NEW TYPES OF TASTE BUD FORMATION IN HUMAN GUSTATORY PAPILLAE[1]

by

K. KUBOTA and J. KUBOTA

Department of Anatomy, Tokyo Medical and Dental University, Yushima, Tokyo, Japan

With regard to the developmental correlation between the taste bud and the related nerve fibers, there have been articles reported by HERMANN (1884), TUCKERMAN (1889, 1890) and GRÄBERG (1898). They all have stated that the taste bud arises from basal cells of the *epithelium* covering the *papilla* in close relation to the nerve fibers that come near the *epithelium*. CLARA ('40) illustrated his text book of human embryology with GRÄBERG's schematic drawings and gave entirely the same opinion (fig. 1). These authors, however, did not study the morphological relationship between the taste bud and the nerve fibers by the silver impregnation method. NAKAYAMA ('43a, b and '44) studies the development of the tongue innervation, but did not mention this relationship. Recently, this subject has been studied by K. KITAMURA et al. ('58), KUBOTA et al. ('56b, '57, '60) and H. KITAMURA ('61). K. KITAMURA et al, have confirmed in their study on the rat's tongue that the development of the taste bud is attained only by means of contact of the taste nerve with the basal layer of *epithelium*. The study on mice by H. KITAMURA has revealed that the anlage of the taste bud is observed on the first day after birth. In past investigations concerned with the human tongue, we ('56b, '57, '60) have confirmed that the differentiation of the taste bud takes place in close association with the plexiform intraepithelial penetration of nerve fibers. At that time, very specific types of taste bud formation have been found in the *circumvallate papillae* of the tongue of 6-month-old fetuses as described below. Since that was a quite revelation to this subject, some contributory descriptions and discussion of it should be given in this paper.

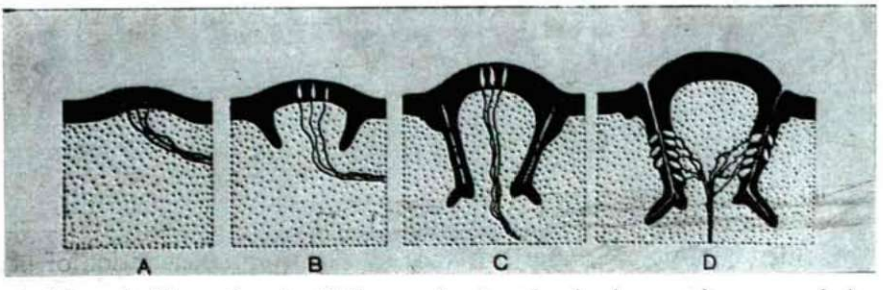

Fig. 1. Schematic illustrations by GRÄBERG, showing the developmental process of the circumvallate *papilla* and taste bud. (Cited from CLARA).

[1] To Professor Dr. AMBRUS ÁBRAHÁM for his seventieth birthday.

Materials and Method

The microscopic preparations examined in this study are frontal and sagittal frozen sections (30—40 microns) of the tongues of human fetuses in various stages: 3-month-old (three), 4-month-old (four), 5-month-old (two), 6-month-old (five), 7-month-old (three), 8-month-old (two) and full-term (two) fetuses. They are stained by the silver impregnation method (KUBOTA et al., '56a).

Observations and Discussion

In the course of the neurohistological study on the development of the lingual *papillae,* we ('57) happened to discover the specific type of taste bud formation in the circumvallate *papillae* of 6-month-old fetuses. In these cases, trench formation in the vallate *papillae* is left unfinished as shown in figures 7, 8 and 9, while it has been already completed in most of the *papillae* in this stage of fetal life. In other words, the indesmolytic portion of *epithelium* partly bars the trench which develops within the common lateral epithelial thickening of the *papilla.* Careful examination of this portion, as shown in figures 10, 11 and 12, reveals that it is composed of cells with large round or ovoidal *nuclei* and is supplied with a great many intraepithelial nerve fibrils which penetrate into it through both basal layers of the common *epithelium.* The detailed drawings of these structures are shown in figures 2, 3 and 4. The nerve fibers are thin, non-myelinated and interlace one another to form a complicated intraepithelial network in which a large characteristic embryonic taste bud is developed. In figures 2 and 3, we can depict other evidences of early development of the taste bud near the large specific anlage, which are characterized by cells with large *nuclei* and the plexiform intraepithelial penetration of the nerve fibrils which have arisen from the well-formed subgemmal *plexus.* These general histological and neurohistological characteristics are quite similar to those of the other embryonic taste buds appearing in the upper *epithelium* of the same *papilla.* From these aspects, it is certain that these indesmolytic portions are a kind of large anlage of the taste bud.

