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Scanning Electron Microscopical Studies of Developing Gustatory Papillae in Humans

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Abstract

Development and morphological changes of human gustatory papillae during postovulatory weeks 6–15 have been studied using scanning and transmission electron microscopy. The first papilla of the tongue appears around postovulatory week 6 in its caudal midline near the foramen caecum. In contrast, the dorsal epithelium of the anterior part of the tongue shows only small hillock- or papilla-like elevations from week 6 on, which comprise an aggregation of 5–20 epithelial cells. From week 7 on, most prominent fungiform papillae develop near the median sulcus and at the margins of the anterior part of the tongue. At their tops, the first primitive taste pores are found around week 10; these are often covered with processes of adjacent epithelial cells. Most pores, however, develop around weeks 14–15. The maturation of taste buds does not coincide with the appearance of taste pores, since taste bud cells are not fully differentiated in the observed period of time. Fungiform papillae are developed before filiform papillae, which do not occur within the first 15 weeks of gestation. Fungiform papillae tend to grow between weeks 8 and 15 of gestation, whereas the size of vallate papillae seems to be constant during this period. **Chem. Senses 22: 601–612, 1997.**

Introduction

In mammals, lingual taste buds occur in three different locations: on the anterior two-thirds of the tongue, taste buds are assembled on fungiform papillae and innervated by axons of the chorda tympani [a branch of the facial nerve, N.VII (Whitehead *et al.*, 1987)]. Most of the taste buds, however, are located on circumvallate papillae (Tomiyama, 1977; Miller and Bartoshuk, 1991) and bilateral foliate papillae. Their gustatory information is carried by the glossopharyngeal nerve (N.IX) to the nucleus of the solitary tract in the brain stem.

Although a subpopulation of taste buds occurs independently of papillary structures, e.g. palatine and epiglottal taste buds (Bradley *et al.*, 1980), the lingual taste buds of adult individuals are usually associated with dermal papillae. It has been shown in various mammals that taste bud formation at the anterior part of the tongue apparently requires a pre-formed papilla (monkey: Zahm and Munger, 1983; rat: Farbman and Mbiene, 1991; Whitehead and Kachele, 1994). Apart from studies concerned with the surface morphology of the human adult tongue (Yamasaki and Takahashi, 1982; Kullaa-Mikkonen and Sorvari, 1985; Myers *et al.*, 1995), there is only one comparable investigation from the first trimester of human development beginning at gestational week 8 (Hersch and Ganchrow,

1980). Therefore, we are particularly interested in the development of gustatory papillae in human embryonic and fetal stages, which have been already examined by transmission electron microscopy (Witt and Reutter, 1996). Although the appearance of gustatory papillae does not necessarily concord with the onset of taste bud formation in early stages, we wanted to compare circumvallate and fungiform papillae using scanning electron microscopy and accom- panying transmission electron microscopy. The particular issues of this study were to: (i) determine the time of the first appearance of papillae; (ii) describe the progression of development of papillae from postovulatory week 6 until postovulatory week 15; and (iii) determine the approximate time at which taste pores open.

One problem in developmental studies in man has been the accurate measurement of gestational age. In order to avoid such uncertainties and inaccuracies in cases in which precise data were not available due to fragmentation of the fetus, we used an independent measure of fetal age, namely the foot length according to Munsick (1984).

Materials and methods

Thirty-two human fetal tongues were obtained from legal and spontaneous abortions from week 6 to week 15 of gestation (Table 1), performed in the Department of Gynecology & Obstetrics of the Medical Academy at Dniepropetrovsk (Ukraine). The specimens were obtained in accordance with the regulations published in the 'Declaration of Helsinki' (1995).

Whole tongues were fixed with 0.1 M cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde for 6–48 h at 4°C. After washing with 0.13 M cacodylate buffer, the tongues were osmicated for 2 h with 1% OsO_4 in cacodylate buffer. Large tongues (postovulatory week 14) were median-sagittally cut. One half was processed for scanning electron microscopy (SEM), the other for transmission electron microscopy (TEM). For the SEM, the specimens were gradually dehydrated with ethanol, critical-point dried, mounted on aluminum stubs and sputtered with gold–palladium (8 nm). The tongues were examined with a Hitachi-S 800 SEM. For the accompanying TEM, small tongue pieces (1–3 mm^3) of the same range of development were prepared using a standard electron microscopical protocol (Witt and Reutter, 1996).

Results

Postovulatory weeks 6–7

The surface of the developing early tongue (week 6,

Table 1 Total number of tongues used for scanning electron microscopical studies on development of gustatory papillae and taste buds

Postovulatory week	6	7	8	9	10	11	12	13	14	15
Tongues (*n*)	3	6	3	4	6	3	2	–	3	2
Foot length (cm)[a]		0.48	0.57	0.71	0.89	1.15	1.40	1.66	2.09	2.41

[a]Data from Munsick (1984), which have been used in part to determine the gestational age of the fetus.

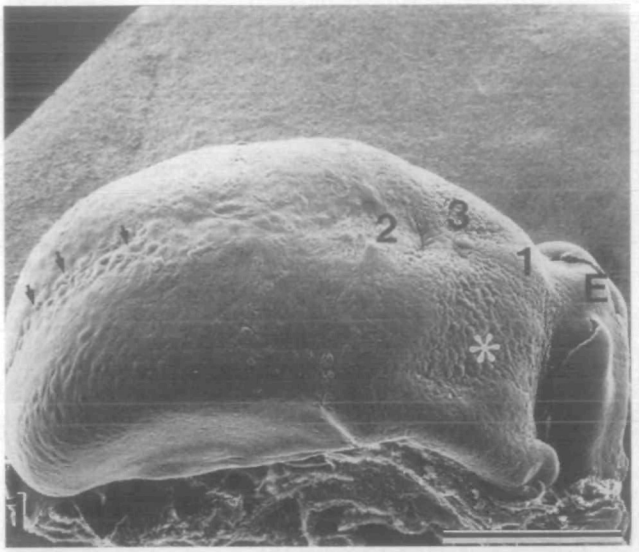
Figure 1 Postovulatory week 6. The surface of the tongue is almost flat, apart from smooth elevations along the midline (arrows) and the margins. Asterisk: root of the tongue. 1 = Hypobranchial eminence (copula of His), 2 = tuberculum impar, 3 = median circumvallate papilla, E = epiglottis. Bar = 500 μm.

