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The development of the human taste bud during the foetal period

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INTRODUCTION

It is now 300 years since the lingual papillae were described by Malpighii (1664) and 100 years since Schwalbe (1867) and Lovén (1867) discovered the human taste buds.

It was not until 1874 that the embryology of the taste bud was described by Hoffmann. He examined human tissue ranging in age from 3·5 months (in utero) to 60 years of age. Later Lustig (1884), and Stahr (1902) did similar studies. Tuckerman (1889) was of the opinion that taste buds were of neural origin, but Gråberg (1898) described them as originating from a local enlargement of the epithelial basal cells. Marchand (1902) agreed with Gråberg, and was of the opinion that the basal cells elongated under neural influence. The most comprehensive study was that of Hellman in 1922. He used a large sample of tongues (43) and studied both the embryology of the buds and the nerves. He maintained that a primitive bud formed and lasted for a short period of time but then disappeared, to be replaced later by adult buds. He was also of the opinion that the buds formed under the direct influence of the nerves. The text-books of embryology essentially agree with Gråberg's finding. Authors disagree on the time the taste buds first appear in the tongue epithelium. Table 1 summarizes their findings.

Table 1

Author	Date	Intra uterine ages
Hoffman	1874	4·5-month foetus
Lustig	1884	7-month foetus
Tuckerman	1890	14-week foetus
Gråberg	1898	3-month foetus
Stahr	1902	5-month foetus
Marchand	1902	5-month foetus
Hellman	1922	7+-week foetus*
Patten	1953	8-week foetus
Arey	1954	2-month foetus

* Hellman gives 16 mm neck-rump length from which the age was estimated using data of Mall, 1910.

There seems to be disagreement on the time of appearance of the buds, the origin of their cells, and the effect the nerves have on initiation of bud formation. The number of specimens (except for Hellman) used in these studies were small or not given. The present study proposes to examine and to describe the embryologic development of the taste buds.

