-thick layer that extends from the basal lamina to the surface of the oral cavity. Intragemmal cells are immunoreactive for keratins 7, 8, 18, and 19 that are, in various combinations, characteristic of simple epithelia [14, 17, 19, 32]. Thus, taste buds can be considered islands of simple epithelium embedded in a sheet of stratified squamous epithelium.

The observation of keratin-7-like immunoreactivity in taste buds is novel. We observed that intragemmal cells at all six taste bud sites in the rat oral cavity were immunopositive with MAbs *RCK105* and *Q3CK7*, each specific for keratin 7. Ramaekers et al. [30] generated MAb *RCK105*, examined its reactivity with two-dimensional (2D) gels, and surveyed its histological pattern in human tissues. In 2D gels *RCK105* was specifically reactive to keratin 7; it did not cross-react with keratins 8, 18, or 19. In tissue sections *RCK105* reacted with simple

epithelia and various ducts including bile ducts, collecting ducts, mammary ducts, pancreatic ducts, prostate ducts, and sweat ducts. While we demonstrated that taste buds in human fungiform and vallate papillae were immunoreactive with the keratin-7-specific MAb *LDS68*, the possibility remained that the keratin 7 MAbs stained rat taste buds by cross-reacting with keratins 8, 18, or 19. However, the keratin 7 MAbs, *RCK105* and *Q3CK7*, did not recognize keratin 8 in serous glands. Selective recognition of just keratin 18 is ruled out because MAbs *RCK105* and *Q3CK7* stained all intragemmal cells, yet fewer than half were keratin-18-positive. It was shown earlier that some rat thymic cells are keratin-18-positive but keratin-7-negative [5]. Many cells in the rat inner ear [11] react with MAb *LP2K* (keratin 19) but not with *RCK105* (keratin 7). Moreover, during inner ear development keratin 7 is up- and down-regulated in temporal and spatial patterns distinct from those for keratin 19 [11]. This differential staining for keratin 7 vs. keratins 8, 18, and 19 in rat tissues indicates that MAb *RCK105* probably reacted with a keratin-7-like peptide in intragemmal cells of rat taste buds.

Intragemmal cells are not the only cells in vivo that have keratins 7, 8, 18, and 19. These four keratins are also present in type-II alveolar cells [10] and in salivary duct cells. Various combinations of two or three of these four keratins are found with epithelial cell populations in thymus, lung, and inner ear [5, 10, 11]. Among intragemmal cells more than half were immunopositive for keratins 7, 8, and 19, but not 18. A report has been submitted on the subset of intragemmal cells that are keratin-18-positive (Zhang et al.).

Unlike intragemmal cells, perigemmal cells and basal cells were negative for keratins 7, 8, 18, and 19. Perigemmal cells were identified by their lateral location and moderate elongation; basal cells were identified by their basilateral location, their polygonal shape, and their specific keratin-14-like immunoreactivity, (MAb *CKB1*). It is the prevailing view that, while intragemmal cells, and probably perigemmal cells, are nerve-dependent, some basal cells survive denervation, thereby allowing taste buds to be re-formed after nerve fibers reinnervate the gustatory epithelium [21, 24, 27, 31]. Hence, denervation of the vallate papilla provided additional evidence for the absence of keratin 7-, 8-, 18-, or 19-like immunoreactivity in the basal cells that are precursors of gustatory cells.

Taste buds exist in disparate environments. Those in the epiglottis lack protective cornification; fungiform taste buds reside in a more keratinized epithelium, while foliate and vallate taste buds are sheltered in trenches. In addition to varying degrees of physical protection, there is regional variation in the composition of saliva that bathes surfaces in the oral cavity [36]. Yet, the consistent staining pattern suggests that to a first approximation keratins 7, 8, 18, and 19 are similar across taste buds. That is, the keratin profile of taste buds was uncorrelated with the presence or absence of a trench, uncorrelated with the extent of keratinization in the surrounding tissue, and uncorrelated with local variation in saliva composition. Nonetheless, there must be subtle variation

among taste cells in different sites. For example, *Q3CK7* was as effective as *RCK105* with fungiform taste buds but was less effective with foliate and vallate taste buds.

Epithelial morphogenesis entails changes in cell shape. For example, in taste buds orderly clusters of elongated intragemmal cells arise from polygonal, immature taste cells [8]. Although keratins 7, 8, 18, and 19 are common in the elongated cells of simple epithelium, their presence alone does not guarantee a fusiform shape since these four keratins are also characteristic of the polygonal cells of salivary ducts (Fig. 5). Nor are these keratins necessary for cell elongation; perigemmal cells elongate without these keratins. Such observations suggest that while fusiform taste cells may be stabilized by keratin filaments, the filaments are neither necessary nor sufficient for the development of a fusiform shape. This agrees with the extant view that keratins maintain the integrity of epithelia, and help preserve cell shape created by actions of other filaments like actin [3]. The biologic utilities that favor keratin gene diversity are unknown. We speculate that keratins 7, 8, 18, and 19 are particularly suited for the polarized cells, like taste cells, that characterize simple epithelia.

In summary, thanks to their diversity, keratins remain the best set of differentiation markers for the oral epithelium [17, 20, 32]. The present study indicates that keratins 7, 8, and 19 are useful markers of specialized oral receptor cells, the intragemmal cells of taste buds. A survey of rat taste buds in six sites indicated that intragemmal cells characteristically contained keratins 7, 8, and 19, whereas keratin 18 was detected in fewer than half of the intragemmal cells. These four keratins were not detected in the stratified lingual epithelium or in the basal and perigemmal cells of taste buds.

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