THE DISTRIBUTION OF GEMMA GUSTATORIA IN SHEEP

by

BUSONO

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INTRODUCTION

The first authors who described the taste buds of sheep were Lovén and Schwalbe (1867-1868), but little was done about the distribution. Schwalbe (1968) doubted whether taste buds could be found on the surface of the fungiform papillae of sheep. Lovén (1868), however, made a statement that the fungiform papillae indeed had taste buds on their surface. Trautmann and Fiebiger (1931) were of the opinion that taste buds were found on the fungiform and circumvallate papillae of sheep and swine. Furthermore, most of the authors agreed that all circumvallate papillae have taste buds on the side wall, either on the entire area or on only part of it. They mentioned that taste buds could be found in fungiform, foliate and circumvallate papillae, the soft palate, epiglottis, and the outer rim of the ligamentum vocale, but the animal species were not denoted.

Since sufficient literature on this subject was not available the author felt that additional information was needed in regard to the morphology and distribution of the taste buds on the adult and embryo sheep. In addition, a new simple staining method called "contour staining" was used.

This research is far from being complete and could be extended by other colleagues to include the number of taste buds for the entire tongue with age variations.

MATERIALS AND METHODS

Three entire embryos and two heads of adult sheep with their cavum oris still intact were procured. Parts of the heads that were examined consisted of the tongue, lips, hard palate including the papilla incisiva, conical papillae lateral to the tongue, soft palate, oro-pharynx, naso-pharynx, aditus laryngeus, and aditus oesophageus.

The tissues were previously fixed in their formalin preservative so further fixation was unnecessary. Dehydration was performed by the use of acetone for one hour in each series of two so that this process covered two hours. The specimens were placed several millimeters above the bottom of the dehydration jar, otherwise water extraction would be poor and contamination of the specimens with extracted water would annihilate dehydration, since it is well known that in dehydration, water is always heavier than the dehydrating solution and occupies the bottom of the jar. To elevate the tissue, plastic acetone-proof perforated capsules were used, which raised the specimens to the required height. Thorough dehydration was checked by placing the specimens on filter paper. If, after evaporation of the acetone, water was still detected, dehydration should be continued.

Clearing was carried out with benzene under the same time requirement, i.e., one hour in each clearing jar of the series. Complete clearing was accomplished within 2 hours, with the specimens becoming transparent; otherwise clearing was considered insufficient. This procedure made the specimens easily

infiltrated by paraffin. Paraffin infiltration was done in a vacuum oven adjusted to 12 pounds pressure. The first infiltration, lasting half an hour, was intended to eliminate the benzene and the second was the actual paraffin penetration. Blocking was done in the usual manner and left at least two hours at room temperature.

The whole mapping technique depended on the sectioning and sampling of specimens. The cutting was divided into two methods, serial and spacing. The author used the serial sectioning in anatomical regions where taste buds were expected to be located and where buds were numerous, e.g., in regions of circumvallate and fungiform papillae. In regions where taste buds were scarce, a spacing method was carried out and the distances between the sections were determined to be between 50 and 100 microns (micra). During paraffin sectioning, either by the spacing or serial method, recording of the handling was carefully taken into consideration to enable the author to reconstruct the actual arrangement of the taste buds.

A total of 1369 slides and 2780 sections were made. The number of slides did not correspond to the number of sections, there being in one slide 2 to 5 sections in serial paraffin cutting. Cutting the sections and making the spatial calculations were facilitated by cutting tissues 10 microns thick.

The first 328 slides were stained by the Harris hematoxylin-eosin method , but realizing that this method was time-consuming, the author tried out other dyes to obtain a mono-staining method good enough to show the taste buds. Gentian violet was tried,

but was found to be not simple enough for this purpose. Finally, cresyl violet was used as a 0.25% solution in distilled water and it proved excellent for this study. The staining procedure is explained as follows:

After deparaffinization in 2 consecutive xylene solutions, sections were consecutively immersed in decreasing dilutions of alcohol: 100%, 95%, 80%, and 40% and finally in distilled water using 1 minute for each dip. Dipping in cresyl violet lasted 15 to 45 seconds, depending upon the age of the staining solution. The older the staining solution, the longer the dipping period. In a freshly prepared cresyl violet solution 15 seconds were sufficient, but after a month or two 45 seconds or even a minute was needed. The intensity of staining could also be ascertained after rinsing in tap water for ½ minute. Ascending percentages of alcohol solutions: 50%, 70%, 95%, and 100% were then used for dehydration previous to the final clearing and covering of sections.