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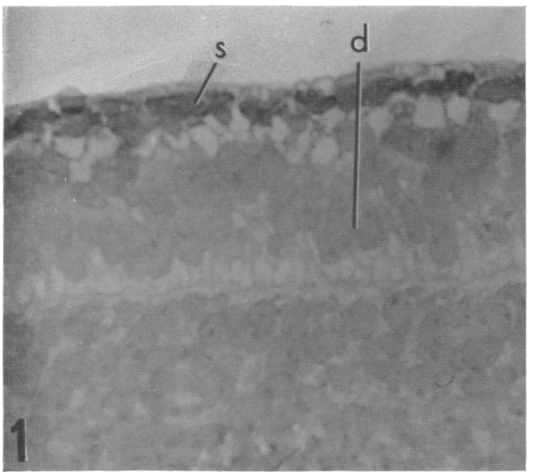
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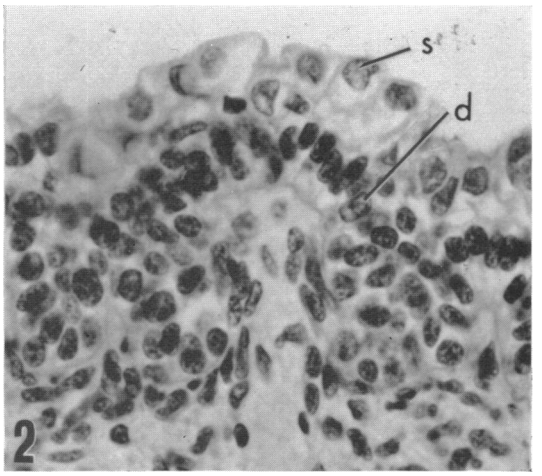
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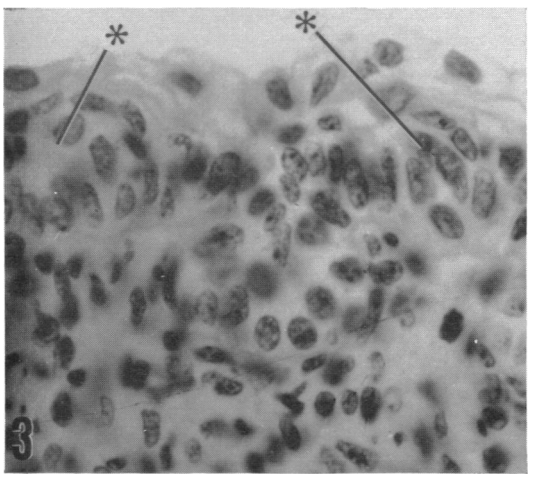
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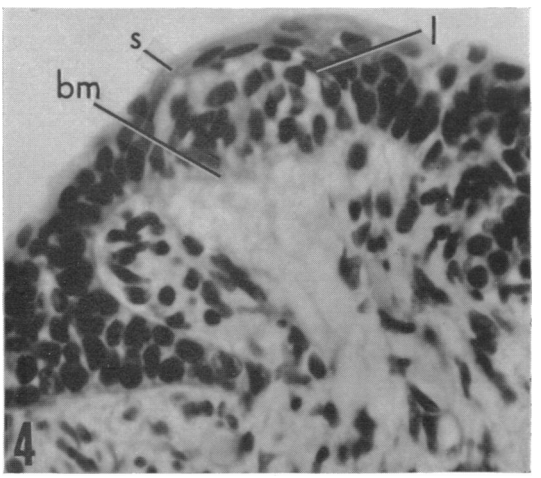
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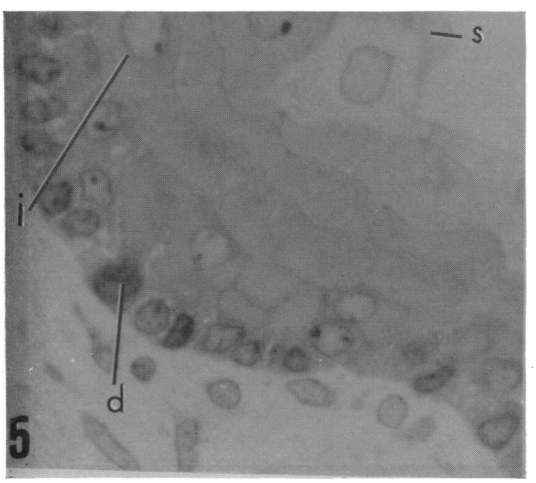
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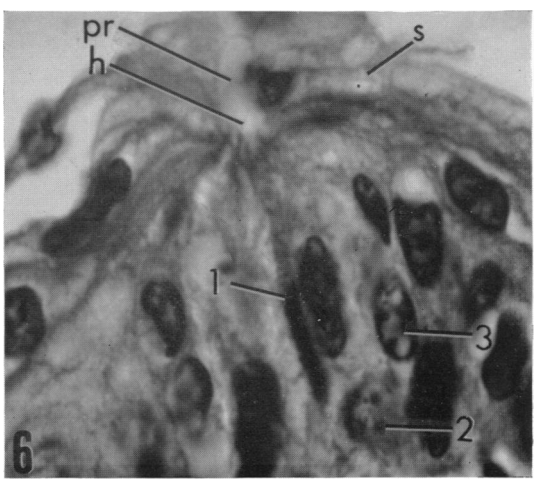
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MATERIALS AND METHODS

Seventy specimens of human foetal tongue material were used in the study. Their distribution is summarized in Table 2.

Table 2

C.R. length in (mm)	No. in group	C.R. length in (mm)	No. in group
20–40	3	140–160	10
40–60	2	160–180	10
60–80	4	180–200	4
80–100	8	200–220	3
100–120	8	220–240	1
120–140	14	240–260	2
		260–280	1

The ages were estimated from C.R., length using a table supplied by Shepard, 1965.

The tongues were removed whole and treated in one of the following ways:

(1) In the case of the majority of the specimens the tongues were cut in half longitudinally and one half used. This was fixed in 10% neutral buffered formalin, embedded in paraffin, serially sectioned at 5–6 μm and stained with haematoxylin and eosin.

(2) Some specimens which had been fixed in 5% glutaraldehyde were either (*a*) post-fixed in 1% osmium tetroxide (Palade, 1952), embedded in epoxy resin (Luft, 1961), sectioned with glass knives at 1·5 μm on a Porter-Blum MT-2 microtome and stained with 0·5% toluidine blue, or (*b*) post-fixed in 10% neutral buffered formalin and prepared as in (1).

(3) When fresh abortion material was available, it was immediately fixed in cold 1% osmium tetroxide and treated as in (2*a*). Portions of the fresh material were taken and prepared by procedure (1).

Fig. 1. Dorsal tongue epithelium of a 23 mm (6·7 weeks) embryo. The epithelium consists of deep (*d*) and superficial cells (*s*) which rest on a basement membrane (*bm*). Toluidine blue stain, ×400, 1·5 μm section.