Why does this taste bud development occur? With regard to the morphological relation of taste nerves to taste bud formation, many investigators have stated the following: The taste bud and its nerve fibers develop in a close anatomical relation to each other. The taste bud differentiates from basal layer cells of the *epithelium,* since remarkable strands of nerve fibers are usually seen beneath the embryonic taste bud in the ordinary (not impregnated) preparations (HERMANN, 1884; TUCKERMAN, 1889, 1890; GRÄBERG, 1898; CLARA, '40). LATELY, K. KITAMURA et al. ('58) studied the development of the taste bud in the rat tongue. According to them, in 17-day-old embryos there was neither intraepithelial nerve fiber nor taste bud anlage, while there were rich nervous elements in the vallate *papilla.* In 21-day-old embryos, however, there was the anlage of the taste bud with intraepithelial penetration of nerve fibers. Contrary to this, H. KITAMURA ('61) has noted that the anlage of the taste bud appears first in the 18-day-old mouse embryos, but it is not in direct connection with nerve fibers. However, the definite association of the nerve fibers with the taste bud is present in 1-day-old mice. In this regard, KUBOTA

and KUBOTA ('56b, '57 and '60) have shown evidence that the differentiation of the taste bud is closely related to the plexiform intraepithelial penetration of nerve fibers, i. e., the taste bud differentiates in the *epithelium* after it has been penetrated by the network of the nerve fibrils. GRÄBERG's drawings introduced as fig. 1 show the correlation of the nerve fiber and the taste bud in the growing circumvallate *papilla*. They suggest that the development of the taste bud in the trench walls occurs after the completion of the trench.

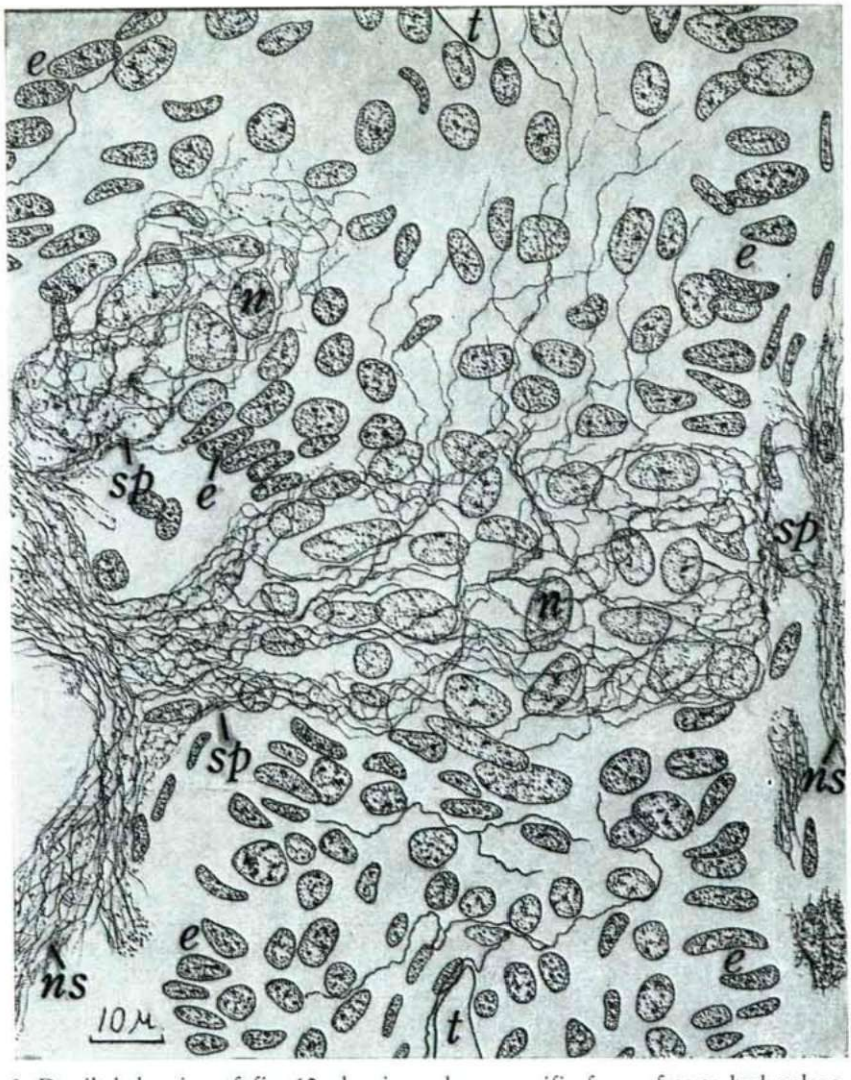

Fig. 2. Detailed drawing of fig. 10, showing a large specific form of taste bud anlage, which is innervated with plexiform intraepithelial nerve fibrils extending from both sides of the *epithelium*. There is evidence of early development of another taste bud, in close association with a plexiform intraepithelial penetration of nerve fibrils, in the upper left quandrant of the picture. ×1000.