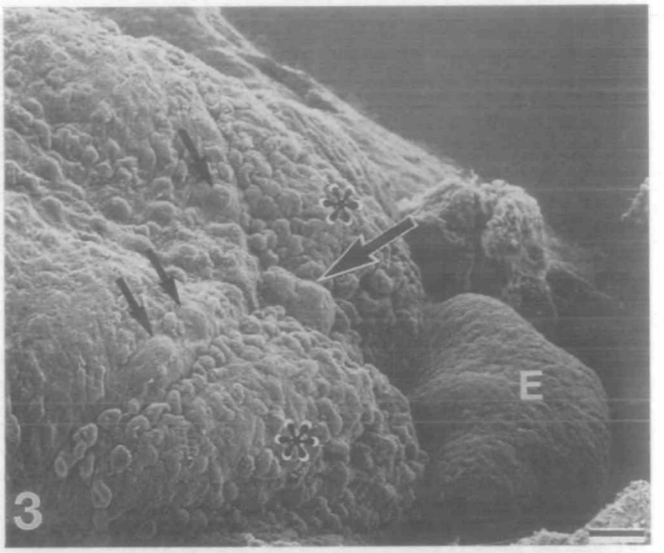
Figure 2 Week 7, dorsal view of the tongue. Mostly the anterior parts (above) and the margins are occupied by often irregular epithelial elevations, presumed early papillae. The sulcus terminalis delimits the root of the tongue from its body (white arrows). Immediately anterior to the *sulcus terminalis* a V-like lane represents a smooth surface for developing circumvallate papillae. These papillae (black arrows, see also Figure 3) are large in comparison to fungiform ones of the same age. Asterisk: remnant of the tuberculum impar. Bar = 500 μm.

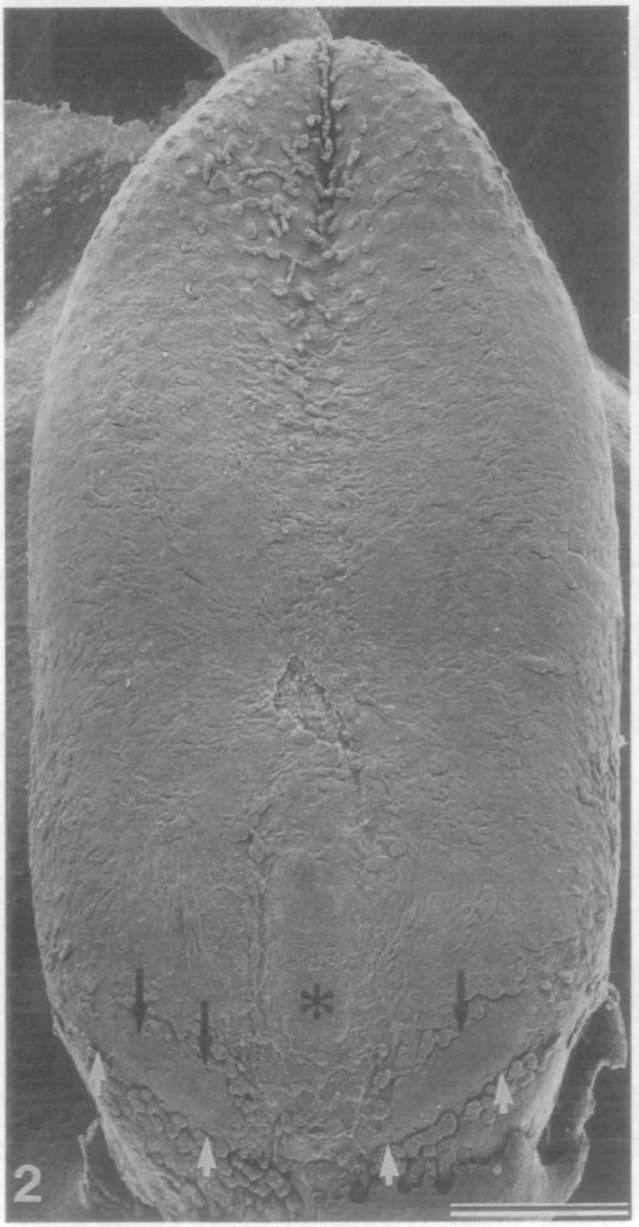
Figure 3 Week 7. Posterior part of the tongue. A median circumvallate papilla (large arrow) is located between the two 'wings' of the V-like sulcus terminalis (not marked), immediately beyond the ridge where circumvallate papillae (small arrows) arise. E = epiglottis. Bar = 100 μm.

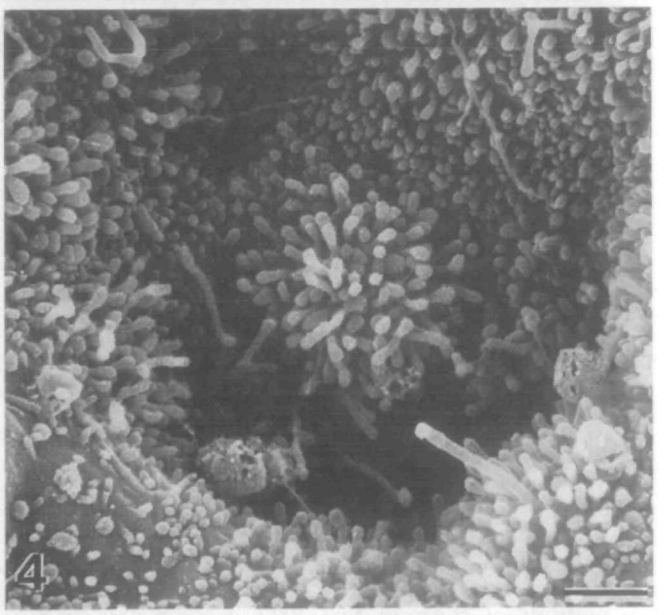
Figure 4 Week 6. Pore-like structure on top of the median circumvallate papilla shown in Figure 3. The microvilli in the center of the taste pit belong to adjacent epithelial cells and not to typically differentiated taste cells. Bar = 1 μm.