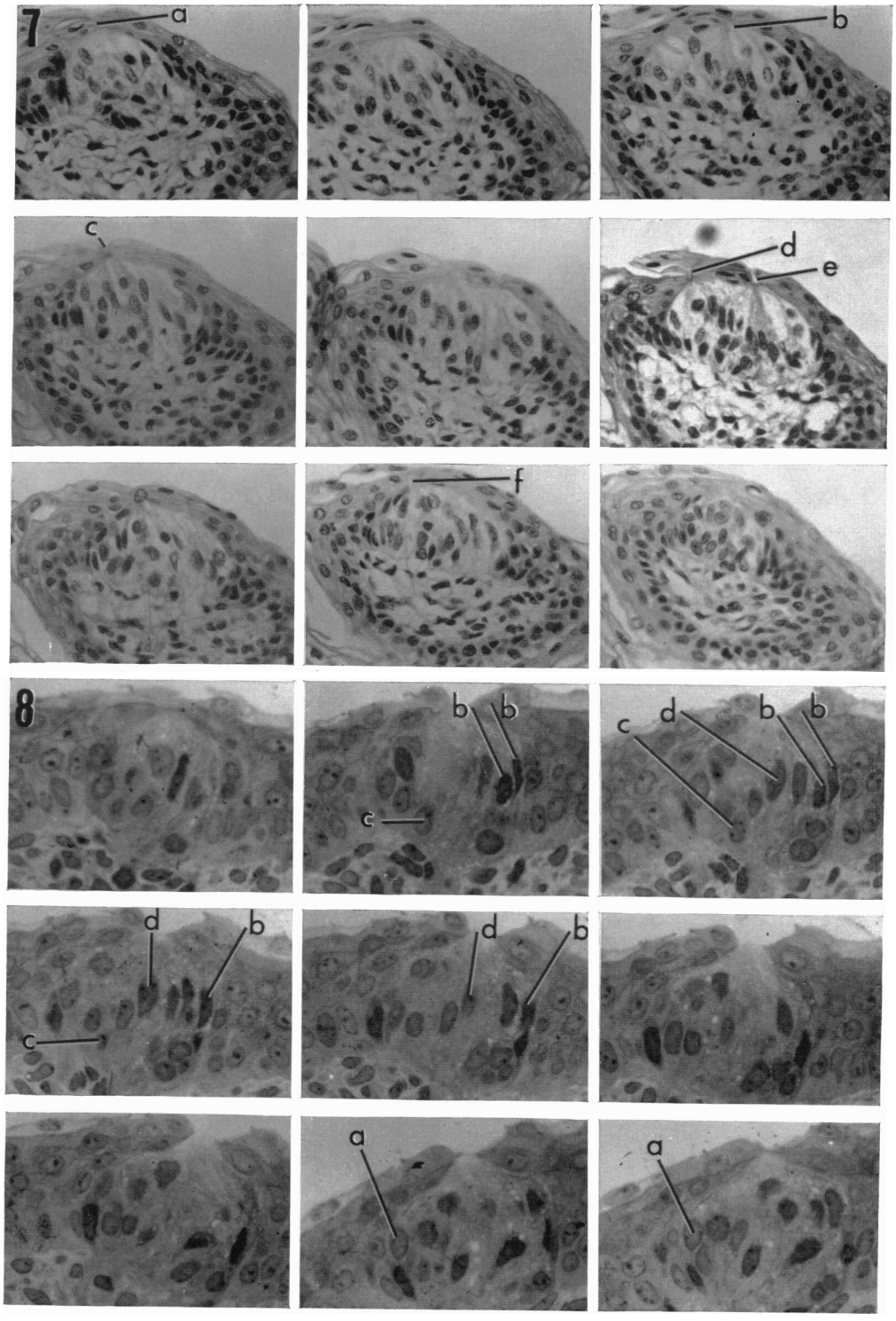
Fig. 2. Early papilla with connective tissue core, from a 53 mm (9·5 weeks) foetus. The epithelium covering the papilla is made up of superficial (*s*) and deep cells (*d*). H. and E. stain, ×400.

Fig. 3. Two cell collections (early bud forms) in the dorsal epithelium of a 53 mm (9·5 weeks) foetus. These are seen at the asterisk. H. and E. stain, ×400.