The above staining method distinguished between a taste bud and a papilla of the runica propria, a difficulty found with hematoxylin-eosin staining. With cresyl violet, the image of a taste bud was clear and a papilla of connective tissue appeared bluish-grey. The author did not conclude that this staining procedure was a standard taste bud-stain. However, it was useful as a tracing or mapping tool to discover the distribution of taste buds and as a general "contour stain." Cresyl violet has previously been used primarily for staining Nissl granules. The 0.25% concentration in addition to being a contour stain has the

following staining characteristics for other tissues:

1. Muscle : blue
2. Nuclei : violet
3. Epithelium : violet
4. Reticular fiber : red to violet
5. Cartilage : red
6. Erythrocyte : grey
7. Lingual glands : red to violet
8. Pharyngeal glands :
   a. Serous : blue
   b. Mucous : red to violet

REVIEW OF LITERATURE

The first person who described taste papillae was Malpighi (1664). He called them lingual papillae but did not recognize the taste buds.

The oldest literature concerning taste buds of sheep was that of Lovén (1868) and Schwalbe (1867-1868). Schwalbe (1867), in his preliminary paper, stated that M. Schultze and F. E. Schultze had observed holes on the free surface of the vallate papillae corresponding to the secondary papillae in the pavement epithelium at the base of which fibrillate-tapered elements ended. Later, M. Schultze could not find any confirmation of these observations, and did not pursue the matter further.

Schwalbe took this matter up again and gave the following results.

His observations were based on the tongue of sheep, ox, horse, rabbit, dog, and cat. According to him, in the consideration of the circumvallate papillae, one must separate the free surface facing the oral cavity from the sides that turned to the

circular ridge. In the sheep, on the surface which faces the oral cavity, numerous secondary papillae were found which were absent at the points turned toward the circular ridge. The slope of the papillae protected by the pavement epithelium was composed of considerably smaller spinous cells. This thin epithelial layer was dotted with strange formations in a straight line from the fibrous basic layer up to the surface facing the circular groove. These formations were mounted on the fibrous stroma with an almost circular base. From the base towards the exterior they were cylindrical and gradually increased in thickness to reach its greatest diameter below the upper surface of the epithelium, after which it tapered rapidly below an opening in the pavement epithelium. This was the first description of the taste bud made by Schwalbe in mammals or vertebrates and he concluded that on the opposite side of the ridge and also in the papillae fungiformes of the animals so far examined, these formations (taste buds) could not be found.

Lovén (1868) cited the investigations of Billroth and Axel Key on the frog's taste cells and their relations to nerves. According to Kölliker (as quoted by Lovén) the epithelium of the gustatory papillae (taste buds) in higher animals offered no peculiarities from which could be drawn conclusions of similar relation, as in the frog. To fill this gap, Lovén made further examination on the vallate papillae and fungiform papillae of the calf's tongue. He found taste buds only on the neck of the papillae, which is that part of the papilla directly adjacent to the circular groove. They are situated with a narrower neck

directly on the mucous membrane, swelling up club-like at the outer part of the epithelium, coming promptly to a point and ending in or immediately within the holes occurring on the surface. This statement was made in the month of June, 1868, and three months later Lovén continued his investigations on the sheep, pig, dog, horse, rabbit, and rat. He was of the opinion that in sheep, calf, and man, both the circumvallate papillae and some of the fungus-shaped papillae were provided with taste buds. In the rabbit and rat these formations were observed on all fungiform papillae.

Schwalbe (1868) made further observations on the tongues of sheep, cattle, deer, pig, horse, cat, dog, rabbit, hare, and guinea pig. In addition, he was fortunate to be able to examine some human tongues within two to five hours after death. He divided the mammals into three divisions according to the number and arrangement of the vallate papillae. In sheep, cattle, and deer the occurrence of circumvallate papillae is limited to two longitudinal positions situated at the caudal portion of the back of the tongue. Here are found from 10 to 15 papillae of various sizes located at various distances from one another. In the horse, pig, and the rodent, only two large diameter papillae are found. They are of equal size and are located one on each half of the body of the tongue. In the horse, another smaller papilla could be found caudal to the large papillae in the position corresponding to the foramen caecum of the human. In carnivores and man, more than two papillae are present. In the dog and cat, there are usually 3 papillae on each side of the tongue although

cats sometimes have only 2 papillae present, In man, the usual number is known to be 7 to 9. An interesting complication of the deer's circumvallate papillae was mentioned. On the upper surface of a regularly formed vallate papilla, a fungiform papilla seemed to be inserted. On the surface of human vallate papillae, small pores were frequently seen with the naked eye. These correspond to the excretory ducts of acinose glands and this situation was also observed in all mammals. Schwalbe confirmed that these glands were completely absent in the neighborhood of the true fungiform papillae. During Schwalbe's examination of various sheep tongues, the occurrence of ramified pigmented cells within the deep layers of the epithelium of the vallate and fungiform papillae were detected. These pigmented cells could be observed macroscopically as black spots. Schwalbe made the following table of the greatest lateral diameter of the taste buds in:

Man	0.0396 mm	Deer	0.0408 mm
Dog	0.0306 mm	Ox	0.0480 mm
Cat	0.0324 mm	Pig	0.020 to 0.0521 mm
Hare	0.0324 mm	Sheep	0.0360 to 0.0540 mm

Schwalbe said that he was not able to detect taste buds outside the region of vallate or fungiform papillae. He had examined in vain the palatine mucous membrane and the arcus glossopalatinus. He was also unsuccessful in observing the structures in question on genuine fungiform papillae. According to Schwalbe, the number of taste buds in a sheep was 9600, in an ox 35,200, in a pig 9520. Finally, he described, in detail, the morphology, histology, and

innervation of taste buds.

Trautmann and Fiebiger (1931), 63 years after Lovén and Schwalbe's statement, stated that taste buds are found in the fungiform papillae of sheep and swine, are less numerous in carnivores, and are rare in the horse and ox. In circumvallate papillae, the side walls were occupied by taste buds. The superficial part of the papillae and the surrounding wall were mostly devoid of taste buds. In the horse, swine, and dog, but not in cats, foliate papillae also had taste buds. Traumann and Fiebiger had the following opinion concerning the distribution of taste buds: Taste buds can be found in fungiform, foliate, and vallate papillae as well as in the soft palate, epiglottis, and ligamentum vocale. They did not mention the species of animals involved.

Arey, Tremaine, and Mozingo (1935) described the numerical and topographical relations of taste buds to human circumvallate papillae throughout the life span. A total of 51 human cadavers and 152 vallate papillae were studied using serial sections made at 20 microns in thickness and stained by the hematoxylin-eosin method. Including Heiderich's experiment the following data were obtained:

Number of individuals	Age (yrs.)	Number of vallate papillae obtained
38	20-70	106
13	74-85	46
48	0-20	96 (Heiderich)

A summary of their observations was as follows:

1. The mean number of taste buds on a papilla.
    a. Newborn to maturity (0-20 yrs.) 245
    b. Maturity to old age (20-70 yrs.) 208
    c. Extreme old age (74-85 yrs.) 88
2. The mean number of taste buds on a trench wall.
    a. At first postnatal year 10
    b. Next two years 18
    c. Between the 4th and 20th yrs. 74
    d. Between maturity and old age 48
    e. At extreme old age 13
3. Taste buds were not found on the superficial surface of the papillae.
4. Involution occurred in the papillae and initially involved the more laterally situated papillae. The most resistant part of the papillae was the central part.

Sisson and Grossman (1953 and 1961) stated that taste buds occurred especially in the foliate, fungiform, and vallate papillae, in the free edge and anterior pillar of the soft palate and in the oral surface of the epiglottis. Nothing was mentioned about the age and species of animal involved.

Lalonde and Eglitis (1961) discussed the number and distribution of taste buds on the epiglottis, pharynx, larynx, soft palate, and uvula in a newborn human. A total of 2583 serial sections were cut at 10 microns in thickness and stained by Delafield's hematoxylin-eosin method. Their findings were as follows:

Region	Number of taste buds
1. Soft palate	419
	254 in stratified squamous epithelium on the oral surface.
	165 on posterior free edge, lateral to uvula.
2. Surface of uvula	none
3. Epiglottic valeculae	48
4. Glossoepiglottic fold	3
	near attachment to dorsum linguae.
5. Epiglottis	949
	3 at the very tip of anterior surface.
	946 scattered on its posterior surface.
6. Oral pharynx	267
	32 in posterior wall along mid-dorsal line.
	135 in right lateral wall.
	100 in left lateral wall.
7. Laryngeal pharynx	656
	26 scattered along length of posterior wall.
	10 in right lateral wall.
	7 in left lateral wall.
	613 in anterior wall.
8. In triangular shaped vestibule of larynx	241
9. Lips of inlet of larynx	213
10. In vestibule of larynx on left ventricular fold	1
11. In ventricular recesses of laryngeal cavity	none