Figure 5 Week 7. Anterior detail from Figure 2. Epithelial elevations mainly along the median sulcus (along the right border) are forming presumptive fungiform papillae, the tops of many of them direct caudally (arrows). The definite papillary structure has not yet been developed. Bar = 100 μm.









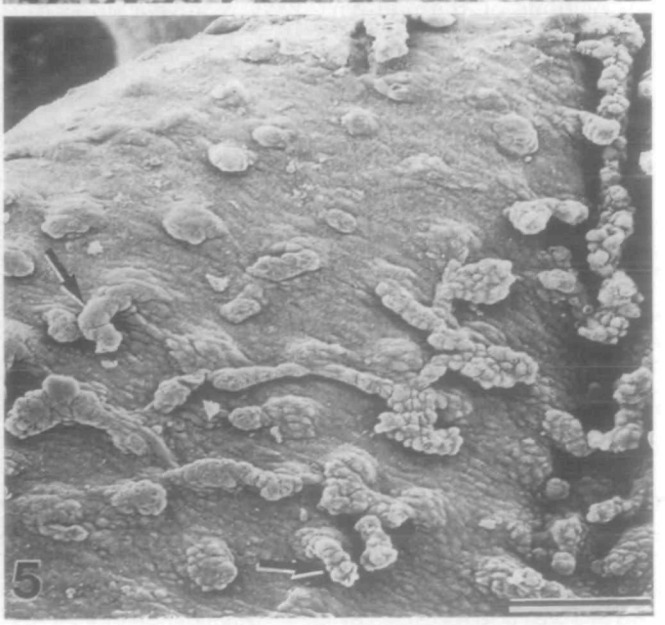


Figure 1) is covered by nearly flat epithelium. The first circumvallate papilla, already with a central pore, develops in the dorsal midline (Figures 3 and 4) in the vicinity of the foramen caecum. The V-like sulcus terminalis, delimiting the root from the body of the tongue, becomes more prominent during week 7. Smooth elevations, the anlagen of the circumvallate papillae, are clearly evident (Figure 2). Around week 7, the earliest forming fungiform papillae appear in the midline of the tongue and at the anterior margins (Figure 2). Droplet-like protrusions or soft elevations consist of ~5–20 epithelial cells (Figure 5). The tips of these protrusions point caudally. Some ciliated cells are scattered across the dorsal lingual surface.

Postovulatory weeks 8–9

The epithelial thickenings at the anterior part of the tongue enlarge and resemble later fungiform papillae (Figure 6). The early circumvallate papillae are now distinctly visible along the sulcus terminalis. They have no complete furrows yet, thus resembling large fungiform papillae (Figure 9). At this stage, the top surface of the circumvallate papillae usually contains a taste pit partly filled with microvilli of presumed underlying taste bud cells (Figure 10) or, in more advanced stages, with stereocilia-like processes (not shown).

Postovulatory weeks 10–11

In this period, numerous fungiform papillae appear predominantly on the lateral margins and the tip of the tongue, and to a lesser extent across the mid-surface. (In semithin sections of the mid-part, only relatively smooth elevations of the epithelium are found.) The large circumvallate papillae often are asymmetrical, i.e. the developing furrow does not surround the papilla entirely, but builds a half-moon-like groove around one side of the papilla.

Fungiform papillae often show slight depressions on their apical epithelia, but no mature taste pores (Figures 7, 8 and 11). Inside the depressions bulbous elevations occur, occasionally pierced by brush-like arrangements of microvilli or even stereocilia. Taste pits, in which no typical projections of presumptive taste cells can be seen by SEM, are covered by thin processes of adjacent epithelial cells, as revealed by TEM (Figure 14). The diameter of the papillae at this stage ranges from ~50 to 80 μm.

Postovulatory weeks 12–13

The fungiform papillae continue to grow especially on the tip and the margins of the tongue. Papillae are scarce in the mid-portion of the tongue. Pores on fungiform papillae occur only rarely. These papillae still have an irregular shape; sometimes they appear oval, sometimes ellipsoid (Figure 12). Some papillae seem to divide themselves showing two stem domes with a connecting epithelial portion (Figure 12).

Postovulatory weeks 14–15

Fungiform papillae are rare in the middle part of the tongue, but relatively numerous on the lateral margins (Figure 15). They are considerably larger in diameter (~70–150 μm; Figure 16) than those of previous stages (30–50 μm, week 7; 40–70 μm, week 9) and have up to two taste pores (Figures 16–18). If present, taste pores are larger

Figure 6 Week 9. Fungiform papillae from the anterior margin of the tongue. Two of them are apparently dividing (arrows). Taste pores are not present in this aspect. Bar = 100 μm.