According to our microscopic observation, however, most of the taste buds in the future trench walls develop in the common lateral epithelial thickening before the beginning of the trench formation, standing in close association with rich plexiform intraepithelial penetration of nerve fibrils.

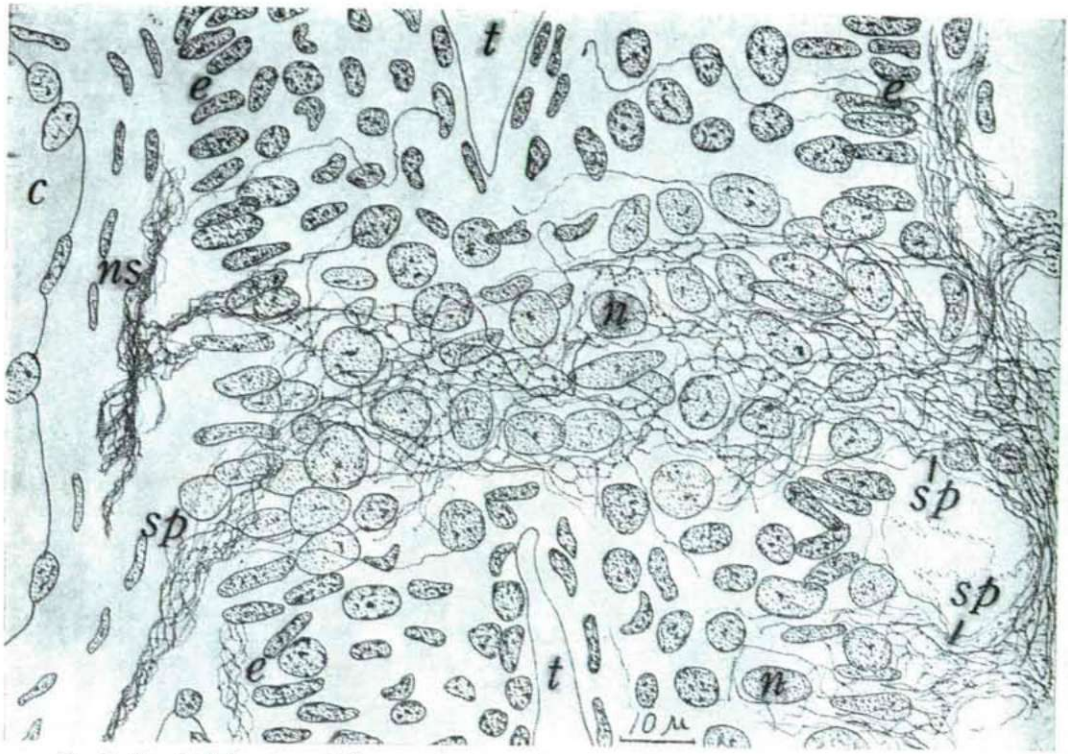

Fig. 3. Detailed drawing of fig. 11, showing a large specific form of taste bud anlage, which is innervated with plexiform intraepithelial nerve fibrils extending from both sides of the *epithelium*. There is evidence of early development of another taste bud, in close association with a plexiform intraepithelial penetration of nerve fibrils, in the lower right quandrant of the picture. ×1000.

Figure 15 shows a growing vallate *papilla* in the tongue of a 5-month-old *fetus*. In the common lateral epithelial thickening of the *papilla* there is no signs of trench formation and there is an advanced developmental stage of many taste bud anlages, which have various shapes and sizes. Especially, on the left of this figure there are seen two anlages in contact with each other (at arrows). Figures 13 and 14 show the common lateral epithelial thickening of the foliate *papillae* in the tongue of 5-month-old fetuses. These thickenings are supplied with abundant nerve fibrils which penetrate into them from each side of the *epithelium*. These nerve fibrils form a complicated intraepithelial network. In this network there are many signs of early development of the taste bud. Also in figure 13 a few nerve fibers are seen running through the embryonic taste bud, which develops through the entire thickness of the

epithelium (at arrows). These above mentioned findings are usually observed in the tongue of the 5-month-old *fetus* when the adjoining two *papillae* develop with a rich nerve supply. At this point, it may be concluded that this peculiar development of the taste bud occurs only in situations as mentioned above.