Kinziro Kubota et al. (1962) made anatomical and neuro-histological observations on the tongue of the Great Anteater (Myrmecophaga Jubata Linn) and the Pangolin (Manis pentadactyla Linn.), and detected some peculiarities. In the anteater, on the dorsal surface of the tongue, 2 papillae of the circumvallate type were found. In the epithelium of the lateral wall of those papillae and the outer wall of the trench, taste buds of various sizes were seen. Most of the taste buds were located around the termination of gland ducts which open into the trench. This correlation between gland ducts and taste buds also was traced in the Kangaroo's tongue, namely around the ducts of serous glands in the postero-lateral region. In the mouse, a few taste buds were detected at the openings of the submandibular, sub-lingual, and palatine arch glands (Nakamura, 1960). In the anteater, the vallate papillae were primarily secretory rather than concerned with the sense of taste. The pangolin's tongue had three circumvallate and poorly developed filiform and fungi-form papillae on its dorsum. The taste buds were located only in the lateral wall of the papillae and were closely associated with the subgemmal plexus. The serous type lingual glands were present only deep within the muscle and not within the papillae. On the apex of the tongue, fungiform papillae of a primitive type were developed. These papillae were richly supplied with nerve fibers which penetrated the thickened epithelium. This indicated a primordial form in the differentiation of taste buds.

In addition to the above literature, other supporting data needed by the author during his histological work are included.

For better understanding of the taste buds, works on the physiology of the taste buds were considered necessary.

Abercrombie (1946) discovered a method to estimate nuclear population in microtome sections. This was also of importance in the investigation of the cellular density of taste buds. He introduced the equation:

$$P = A \frac{M}{L + M}$$

in which P = average number of nuclear points per section

A = crude count of number of nuclei seen in section

M = thickness (in microns) of nuclei

L = average length (in microns) of nuclei.

By nuclear points, he meant any geometrical point of the same relative position in all nuclei at right angles to the microtome section. Agduhr (1941), cited by Abercrombie, used another formula:

$$P = A \frac{2 S M - L}{S M (S+L)}$$

in which P = average number of nuclear points per section

A = crude count of number of nuclei seem in section

S = whole number of sections through or into which average nucleus extends

L = average length (in microns) of nuclei

M = thickness (in microns) of section.

Dornfeld, Slater, and Scheffe (1942) devised a method for determining the volume and cell number in small organs, and the formula used was:

$$V = \left[\frac{P \times Y\ (645)}{M^2}\right]\left[\frac{t}{1000}\right]$$

where V = volume of organ in cubic millimeters

P = planimeter reading in square inches

M = linear magnification of the camera lucida projection

t = thickness of the sections in microns

X = number of sections per horizontal inch on the graph

Y = square inches per vertical inch on the graph.

The first bracket gives the sum of the areas of all sections in the serial reduced to square millimeters. The planimeter reading (P) multiplied by XY gives the sum of the areas in square inches and includes the camera lucida magnification. The constant 645 is the number of square millimeters in 1 square inch. The magnification of the areas of the sections produced by the camera lucida projection is eliminated in dividing by M^2. Finally, multiplication by the second bracket, which is merely the thickness (t) reduced to millimeters, gives the volume of the organ.

Dempster (1942) explained the mechanics of paraffin sectioning by the microtome and mentioned that for the intelligent use of the microtome knife, 5 or 6 factors were important:

a. bevel angle
b. sharp knife
c. facet polish
d. clearance angle
e. rake angle

A special chart for determining clearance angle was constructed. A wide rake angle would give less compressed sections. Clearance angles as low as 1^{o} were effective if the inner facet was kept

clear of gummy accumulations of paraffin.

Read and Johnstone (1961) gave an example of how to work out the distribution of parietal cells in the gastric mucosa of a cat. The stomach was stretched and kept in formalin a minimum of 10 days. The greatest length and width of the stomach was 15 cm. Thirty round samples (0.7 cm in diameter) were collected by the use of a sharpened cork borer and later mounted for sectioning according to the usual procedure. From each sample, 4 sections at 6 microns in thickness were cut and stained with hematoxylin-eosin. In each sample, 5 random high dry views were selected and the total number of parietal cells observed in each field was counted.

Some facts about the physiology of the taste buds were needed among other things.