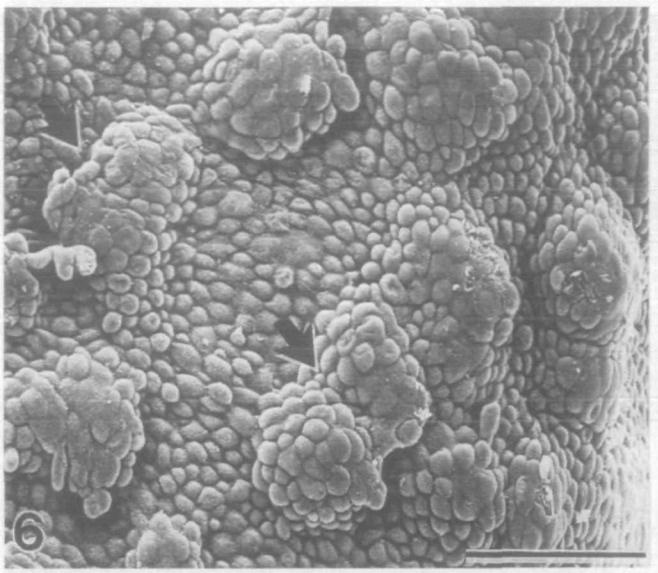
Figure 7 Week 10. Apical part of a fungiform papilla. A soft depression of the surface epithelium gives the aspect of a presumptive taste pore with an underlying taste bud primordium (cf. Figure 14). The microvilli in the center of the pore belong to non-sensory epithelial cells. Bar = 1 μm.

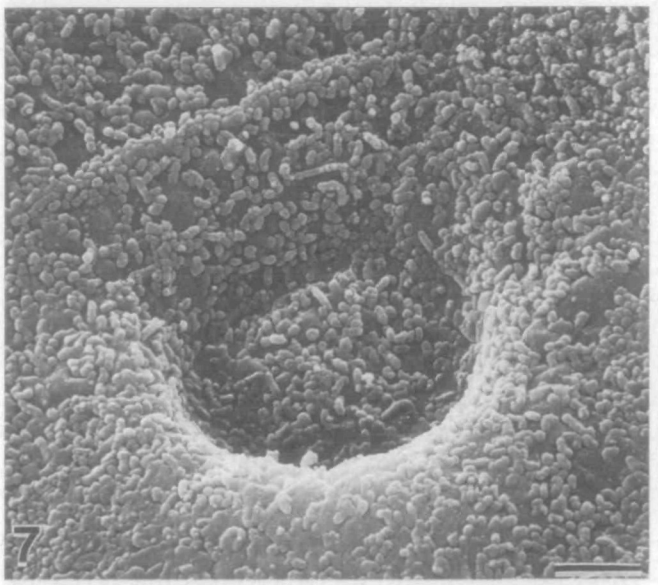
Figure 8 Week 10. Papilla adjacent to that depicted in Figure 7. In the center of the flat epithelial depression a tuft of microvilli is to be seen which possibly belongs to one or more taste cells underneath. Bar = 1 μm.

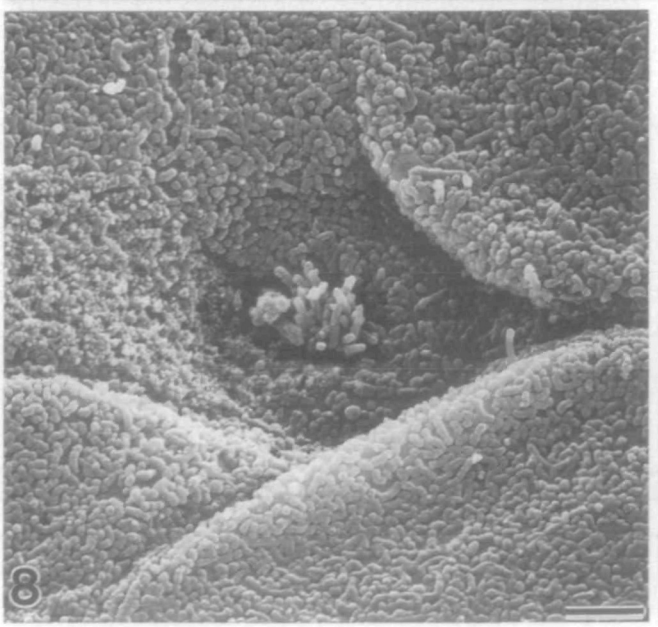
Figure 9 Week 9. Median circumvallate papilla. On its right side, the trench is not yet developed. The arrow marks a putative taste pore enlarged in Figure 10. Bar = 50 μm.

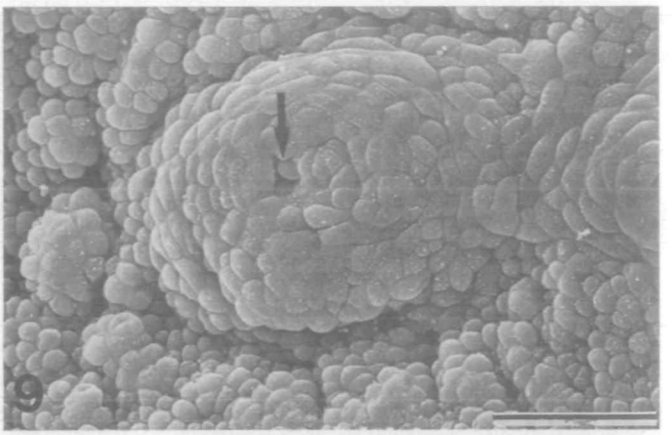
Figure 10 Week 9. Higher magnification from the taste pore shown in Figure 9. In the fissure between two superficial epithelial cells the tips of microvilli are seen (arrows). They obviously belong to taste bud cells which are ready to break through and to reach the surface. Bar = 1 μm.