What changes will these peculiar taste bud anlages undergo thereafter? They were never found in specimens of 7-month or older fetuses. The structure in figure 11 seems to indicate division at the middle plane. Taking these facts into consideration, as illustrated with schematic drawings in figures 5 and 6, it is highly conceivable that they will become separated from each other before the 7th month at the middle plane (on the dotted line in fig. 6e) of the taste bud anlage (white square); one half going to the lateral wall of the *papilla* and the other to the outer wall of the trench (fig. 6f). In this way, which may be called *„transverse division"* of the taste bud anlage, two embryonic taste buds are formed simultaneously from one large peculiar anlage in the common epithelial thickening of the growing vallate and foliate (fig. 6f). This may be understood better from the schematic drawings in figures 5 and 6, in which normal and specific developmental processes of the taste buds in the common epithelial thickening of both vallate and foliate *papillae* are shown. HEIDEN-

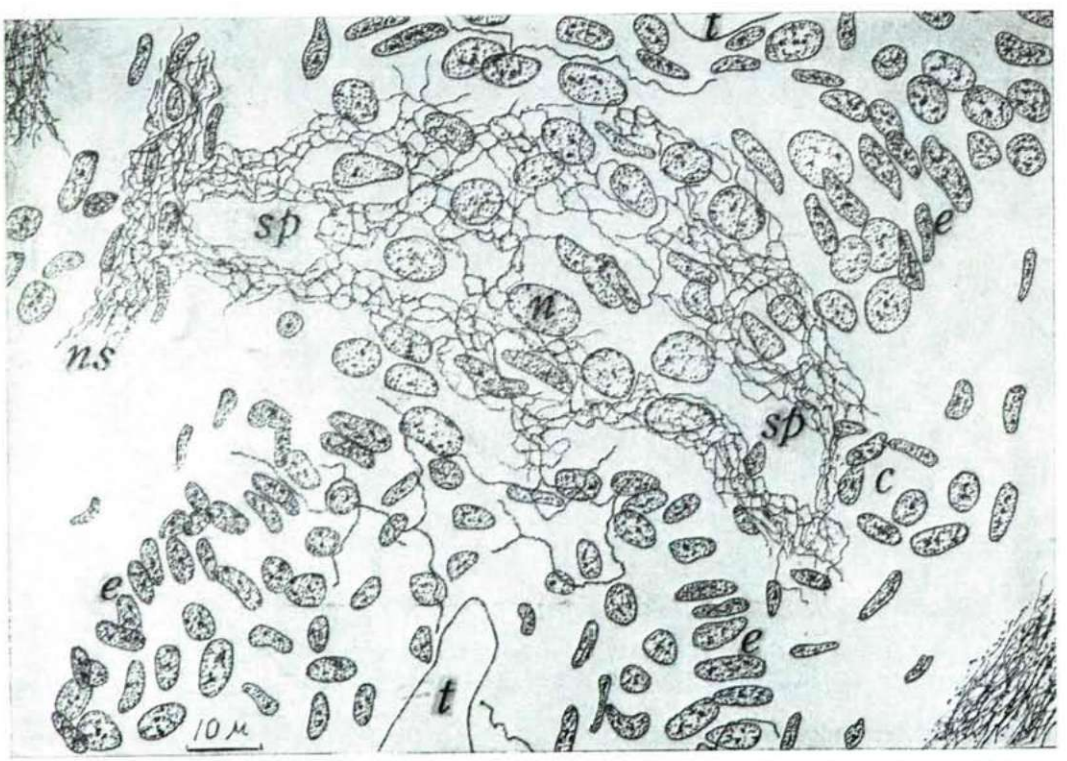

Fig. 4. Detailed drawing of fig. 12, showing a large specific form of taste bud anlage, which is innervated with plexiform intraepithelial nerve fibrils extending from both sides of the *epithelium*. ×1000.