Beidler (1954) was of the opinion that the response of a taste receptor to Na-salt stimulation clearly indicated that the ions of chemical stimulus were loosely bound to some substance. This could be thought of as an initial reaction which ultimately led to the stimulation of the receptor and an eventual depolarization of the associated sensory neuron.

In his biophysical approach to taste, Beidler (1961) revealed the theory of receptor stimulation and the dynamic character of taste cells. He suggested a possible mechanism of receptor stimulation. The cells of taste buds were normally electrically charged, the interior being negative with respect to its environment with a difference in concentration of ionic constituents between the cell interior and exterior. Adsorption of

the stimulus (electrolyte or non-electrolyte) to the site of the receptor surface of the taste cell might result in a slight change in the spatial configuration of the receptor molecule in such a way that a hole was formed large enough for certain ionic species contained within the cell to escape to the exterior and thus decrease the potential across the receptor membrane. Spread of local depolarization over the rest of the cell surface might stimulate the innervating nerve. It was found that no two receptors had identical sensitivities. The quality discrimination of taste had been established and rodents were supposed to respond much better to NaCl than KCl. The reverse was true in carnivores. The hamster and guinea pig responded quite well to sugar, while the rat and dog did not respond well and the cat not at all.

Most of the receptor cells, if destroyed, could not be replaced, but this was not the case with taste buds. The cells surrounding the taste bud, which were ordinary epithelial cells, continually divided and the daughter cells passed into the taste bud. These were then innervated and were transformed into taste cells with special microvilli and sensory functions. The average life of such a cell was about 300 hours. Experiments were carried out by injecting colchicine and triated thymidine into animals.

OBSERVATIONS AND DISCUSSION

Schwalbe (1868), in his experiments, used iodine serum, super osmic acid sol (1%), or potassium bichromate (0.25%) for fixation of his specimen. This method was excellent for cellular

examination but difficult for topographical study.

Lalonde and Eglitis (1961) fixed their embryo in Heidenhain's "Susa" and stained it with Delafield's hematoxylin-eosin. This procedure was good for mapping, but excessive time was needed. Ordinary hematoxylin-eosin staining needs at least 25 to 27 minutes before mounting.

Arey et al. (1935) preserved their specimens in Bouin's fluid and stained them with hematoxylin-eosin. This procedure was time consuming.

The cresyl violet method utilized by the author was originally a stain for the Nissl substance. The standard solution consisted of cresyl violet 0.5 Gm, distilled water 100 cc. In using this, a regressive staining method should be performed. However, the author used this stain as a progressive stain and the entire process required only 19 minutes. In comparison with the hematoxylin-eosin method, this meant a difference of 8 minutes for each slide. Another advantage of this cresyl violet method was the ease of microscopic observation (compare Plate V, Figs. 1 and 2). The percentage of cresyl violet solution was decreased from 0.5 to 0.25% after some trials had been performed in order to establish an ideal percentage.

Previous to embedding in paraffin, dehydration and clearing procedures were performed with acetone and benzene rather than with alcohol-chloroform because less time was necessary. The latter method required a minimum of 40 hours, whereas the acetone-benzene method required only about 4 hours. The disadvantage of the acetone-benzene method was the approximately 10-15% shrinkage

of the specimen. For mapping purposes, however, this shrinkage was not serious. The sampling and result of these efforts of mapping were summarized as follows:

Sheep	Samples	Result
Sh.I (fetus)	1. Apex linguae	Taste buds were found on the fungiform papillae.
	2. Radix linguae	No taste buds were found.
	3. Circumvallate papillae	Many taste buds on the side walls of papillae and few on the oral surface.
	4. Corpus linguae (median part)	Not all fungiform papillae had taste buds.
	5. Palatum molle	No taste buds were found.
	6. Nasopharynx	" " " " "
	7. Oropharynx	" " " " "
	8. Larynx	" " " " "
	9. Labium inferius oris	" " " " "
	10. Anterior part of oesophagus	" " " " "
Sh.II (adult)	1. Apex linguae	Taste buds were found on the fungiform papillae.
	2. Radix linguae	No taste buds were found.
	3. Circumvallate papillae	On the side walls, taste buds were found and none on the oral surface.
	4. Median part of corpus linguae	Not all fungiform papillae had taste buds.
	5. Palatum molle	No taste buds were found.
	6. Palatum durum	" " " " "