Fig. 4. Presumptive bud in a fungiform papilla from a 77 mm (11·2 weeks) foetus. The bud is bounded by flattened cells superficially (*s*) by stave-shaped cells laterally (*l*) and below by a basement membrane (*bm*). H. and E. stain, ×400.

Fig. 5. Dorsal tongue epithelium of a 134 mm (15·3 weeks) foetus. The epithelium consists of three layers—superficial (*s*), intermediate (*i*) and deep (*d*). Toluidine blue stain, ×400. 1·5 μm section.

Fig. 6. Taste bud from a 115 mm (14 weeks) foetus. H. and E. stain, ×100. *s* denotes superficial cells; *h*, the taste hairs; *pr*, the taste pore; and 1, 2, and 3 are numbers representing shapes and staining of various nuclei.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OBSERVATIONS

Six to seven weeks

Between the age of 6 and 7 weeks the tongue consists of a mass of closely packed cells penetrated by blood vessels and occasional strands of muscle.

The epithelium of the dorsum is approximately 40 μm in height and consists of two layers—a superficial and a deep layer.

The cells of the superficial layer which are squamous in appearance are approximately 10 μm in height and 5 μm in width (Fig. 1, *s*) and have irregularly shaped, relatively flat, dark staining nuclei (i.e. with toluidine blue stain). The cytoplasm of these cells appear structureless and stains lightly; however, the cytoplasm adjacent to the surface is darker staining.

The cells of the deep layer (Fig., 1, *d*) which are 30 μm in height and 5 μm in width are columnar pseudostratified, with large pale staining nuclei. These nuclei occupy the apical two-thirds of the cell. Below these cells there is a basement membrane which is distinct and scalloped in form.

Seven to nine weeks

At 7–9 weeks the epithelium of the dorsum of the tongue is made up of two epithelial cell types (Fig. 2, *s* and *d*) as in the 6- to 7-week period.

The deep layer which is two–three cells in height (*ca.* 30 μm) accounts for half the vertical epithelial dimension. The cell outlines of this layer are often obscure. The nuclei are large and elongated and their chromatin stains distinctly (Fig. 2, *d*).

In contrast the cell outlines of the superficial cells are more distinct (Fig. 2, *s*). The cells of this layer are not as closely packed as the deeper cells and the ratio of nucleus to cytoplasm is about equal. The nuclei are pale staining and more rounded.

Collections of cells resembling taste buds are first seen in this time period (Fig. 3, *). These cell collections will be referred to as presumptive buds. The presumptive buds are at the tips of papillae. They occupy three-quarters of the thickness of the epithelium and consist of elongated cells which stain *similarly* to the cells of the deeper layers. The cells are *ca.* 30 μm in height. The superficial cells overlying these are squamous in form. Presumptive buds are separated from the subepithelial tissue by a basement membrane. No taste pores can be demonstrated.

Ten to twelve weeks

The epithelium of the dorsum is four–five cells thick and is made up of deep cells and superficial cells. The deep layer is a single cell in height, while the superficial layer is three–four cells in height (Fig. 4). The deep (*d*) and superficial (*s*) cells resemble those of the 7- to 9-week period.

Fig. 7. Serial sections of a taste bud from a 146 mm (16·2 weeks) foetus. Note that this bud is asymmetrical, and has six taste pores (*a*, *b*, *c*, *d*, *e* and *f*).

Fig. 8. Serial sections of a taste bud from a 142 mm (16·0 weeks) foetus. The letters represent corresponding nuclei which have been traced from section to section. Toluidine blue stain, $\times 400$, 1·5 μm section.

At the tongue tip are numerous papillae. These contain presumptive buds which at this age (unlike those at 7–9 weeks) stain *differently* from the surrounding epithelium.

The presumptive buds are approximately 30 μm high and 40 μm wide and consist of similarly staining cells whose individual outlines are indistinct. These cells have a lighter staining cytoplasm than the surrounding epithelium. The nuclei, however resemble the nuclei of the deep layer with the exception that their axial inclination is irregular rather than perpendicular to the basement membrane (Fig. 4, *bm*). The bud is covered by flattened superficial cells (*s*) and is bordered by cells of the deeper layer which are stave shaped (Fig. 4, *l*). In addition to the round presumptive bud, tear-shaped and buds whose apices project between the superficial cell junctions are seen.