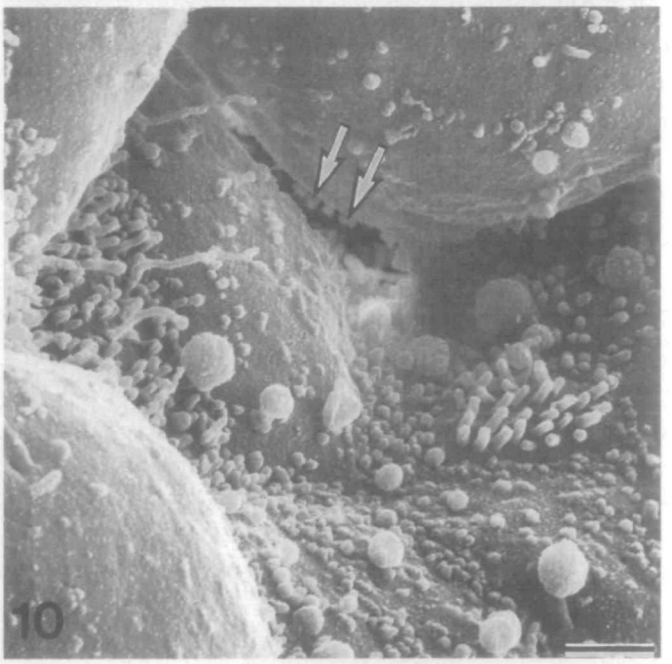
Figure 11 Week 10. Fungiform papilla. At the margin of the papilla there are two ciliated cells and in the central area a taste-pore-like depression is located (arrow) within a superficial epidermal cell. Bar = 10 μm.











than in earlier stages and some of them seem to divide (Figure 13). Rarely, ciliated cells may project into the taste pit (Figure 19). Circumvallate papillae have increased in size, but are usually not as clearly distinguished from the lingual root as in previous stages. Filiform papillae have not yet developed.

Summary—time course of taste papilla development

The first evidence of fungiform papillae is a series of epithelial swellings in the anterior part of the tongue during week 7. By week 10, many fungiform papillae exhibit depressions in their apical surface; occasionally microvilli or stereocilia can be observed extending through the thin epithelial cells covering the bottom of these depressions. Within 2–3 weeks, these thin epithelial cells disappear, revealing the microvillous apices of underlying taste bud primordial cells.

The first circumvallate papilla appears in week 6 as a single median papilla, just caudal to the converging wings of the V-shaped sulcus terminalis. In the course of the next 2–4 weeks, four or five circumvallate papillae appear on each side of the sulcus terminalis. They are considerably larger than fungiform papillae of the respective age, but do not tend to grow proportionally.

Discussion

In this study, we investigated the developing papillae of the anterior part (fungiform papillae) and the posterior part (circumvallate papillae) of the human tongue by means of SEM. In contrast to the situation in other mammals, e.g. the rabbit, the bilateral foliate papillae in humans are usually hardly developed, and due to their late and irregular appearance (Hellman, 1922), are not considered here.

The staging problem

One of the major problems concerning the comparison of our results with those of earlier authors is the lack of a uniform measurement and staging system for human embryos and fetuses. In contrast to the embryonic period that covers the prenatal age up to postovulatory week 8 [commonly known as Carnegie stages (O'Rahilly and Müller, 1987)], attempts to work out an analogous staging system for the fetal period have failed until now (O'Rahilly and Müller, 1994). Thus, our data and determinations of the respective ages are sometimes difficult to compare with those of earlier authors, especially when data (e.g. fetal length) have not been assigned to respective 'ages' in previous studies. Another problem is that the determination of the greatest length of the embryo or fetus was often very difficult due to the lack of ultrasound diagnosis or to fragmentation of the embryos or fetuses during curettage or vacuum extraction. As a consequence, in these cases we estimated the approximate age from measurements of the lower extremities (Munsick, 1984; see Table 1).