Sheep	Samples	Result
Sh.II (adult)	7. Transition from lower jaw mucosa to ventral side of tongue plus conical papillae	No taste buds were found.
	8. Transition from hard palate to cheek mucosa plus conical papillae	" " " " "
Sh.III (fetus)	1. Apex linguae	Taste buds were found on the fungiform papillae.
	2. Labium inferius	No taste buds were found.
	3. Circumvallate	On the side walls, taste buds were found and a few on the oral surface.
	4. Pharynx	Taste buds were found on 50% of the sections and they were larger than those found on the tongue.
	5. Epiglottis	Taste buds were found.
	6. Radix linguae	No taste buds were found.
	7. Palatum molle	" " " " "
	8. Palatum durum	" " " " "
	9. Papilla incisiva	" " " " "
Sh.IV (fetus)	1. Pharynx, larynx, esophagus, and radix linguae (one sagittal section)	On the pharynx, taste buds were found near the larynx. Epiglottis had taste buds on the cranial and caudal sides. That part of the pharynx caudal to the radix linguae was devoid of taste buds.
	2. Apex linguae	Taste buds were found on the fungiform papillae (1 or 2 taste buds on each papilla).

Sheep	Samples	Result
Sh.IV (fetus)	3. Circumvallate region	On the side walls, taste buds were found and a few on the oral surface.
	4. Median part of corpus linguae	Not all fungiform papillae had taste buds.
	5. Palatum durum	No taste buds were found.
	6. Papilla incisiva	" " " " "
Sh.V (adult)	1. Apex linguae	Taste buds were found on the fungiform papillae.
	2. Epiglottis	Some taste buds were found.
	3. Oesophagus	No taste buds were found.
	4. Pharynx	A few taste buds were found.
	5. Circumvallate papillae	On the side walls, taste buds were found and none on the oral surface.
	6. Radix linguae	No taste buds were found.
	7. Palatum molle	" " " " "

After studying all the sheep heads, the general distribution of taste buds was found to be:

In an embryo or an adult sheep, the fungiform papillae on the apex usually have 1 or 2 taste buds. On the corpus linguae or dorsum linguae, some of the fungiform papillae bore taste buds. Macroscopically, some of these fungiform papillae were blackish-grey due to the occurrence of ramified pigmented cells described by Schwalbe (1868). This was not related to the presence or absence of taste buds. This finding is in conformity with that of Trautmann and Fiebiger (1935).

Most of the taste buds were situated in the circumvallate papillae. In an adult sheep, taste buds were mostly on the side walls of the vallate papillae, but in a fetus the oral surface often bore taste buds.

The pharynx of a sheep embryo had taste buds and they were regularly larger than those on the papillae. Most of these buds were situated near and around the larynx and on the ventral wall. In an adult sheep, remnants of these buds could be found so that the younger the animal, the more buds were present.

The epiglottis, the aditus laryngis in general, and the aditus oesophagi, had taste buds during fetal and neonatal life. With age, the taste buds diminished in number and in an adult, these formations were difficult to find.

Lalonde and Eglitis (1961) were able to find taste buds on the soft palate of a newborn human and on the posterior free edge lateral to the uvula. In this study, no taste buds were found on the soft palate. More tedious experiments will have to be done to substantiate this observation.

The hard palate, including the papilla incisiva, was devoid of taste buds in both the fetus and the adult.

The labium superius and inferius, buccae, and the conical papillae also were devoid of buds. Investigations of these structures were based on the statements of Kinziro Kubota et al. (1962) that taste buds could be found in areas close to the gland ducts as in the kangaroo.

SUMMARY

A study of the distribution of taste buds in sheep was made using 1396 slides with a total of 2780 sections, derived from 5 sheep heads (3 feti and 2 adults). In addition, a staining method was developed using cresyl violet (0.25%) as a progressive stain. Cresyl violet is often used as a stain for the Nissl granules of neurons and ordinarily is used as a regressive stain. This staining method was effective for the microscopic observation of taste buds. Less time was required for staining each slide (8 minutes) than with the hematoxylin-eosin method. Dehydration and clearing were accomplished with the acetone benzene method, which proved more rapid than the alcohol-chloroform method.

The general findings were:

The circumvallate papillae always bear taste buds on the side walls in an adult sheep and also on the oral surface in a fetus or newborn lamb.

Taste buds have been found in the fungiform papillae but not in all of them. The apex linguae's fungiform papillae have more taste buds than those papillae on the dorsum linguae.

The pharynx and its neighboring structures: the epiglottis, the anterior region of the esophagus, and the aditus laryngis have scattered taste buds during fetal life up to a few months after birth. In the adult, these buds atrophy and vanish.