Thirteen to fifteen weeks

At this stage in development there are three cell types distinguishable in the dorsal tongue epithelium. The deeper (Fig. 5, *d*) and superficial cells (Fig. 5, *s*) are similar to those described in the 7–9 weeks age period. There is also an intermediate layer two cells in height. These are large cells with pale staining nuclei (Fig. 5, *i*), and essentially the tongue epithelium has become stratified squamous.

Tongue papillae contain buds which are made up of cells whose long axis (*ca.* 50 μm) extends the whole height of the epithelium and bulges into the connective tissue core of the papilla. The nuclei occupy the lower two-thirds of the cells and the apical one-third of the bud consists of the cytoplasm, i.e. distal ends of the cells. The nuclei are elongated and stain intensely (Fig. 6, *l*), others are elongated and stain lightly (Fig. 6, 3), whilst still others are rounded and lightly staining (Fig. 6, 2) although these differences may in reality relate to orientation of plane of section and functional structure of the cells. The apical ends of the bud cells communicate with the oral cavity through a pore (Fig. 6, *pr*). The pore is a cylindrical channel, whose walls are made up of superficial epithelial cells (Fig. 6, *s*). The pore is approximately 4 μm in diameter and 10–12 μm in length. At the base of the pore there are specializations of the apical ends of the bud cells, which resemble hair-like structures. These extend from the apex of the bud cell into the basal end of the pore (Fig. 6, *h*). The cell surface between adjacent taste hairs has a fuzzy appearance. The hairs are approximately 3 μm in height while the fuzzy border is about half that dimension.

Sixteen to twenty weeks

The dorsal epithelium is six–eight cells in height, and is made up of deep, intermediate and superficial cell forms.

Buds similar to that described at 13–15 weeks are present, however, there are interesting variations.

Figure 7 shows 5 μm serial sections through a large bud. It is approximately 65–80 μm in depth and is asymmetrical, apparently consisting of two buds. More interesting than this, however, is the multiplicity of taste pores. Perhaps as many as six pores exist in this complex taste bud (Fig. 7, *a–f*).

Material fixed in osmium tetroxide and sectioned, at 1·5 μm shows differences in bud morphology. Figure 8 is of selected serial sections of a bud whose pore does not show any taste hairs, merely a fuzzy border to the apices of the bud cells. Some of the

nuclei were numbered and traced through several sections. This was to ascertain whether or not the staining density of the nuclei were constant. By and large the light staining nuclei remained light (*a*) and the dark remained dark (*b*), however, nuclei *c* and *d* indicate nuclei which are dark in one section and light in another.

Twenty weeks to term

No essential changes in morphology of the apithelia and buds are encountered in the older specimens. The tongue epithelium is ten to fifteen cells in height and resembles adult stratified squamous epithelium. Towards term the surface cells are very flattened, and contain dark staining nuclei and cytoplasm. Their cell volume is decreased.

DISCUSSION

Hellman's study (1922) was the most comprehensive study of human taste bud development (Table 3).

Hellman's greatest number of specimens comes from the 7 to 9·3 weeks age group and no specimen is older than 13 weeks. In the present study there are seventeen specimens in this age range and the greatest number is between 10·5 and 20·5 weeks (59). The distribution is normal with a maximum between 14·5 and 16 weeks. Hellman's curve is skewed to the right with a maximum at the 7- to 9·3-week period. Ideally one should have the same 'adequate' number of specimens over the whole gestation period, i.e. the distribution should be rectangular.

Table 3

Neck-rump length in mm from Hellman (1922)	Group mean in (mm)	No. of Specimens	C.R. converted in (mm*)
15·4–20·0	15	11	22·3
20·0–30·0	25	14	38·2
30·0–40·0	35	7	54·0
40·0–50·0	45	4	70·0
50·0–60·0	55	4	84·2
60·0–70·3	65	3	100·0

* From data in Scammon & Calkins (1929)

It is not always possible to be sure of the exact age of a specimen because of individual variation. Moreover, many of the specimens were aborted so that there is a likelihood that they were abnormally developed. Therefore, the precise age of a taste bud anlage based on foetal size must be an approximation. When, however, many specimens are employed, an average is obtained which may be more accurate than a single observation.

It is unnecessary to point out that static studies do not indicate dynamic actions such as growth, cell migration, cell differentiation, etc. The use of serial populations, however, permits one to interpret changes between age groups in a more dynamic manner.

This study, together with Hellman's, provides an almost rectangular distribution up to 180 mm C.R. length (*c.* 19 weeks). Definitive observations may be made from such a sampling.