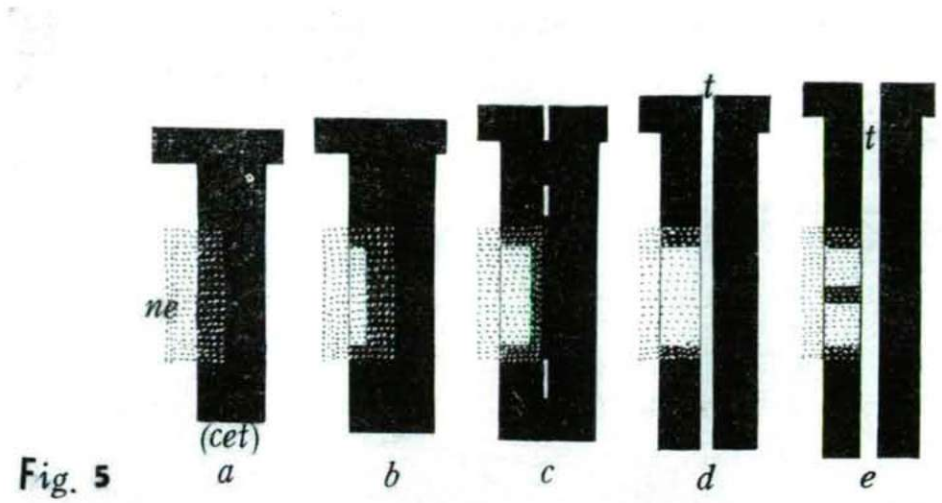

Fig. 5

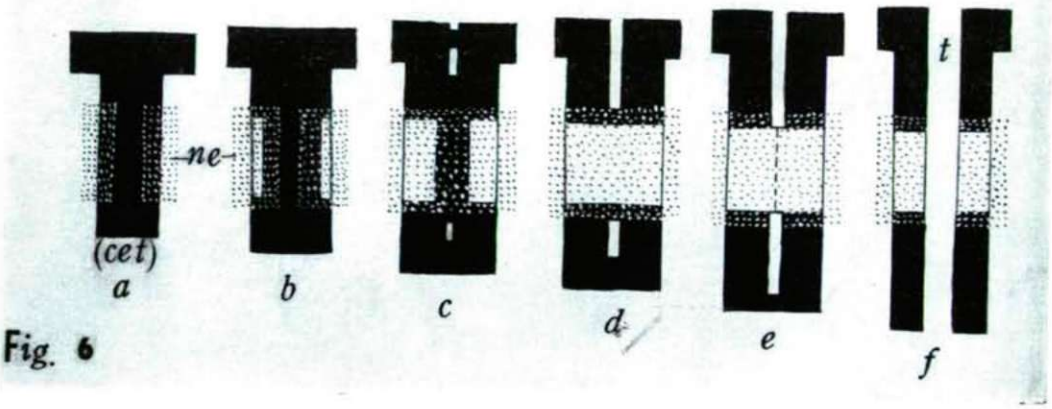

Fig. 6

Fig. 5. Diagrammatic representation showing normal development and relationship of taste bud and nerve fibers in the lateral epithelial thickening of the vallate *papilla*. a; shows the plexiform intraepithelial penetration of nerve fibrils from the papilla side. b; shows an early stage of taste bud formation in the innervated portion of the *epithelium*. c; shows a more advanced stage of taste bud formation and the beginning of trench formation in the *epithelium*. d; shows a large embryonic taste bud white square in the *papilla* wall and the opening of the trench. e; shows a longitudinal division of the embryonic taste bud by an epithelial *septum*.

Fig. 6. Diagrammatic representation of peculiar development and relationship of taste bud and nerve fibers in the lateral epithelial thickening of the vallate *papilla*. a: shows the plexiform intraepithelial penetration of nerve fibrils arising from both sides of the *epithelium*. b; shows an early stage of taste bud formation in the innervated portion of the *epithelium*. c; shows the plexiform intraepithelial penetration of nerve fibrils extending from both sides of the *epithelium* and a more advanced stage of taste bud formation within. d; shows a large specific taste bud anlage white square extending over the *epithelium*, preventing the opening of the trench. e; shows that the anlage is divided in the middle (dotted line), as the trench grows. f; shows a transverse division of the anlage and the opening of the trench.

HAIN ('14) described longitudinal division of the growing taste bud in the rabbit's foliate *papillae*. According to him, the taste buds increase in number by means of this division, which is characterized by an epithelial *septum* as shown in figure 5e. This has been known as „Teilkörpertheorie" since that time. Viewed in this light, in the course of the taste bud formation there are two processes; transverse (fig. 6) and longitudinal (fig. 5) divisions. In this manner, the taste buds increase in number and diminish in size.

Summary

In preparations of 6-month-old human fetal tongues, stained by the silver impregnation method, specific types of taste bud formation were found. The taste bud anlage was noted to develop in the common lateral epithelial thickening extending over the *epithelium*. It was supplied with abundant plexiform intraepithelial nerve fibrils which arose from the subgemmal nerve *plexus* on both sides of the *epithelium* through its basal layers. It was noted that during the growth of the *papilla*, it is transversely pulled off into two embryonic buds in the middle; one half going to the *papilla* side, the other to the outer wall of the trench. This was called the transverse division of the embryonic taste bud as opposed to the longitudinal division described by HEIDENHAIN.

Plate 1.