The soft palate was devoid of taste buds. This finding differed with that of Lalonde and Eglitis (1961), who found taste

buds on the posterior and lateral edge of the uvula.

The hard palate, papilla incisiva, lips, cheek, and conical papillae were examined for taste buds but none were detected.

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APPENDIX

Table 1. Specification of materials.

a. Animal material.

Number	Sheep number	Age	Number of slides	Number of sections
1	Sh.I	fetus	330	330
2	Sh.II	adult	275	275
3	Sh.III	fetus	370	743
4	Sh.IV	fetus	251	830
5	Sh.V	adult	170	602
Total			1396	2780

b. Anatomical region.

Number	Region	Total number of sections	Number	Region	Total number of sections
1	Apex linguae	404	9	Palatum durum	213
2	Radix linguae	29	10	Labia	45
3	Corpus linguae		11	Larynx plus esophagus	20
	a. Vallate region	930			
	b. Median area	251	12	Transition from palatum durum to cheek mucosa plus conical papillae	20
4	Palatum molle	301			
5	Nasopharynx	15	13	Pharynx	50
6	Oropharynx	25	14	Pharynx near epiglottis	50
7	Larynx	25			
8	Transition from lower jaw mucosa to ventral side of tongue plus conical papillae	10	15	Epiglottis	162
			16	Pharynx, larynx, esophagus, and radix linguae	230

Table 2. The result of mapping on Sheep I (fetus).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
1	Sh.I#I (1)-(18)	Apex linguae	8	8	Positive on fungiform papillae
2	Sh.I#II (1)-(9)	Posterior part of radix linguae	9	9	Negative
3	Sh.I#III (3)-(19)	Circumvallate papillae area	19	19	Positive
4	Sh.I#IV (1)-(10)	Corpus linguae	10	10	Positive on fungiform papillae
5	Sh.I#IV (11)-(30)	Corpus linguae	20	20	" "
6	Sh.I#V (1)-(10)	Apex linguae	10	10	" "
7	Sh.I#VI (1)-(10)	Palatum molle	10	10	Negative
8	Sh.I#VI (11)-(20)	" "	10	10	"
9	Sh.I#VI (21)-(25)	" "	5	5	Negative to dubious
10	Sh.I#V (21)-(26)	Apex linguae	5	5	Positive on fungiform papillae
	Sh.I#VII (1)-(10)	Palatum molle	10	10	Negative
	Sh.I#V (11)-(20)	Apex linguae	10	10	Positive on fungiform papillae
	Sh.I#VI (26)-(30)	Palatum molle	5	5	Negative to dubious
	Sh.I#VII (11)-(15)	" "	5	5	" "
11	Sh.I#VII (16)-(25)	" "	10	10	Negative

Table 2 (concl.).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
12	Sh.I#VIII (1)-(15)	Nasopharynx	15	15	Negative
13	Sh.I#IX (1)-(25)	Oropharynx anterior to larynx	25	25	"
14	Sh.I#X (1)-(5)	Larynx	5	5	"
15	Sh.I#XI	Larynx (posterior part)	20	20	"
	Sh.I#III (20)-(27)	Radix near circumvallate region	7	7	Positive
	Sh.I#IV (31)-(39)	Corpus (median part)	9	9	Negative
	Sh.I#V (28)-(30)	Apex linguae	3	3	Positive on fungiform papillae
16	Sh.I#XII (1)-(10)	Labium inferius dextrum	10	10	Negative
	Sh.I#XII (11)-(20)	" "	10	10	"
	Sh.I#XIII (1)-(10)	Larynx and esophagus (right half)	10	10	"
	Sh.I#XIII (11)-(20)	" "	10	10	"
17	Sh.I#XIV (1)-(10)	Palatum molle (right half)	10	10	"
	Sh.I#XIV (11)-(60)	" "	50	50	"

Slides Sh.I#V(5), Sh.I#VI(21)-(25), Sh.I#V(21)-(26), Sh.I#VII(16)-(25), Sh.I#VIII(11)-(15), were stained with Gentian violet.

Slides listed under numbers 15 through 17 were stained with cresyl violet.

The remainder were stained with hematoxylin-eosin.