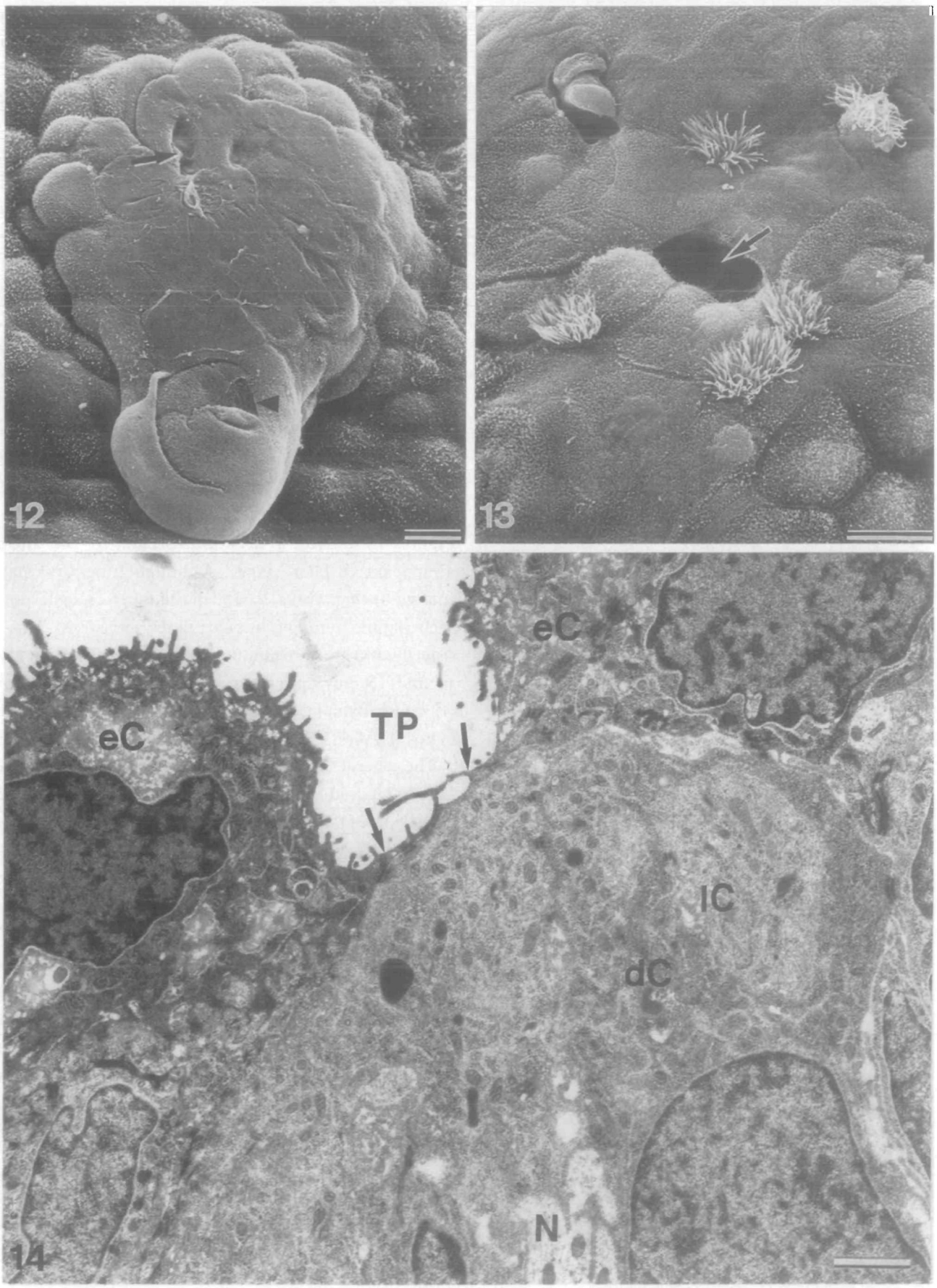
Development of the tongue anlage

During the first five embryonic weeks, the morphogenetic processes which occur in the developing mouth cavity are all prior to the formation of taste papillae and lead to the tongue anlage which is necessary for taste papilla development. The first structure of the anlage is the tuberculum impar which is situated between the first (mandibular) and the second (hyoid) branchial arches. Then, anterolateral to the tuberculum impar, the paired lingual swellings which derive from the medial parts of the mandibular arches join the tuberculum impar (Carnegie stages 14–17; Hinrichsen, 1990). Caudally, marked by the foramen caecum (anlage of the thyroid gland), the tongue's base is formed by the hypobranchial eminence or copula of His (Figure 3) comprising the third and fourth branchial arches. Later, a transverse trench separates the tongue's base from the epiglottis. The border between the tongue's caudal

Figure 12 Week 12. This fungiform papilla is dividing. In its upper part there are two taste pores, one of which (arrow) is apparently dividing. The epithelial discontinuity in the lower part (arrowhead) is an artifact. Bar = 10 μm.

Figure 13 Week 14. Fungiform papilla with one taste pore serving apparently two taste bud anlagen. The pore is separated by a fine septum (arrow). Microvilli lie in the very depth of the taste pit and are not seen here (cf. also Figure 14). Note the ciliated cells near the taste pore. Bar = 10 μm.

Figure 14 Postovulatory week 10. Transmission electron micrograph of a longitudinally cut taste bud primordium. Thin processes (arrows) of dark epithelial cells (eC) cover the site where later projections of dark (dC) and light (lC) taste bud cells will pierce into the definite taste pore (TP). N = nerve fiber. Bar = 1 μm.



part and its rostrally growing body is marked by a V-like rim, the sulcus terminalis. Interindividual morphological differences within even one developmental period are common and known also in other organ systems, giving cause for the introduction of a staging system (O'Rahilly and Miller, 1994). An example of differently timed morphogenetic events is the disappearing tuberculum impar (see the differences between Figure 2 and Figure 3, which show different tongues of the same age). Furthermore, the formation of taste bud anlagen in late embryonic and early fetal periods is more heterogeneous than in later stages or even in renewal processes in adulthood, insofar as early taste buds (weeks 8–10) do not necessarily require a pre-formed dermal papilla (Witt and Reutter, 1996).

Development of circumvallate and fungiform papillae and its implication for maturation of the peripheral taste system

From our observations it is obvious that circumvallate papillae start to develop earlier than fungiform papillae, beginning with one large median papilla. Their size can be recognized easily in early ages (weeks 6–8), when the surface of the tongue is smooth and the irregular surface of the lingual root has yet to form (Figures 1 and 3). This is considerably earlier than reported by other authors: according to Starck (1975), in humans the first circumvallate papillae appear between weeks 8 and 9. Hersch and Ganchrow (1980) observed the first median circumvallate papilla in 8-week-old embryos, which is 2 weeks later than in our findings. The first taste buds appearing on the top of the papilla will be replaced later by those which arise on each lateral side of the papilla when epithelial ridges protrude into the dermis forming the trench and the Ebner glands. We observed first taste pit-like grooves in the apical surface of the circumvallate papilla already during weeks 6 and 7 (Figures 1 and 3), at a time when the taste buds have not yet formed.

On the anterior part of the tongue, it is doubtful whether all cellular elevations and even droplet-like structures seen in the SEM during weeks 7–9 should be termed 'papillae', since serial sections show that only a small number of elevated structures have a real dermal core, which is a typical characteristic of fungiform papillae (Witt and Reutter, 1996). Our SEM studies show that filiform papillae have not yet been formed during the first trimester, whereas fungiform papillae and their precursors are widely distributed. This is in accordance with findings of other authors (Boshell *et al.*, 1977; Hersch and Ganchrow, 1980). Of particular interest are the marginal lingual papillae (Figure 15; Habermehl, 1952; Yamasaki and Takahashi, 1982; Schlechta *et al.*, 1996), which persist only in prenatal and newborn developmental stages. Due to their lacking taste buds (at least in the second trimester) and having a prominent marginal appearance, the marginal papillae have been compared to filiform papillae, and their function has been assigned to assistance in sucking (Habermehl, 1952). Some marginal papillae, however, have (taste) pores (see Figures 15 and16), a structural peculiarity that probably disappears in later stages. Although fungiform papillae enlarge during weeks 10–15, the number of taste pores in each papilla remains approximately constant. This consistency of taste pore numbers between postovulatory weeks 11 and 15 corresponds to results obtained by Arvidson (1979) in postnatal humans and Mistretta and Baum (1984) in postnatal rats.