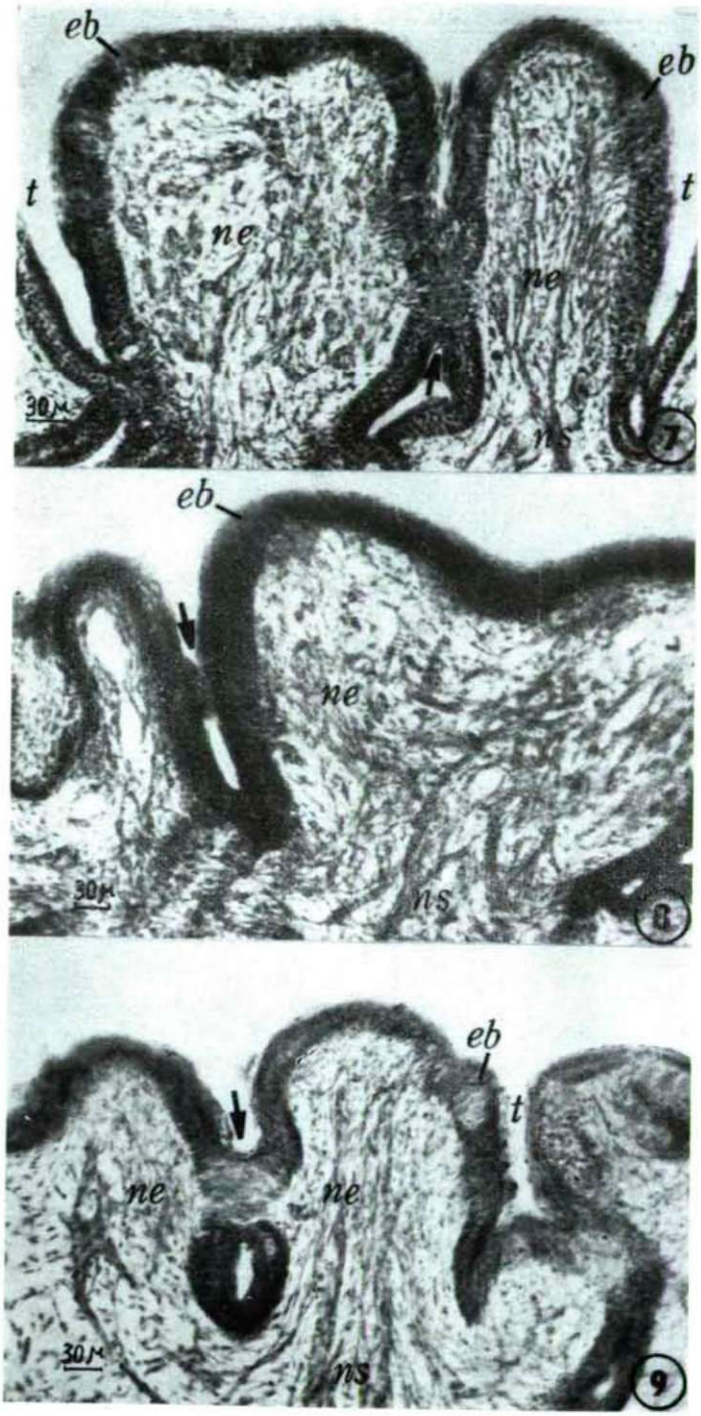

Plate 2.

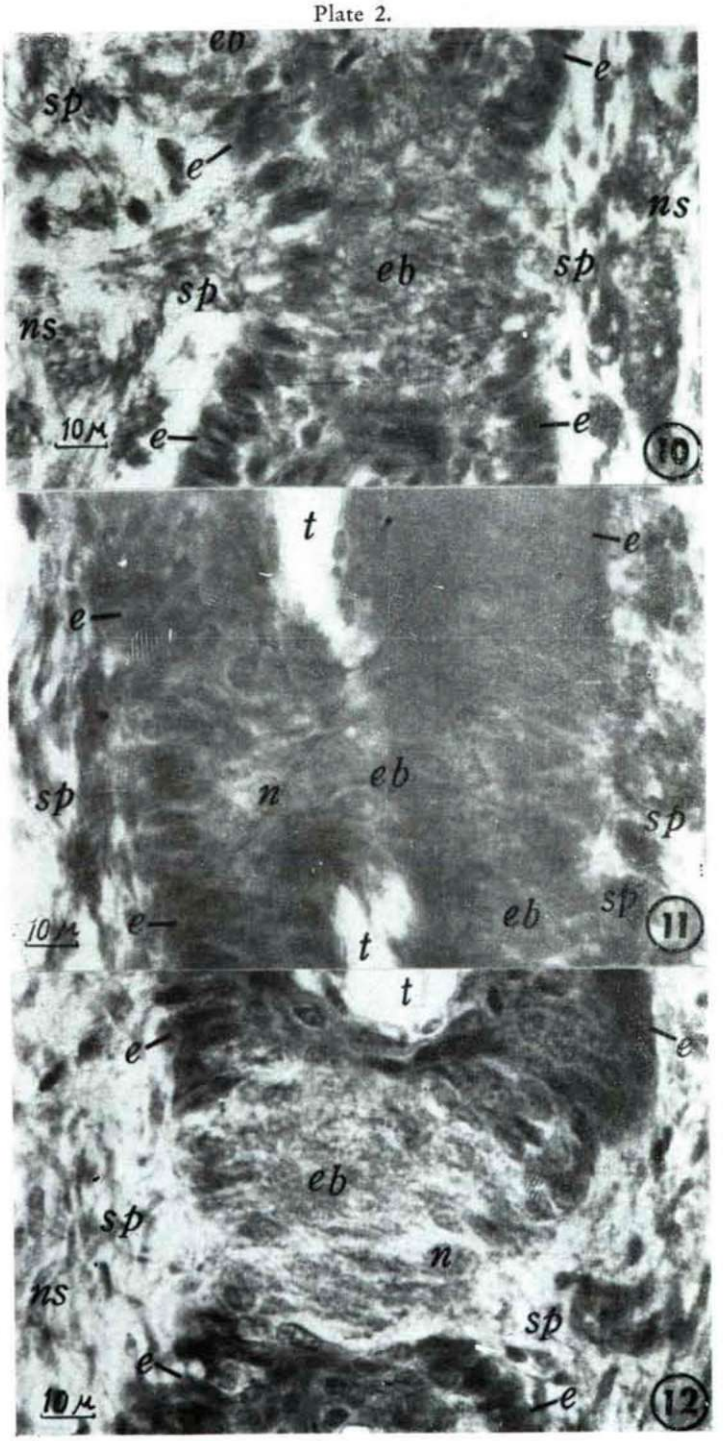
eb
e
sp
e
ns
eb
sp
sp
ns
10μ
e
e
10
t
e
e
sp
n
eb
sp
10μ
e
eb
sp
11
t
t
e
e
eb
sp
n
ns
sp
e
10μ
e
12