The origin of the taste buds and their time of appearance

The cells of the taste bud are epithelial in origin and differentiate from the epithelial cells (Gråberg, 1889; Marchand, 1902; Arey, 1954). There is some disagreement concerning the time of their appearance (see Table 1). In the present study presumptive buds are observed at 11 weeks. However, collections of cells were noted as early as the seventh week. Hellman (1922) gives the seventh week for the appearance of the taste bud. In a static study, it is not possible to resolve the question of whether or not these cell collections are functional entities. Consequently, the difference between the present findings and Hellmann's may be due to a difference in interpretation. Hellman is of the opinion that these early bud forms disappear and taste buds appear later. This observation is not consistent with our findings where an unbroken progression of bud forms (cell collections, presumptive buds and definite taste buds) are found.

The formation of the taste pore

The taste pore forms soon after the bud first makes its appearance. The newly differentiated bud cells elongate, become spindle-form and pierce the surface epithelium. The cell apices then form a fringe-like border. Next the surface epithelial cells surround the apical tuft of bud cells forming the embryonic taste pore (Gråberg, 1898). The fringe-like border becomes the taste hairs. Taste pores and hairs are a consistent feature of the taste bud from 14 weeks on.

In Farbman's (1965*a*) description of the ultrastructure of the developing taste bud in rats, the pore is first seen at about the (twelfth) postnatal day, at the same time that a cell (type II) appears. In his opinion, this cell is involved in pore formation and also pore maintenance.

Some taste buds possess more than one pore. They occur in large buds which in serial section appear to be multiples of single bud units. Heidenhain (1914) describes similar buds in rabbit's foliate papilla. He advances the theory that these multipored buds constitute a unit called a 'taste field', He presumably thought that these units had some physiological significance, but this has never been substantiated by later workers.

Taste hairs are almost always seen when taste buds are examined with the light microscope (Ranvier, 1882; Heidenhain, 1914; De Lorenzo, 1958), but are never seen as such in the ultrastructural studies (e.g. De Lorenzo, 1963; Farbman, 1965*b*). The taste hair as seen in the light microscope is said to be an artifact (De Lorenzo, 1963) probably due to the formaldehyde fixation. The taste hair is not seen when the material is fixed with osmium tetroxide. However, there is a strong likelihood that the taste hairs of light microscopy and the microvilli of electron microscopy correspond.

The function of embryonic taste buds

It is Beidler's (1961) theory that the taste substances interact with the microvilli and thereby initiate mechanisms which give rise to the sensation of taste. As the

taste microvilli of the electron microscopist and the taste hairs of the light microscopist are probably the same structure, and as we have shown that hairs are present during the 13 to 15-week period it could be reasonable to assume that taste could be functional at that time. Using radiographic techniques on pregnant women, Davis & Potter (1946) could demonstrate foetal swallowing as early as 12 weeks.

In the treatment of hydramnios a foetus can be induced to swallow amniotic fluid if saccharine is introduced into the amniotic cavity (De Snoo, 1937; Windle, 1940). De Snoo subsequently injected methylene blue into the amniotic cavity along with the saccharine and showed that this appeared intermittently in the mother's urine, suggestive that the foetus drank at intervals.

One may speculate that because taste buds are present, and foetal swallowing does occur, that foetal taste buds are functional.

SUMMARY

1. Seventy specimens of human foetal tongue from foetuses 20–40 to 260–280 mm C.R. length were examined microscopically.

2. At 6–7 weeks the tongue epithelium consists of a superficial and deep layer of cells. The superficial layer, periderm, is not present after this period. Collections of cells resembling taste buds are first seen in the seventh week. These cell collections occupy three-quarters of the thickness of the epithelium and stain similarly to the adjacent basal cells.

3. At 11 weeks the presumptive bud is formed. Its cells stain differently than the surrounding basal cells.

4. Between the twelfth and fourteenth week (approximately) the cells of the presumptive bud elongate, pierce the surface as a small tuft of cells. The surrounding surface epithelium forms the taste pore.

5. The definite (adult form) taste bud is recognizable at 13–15 weeks. Its cells extend the height of the epithelium and bulge into the connective tissue papilla. Taste hairs are present on the apical end of bud cells and extend into the base of the pore.

6. At 16–20 weeks some buds continue to develop in complexity. Buds with multiple pores and assymetrical forms resembling multiple buds or two or more buds in juxtaposition are seen.

7. There are a number of cell types in the definite taste bud presumably representing different functional states of the cells.

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