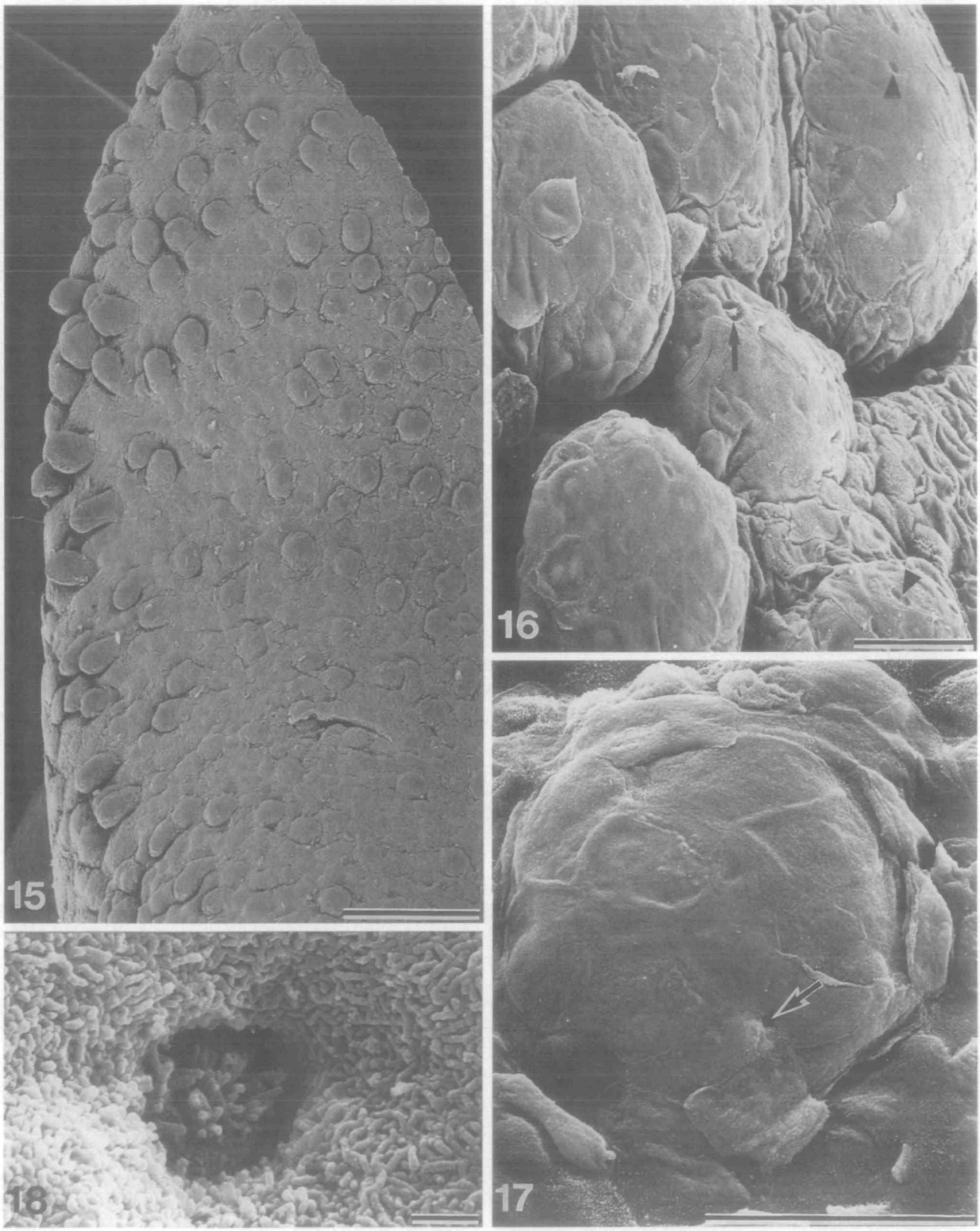
The general surface morphology of the human tongue has been compared to that of monkeys (Kullaa-Mikkonen and Sorvari, 1985), but it seems to be significantly different from that of other mammals, especially rodents (Hume and Potten, 1976), which serve as models for developmental studies in the mammalian taste system (Farbman and Mbiene, 1991; Iwasaki *et al.*, 1997). In contrast to the human system, taste pores in rats do not open until *post*natal day 10 [considered to be a morphological sign of maturation (Mbiene and Farbman, 1993)]. A common morphogenetic event in rat and human development is the

Figure 15 Week 14. Anterolateral part of a median-sagittally sectioned tongue with numerous large fungiform papillae, especially on the margins and its tip. Bar = 500 μm.

Figure 16 Detail from Figure 15. Fungiform papillae from the anterior portion of the tongue. Note the scarce appearance of taste pores (arrow). Two dwellings similar to those depicted in Figure 7 are marked by arrowheads. Bar = 50 μm.

Figure 17 Week 14. Fungiform papilla with one well-developed taste pore (arrow). Bar = 50 μm.

Figure 18 Detail from Figure 17. Taste pore with microvilli which belong to underlying taste bud cells. Bar = 1 μm.