Plate 3.

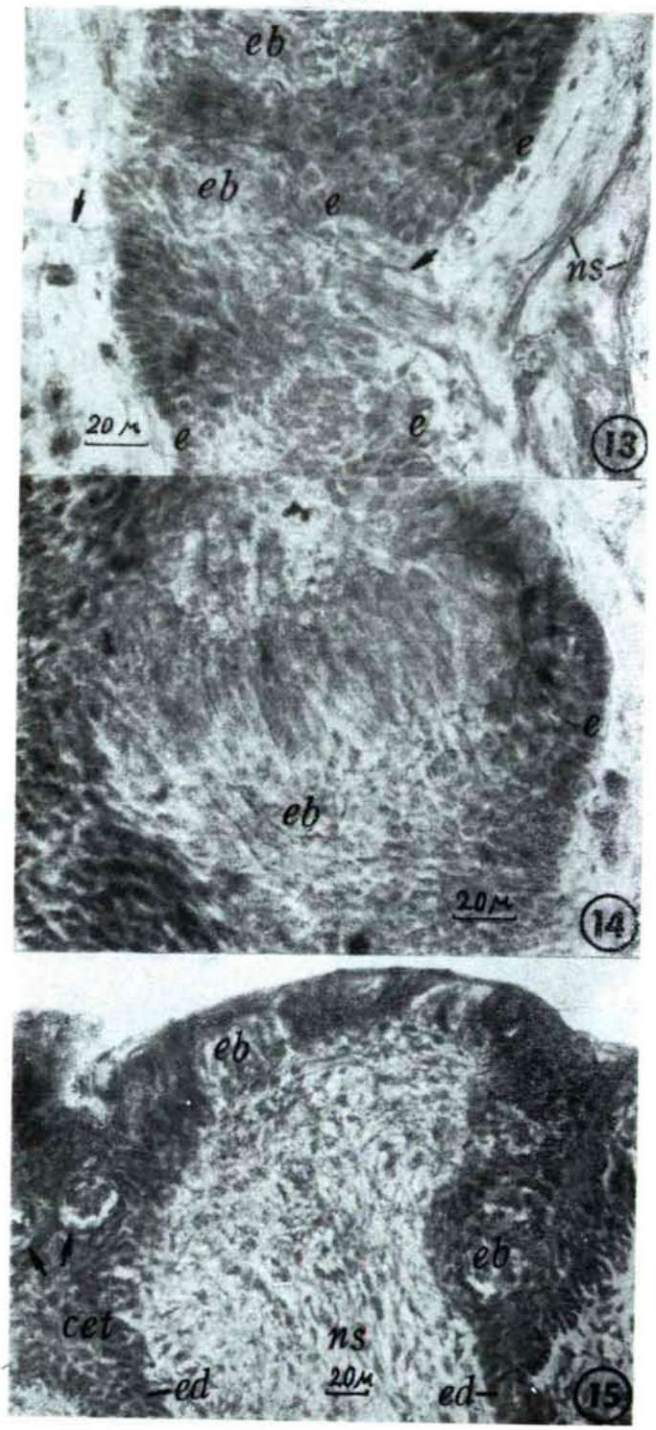

Plate 1.

Explanation of Figures

All photographs were taken from the preparations stained by the silver impregnation method.

7. Photomicrograph of the circumvallate *papillae* on the tongue of a 6-month-old *fetus*, showing the opened outer moat and the unopened inner moat (at arrow). ×200.
8. Photomicrograph of a circumvallate *papilla* on the tongue of a 6-month-old *fetus*, showing the unopened trench (at arrow). ×200.
9. Photomicrograph of a circumvallate *papilla* on the tongue of a 6-month-old *fetus*, showing the unopened trench (at arrow). ×200.

Plate 2.

Explanation of Figures

All photographs were taken from the preparations stained by the silver impregnation method.

10. Magnified picture of the indesmolytic portion of the *epithelium* indicated at the arrow in fig. 7, showing the peculiar taste bud anlage with a rich intraepithelial nerve supply, which extends from both sides of the *epithelium*. ×850.
11. Magnified picture of the indesmolytic portion of the *epithelium* indicated at the arrow in fig. 8, showing that it is a specific form of the taste bud anlage with rich inraepithelial nerve supply which is arising from the subgemmal *plexus* on either side of the *epithelium* and that it seems to indicate division at the middle plane. ×850.
12. Magnified picture of the indesmolytic epithelial portion indicated at the arrow in fig. 9, showing that it is a specific form of taste bud anlage made up of cells with large round or ovoidal *nuclei*. ×850.

Plate 3.

Explanation of Figures

All photographs were taken from the preparations stained by the silver impregnation method.

13. Photomicrograph of a part of the common epithelial thickening of the foliate *papillae* in a 5-month-old *fetus*, showing the plexiform intraepithelial penetration of nerve fibrils and a peculiar taste bud formation within. Note the nerve fibers (at arrow) run through the *epithelium*. ×500.
14. Photomicrograph of a part of the common epithelial thickening of the foliate *papillae* in a 5-month-old *fetus*, showing the plexiform penetration of nerve fibrils and taste bud development within. ×500.
15. Photomicrograph of an embryonic circumvallate *papilla* on the tongue of a 5-month-old *fetus*, showing that the taste bud develops in the lateral epithelial thickening before beginning of trench formation. Note that there are two anlages of taste bud coming into contact with each other (at arrow). ×340.

Abbreviations in Text Figures and Plates

c, blood capillary
cet, common epithelial thickening
e, basal layer of *epithelium*
eb, embryonic taste bud
ed, embryonic duct of EBNER's gland
n, nucleus
ne, nervous element
ns, nerve fibers
sp, subgemmal nerve *plexus*
t, trench

Acknowledgment

The authors would like to thank Mr. FUMIO HIROSE and the staff of the photography department for helping with the photographic work.

Literature Cited

CLARA, M.: 1940 Die Entwicklung der Zunge. Entwicklungsgeschichte des Menschen, S. 300. Leipzig.

GRÄBERG, J.: 1848 Beiträge zur Genese des Geschmacksorgans des Menschen. Schwalbe Morph. Arbeit., *8*:117—134.

HEIDENHAIN, M.: 1914 Über die Sinnesfelder und die Geschmacksknospen der Papilla foliata des Kaninchens. Beiträge zur Teilkörpertheorie III. Arch. f. mikr. Anat., *85*:365—479.

HERMANN, FR.: 1884 Beitrag zur Entwicklungsgeschichte des Geschmacksorgane beim Kaninchen. Arch., f. mikr. Anat., *24*:216—229.

KITAMURA, H.: 1961 Histologic studies on the lingual papilla- and taste bud innervation of the mouse tongue. J. J. S. S., *10*:81—92. (Japanese.)

KITAMURA, K., UENO, M., MAKAIBO, S., DOZONO, K., and IWASAKI, S.: 1958 Microscopical studies of the innervation in the oral tissues of various animals. (Part 1.) On the association between the taste bud and the nerve. Kyushiu Shika Gakkai Zassi, *11*:513—519. (Japanese.)

KUBOTA, K., SATO, Y., and KUBOTA, J.: 1956a Silver-impregnation method of peripheral nerves with frozen sections. J. Jap. Stomatol. Soc. (Kokubyo Zassi), *23*:267—274. (Japanese.)

KUBOTA, K. and KUBOTA, J.: 1956b Developmental studies on the innervation of the human lingual papillae. Ibid., *23*:354. (Japanese.)

— — 1957 A new aspect concerning the development of the taste bud of lingual papilla in man. Acta Anat. Nippon. (Kaibo Z.), *32*:Supplement 10. (Japanese.)

— — 1960 Contribution to nerve development of so-called gustatory papillae in human tongue. Bull. Tokyo Med. Dent. Univ., *7*:475—505.

NAKAYAMA, T.: 1943a On the intraepithelial nerve fibers in the human tongue. (Include the development of the lingual papillae.) Tohoku Med. J., *33*:447—456. (Japanese.)

— — 1943b Histological studies on the innervation of human lingual papillae, especially on the endings of its sensory nerve fibers. Tohoku Med. J., *33*:474—485. (Japanese.)

— — 1944 On the innervation of the taste buds of the human tongue. Tohoku Med. J., *34*:387—395. (Japanese.)

TUCKERMAN, F.: 1889 On the development of the taste-organs of man. J. Anat., *23*:559—582.

— — 1890 Further observations on the taste-organs of man. Ibid., *24*:130—131.