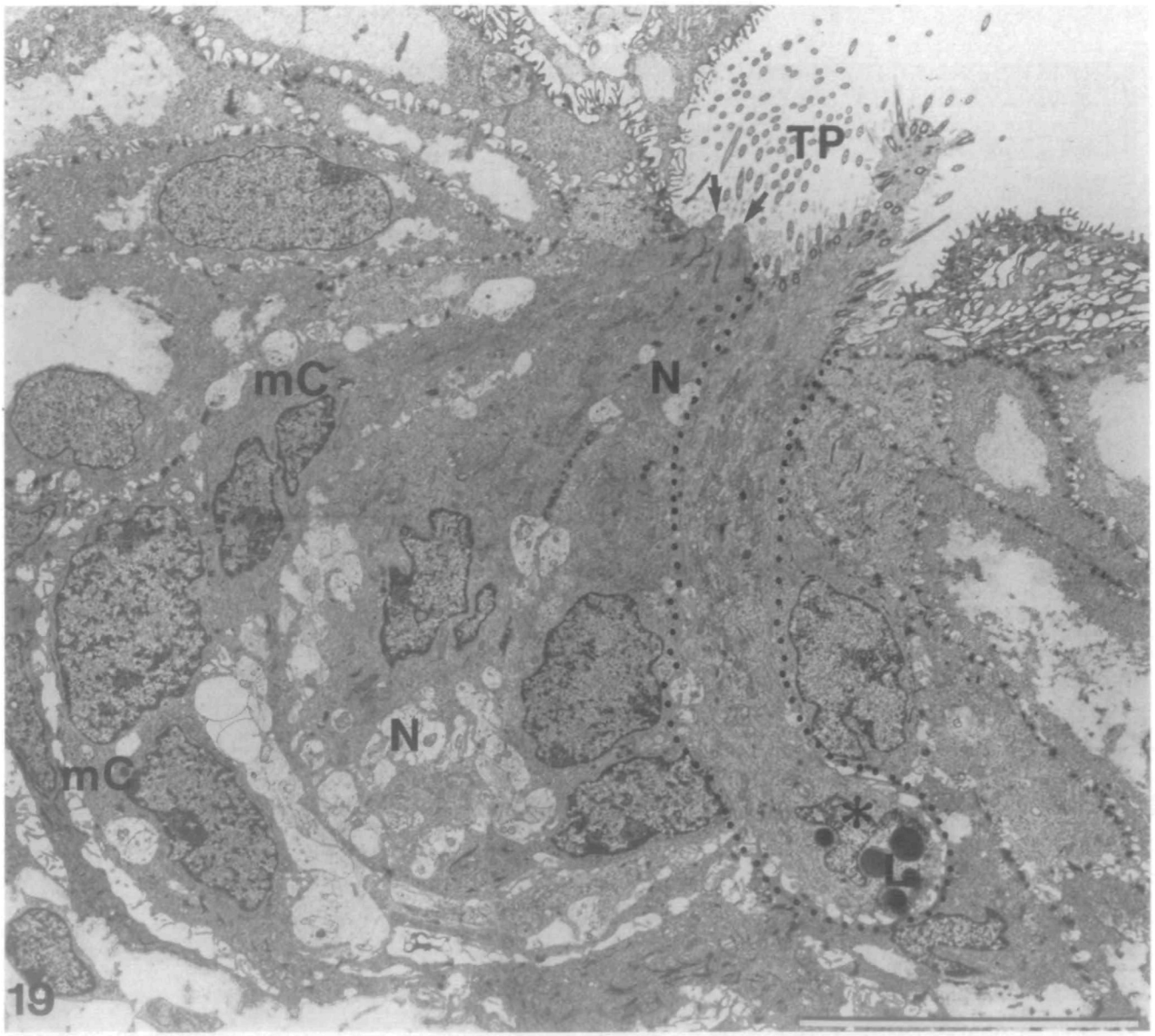


Figure 19 Week 14. A transmission electron micrograph made from the tongue whose left half was prepared for SEM (Figures 15 and 16). The relatively small primordium is clearly delimited by marginal cells (mC). The taste pit is wide and contains two processes of intragemmal cells (left) and one large knob-like process of a ciliated cell. The extragemmal portion of the ciliated cell (marked by dotted lines) contains the nucleus (asterisk), which is surrounded by lysosomes. Nerve fibers (N) ascend up to the apex of the primordium. Bar = 10 μm.

first appearance of the median circumvallate papilla [papilla vallata mediana posterior (Münch, 1896); in mouse by day 13 (Paulson *et al.*, 1985; AhPin *et al.*, 1989) and in rat by E16 (Iwasaki *et al.*, 1997)]. Apparently after the common early appearance of the median circumvallate papilla in humans and rodents, the developmental programs of the two groups diverge. In dogs, fungiform and circumvallate papillae were observed by fetal day 38 (term = 63 days), but taste buds were not seen until day 47, i.e. in the last trimester (Ferrell, 1984), which is relatively later than in humans.

Whereas adult human fungiform papillae may contain up to 15 taste buds (Arvidson, 1979), fetal human fungiform papillae (up to week 15) contain maximally only two taste buds. The functional maturation of the entire peripheral taste system has not been reached therefore, but this is not necessarily true for individual taste buds. However, more than half of the fungiform papillae in human adults have no taste buds (Arvidson, 1979). It is obvious that important prerequisites for taste bud functioning are: (i) the contact of receptor cells to the outer environment, the mouth cavity; and (ii) the synaptic contact of a chemoreceptive (receptor) cell to an afferent nerve fiber. Various reports [e.g. altered

chemical response latencies or nerve responses to NaCl in early development; for a review see Mistretta (1991)] showed that gustatory information is transduced even in developmental periods in which taste pores are not present. The opinion that maturity of a taste bud is accomplished when a taste pore appears seems to be doubtful, since in our SEM and TEM observations even deep pores may represent 'pseudopores', i.e. those whose bottoms are still covered by adjacent epithelial cells prior to the outgrowth of taste cell apices (Figure 14). Even taste bud cells of this or a somewhat more advanced age (postovulatory weeks 10–14) with visible microvilli reaching into a pore may not yet be fully differentiated. Type I cells at this stage are devoid of dark granules typical of mature cells and which are most likely the storage sites for the amorphous glycoprotein-rich material within the taste pit (Witt, 1996). In addition, some cellular projections may be misinterpreted as microvilli of taste bud cells. By TEM analysis, we observed atypical projections within taste pores resembling stereocilia (Witt and Reutter, 1996) or even true kinocilia (Figure 19). These 'aberrant' structures have not been reported in adult taste buds nor in developmental stages of other vertebrates. In humans, kinocilia of the lingual surface disappear completely by week 20 (Tedde Piras and Mazzarello, 1985). They obviously 'survive' in the bottom of the trenches of circumvallate papillae (Mattern *et al.*, 1970: human; Toyoshima and Shimamura, 1979: rat).

In summary, our results show that circumvallate papillae develop earlier than fungiform papillae. The fungiform papillae are smaller, but exhibit a stronger tendency of individual growth and even division. The presence of a taste pore is not always associated with a fully mature taste bud in that the bottoms of early taste pits (weeks 9–10) may still be covered by flat epithelial cells.

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