Table 3. The result of mapping on Sheep II (adult).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
1	Sh.II#I (1)-(10)	Circumvallate region	10	10	Positive
	Sh.II#I (11)-(15)	" "	5	5	"
	Sh.II#I (26)-(45)	" "	20	20	"
2	Sh.II#II (1)-(10)	Anterior part of circumvallate region	10	10	Negative
	Sh.II#II (21)-(30)	" "	10	10	Fungiform papillae dubious to positive
	Sh.II#II (11)-(20)	" "	10	10	Fungiform papillae negative to positive
3	Sh.II#III (1)-(20)	Apex linguae	20	20	Fungiform papillae positive
4	Sh.II#IV (1)-(20)	Dorsum linguae (median part of corpus)	20	20	Negative
5	Sh.II#V (1)-(10)	Transition from lower jaw mucosa to ventral side of tongue (lateral right side) covered with conical papillae	10	10	"
6	Sh.II#VI (1)-(10)	Radix linguae	10	10	" (too many connective tissue papillae)
	Sh.II#IX (1)-(10)	Palatum molle	10	10	Negative
	Sh.II#VI (11)-(20)	Radix linguae	10	10	"

Table 3 (concl.).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
6	Sh.II#IX (11)-(20)	Palatum molle	10	10	Negative
7	Sh.IIXIX (21)-(30)	" "	10	10	"
8	Sh.IIXIX (31)-(40)	" "	10	10	"
	Sh.II#X (1)-(10)	Palatum durum	10	10	"
9	Sh.II#XI (1)-(20)	Anterior tip of hard palate (right half)	20	20	"
10	Sh.II#XII (1)-(20)	Transition from hard palate to cheek mucosa plus conical papillae	20	20	"
11	Sh.II#VII (1)-(20)	Circumvallate region	20	20	Positive
	Sh.II#VII (21)-(45)	" "	25	25	"
12	Sh.II#VIII (1)-(25)	" "	25	25	"

Slides Sh.II#I(1)-(10) were stained with hematoxylin-eosin.

Slides Sh.II#I(36)-(45) were stained with crystal violet during 1/2 min.

Slides Sh.II#I(26)-(35) were stained with crystal violet during 20 sec.

Table 4. The result of mapping on Sheep III (fetus).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
1	Sh.III#I (1)-(25)	Apex linguae	25	25	Positive on fungiform papillae
2	Sh.III#II (1)-(25)	Right half of lower lip	25	25	Negative
3	Sh.III#III (1)-(25)	Anterior part of circumvallate region	25	25	Negative and some positive on fungiform papillae
4	Sh.III#IV (1)-(25)	Posterior part of circumvallate region	25	25	Positive also on oral surface
5	Sh.III#V (1)-(25)	Pharynx (right half)	25	25	Negative to positive, taste buds larger
	Sh.III#V (26)-(50)	" "	25	25	" "
6	Sh.III#VI (1)-(25)	Pharynx near epiglottis	25	25	" "
7	Sh.III#VII (1)-(25)	" "	25	25	Negative to positive
8	Sh.III#VIII (1)-(40)	Circumvallate region	40	127	Positive
9	Sh.III#X (1)-(25)	Epiglottis	25	69	8 slides positive
	Sh.III#X (26)-(55)	"	30	93	Positive
10	Sh.III#IX (1)-(25)	Radix linguae	25	79	Negative
11	Sh.III#XI (1)-(25)	Anterior part of palatum durum plus papilla incisiva	25	78	"
12	Sh.III#XII (1)-(25)	Palatum molle annex posterior part of hard palate	25	97	"

Table 5. The result of mapping on Sheep IV (fetus).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
1	Sh.IV#I (1)-(25)	Left half of pharynx, larynx, esophagus, and radix linguae	25	25	Positive (sagittal section)
	Sh.IV#I (26)-(50)	" " "	25	25	"
2	Sh.IV#II (1)-(25)	Circumvallate region	25	90	Positive
3	Sh.IV#III (1)-(25)	Apex linguae plus fungiform papillae	25	98	"
4	Sh.IV#IV (1)-(25)	Circumvallate region (right half, 4 vallate papillae)	25	98	"
	Sh.IV#IV (26(-(50)	" "	25	92	"
5	Sh.IV#V (1)-(25)	Anterior part of circumvallate region plus 7 black fungiform papillae	25	100	Positive on fungiform papillae
	Sh.IV#V (26)-(50)	" "	25	92	" "
6	Sh.IV#VI (1)-(26)	Dorsum linguae	26	105	Positive on fungiform papillae
7	Sh.IV#VII (1)-(25)	Palatum durum with papilla incisiva	25	105	Negative

Table 6. The result of mapping on sheep V (adult, 4 months).

Number	Code of specimen	Anatomical region	Number of slides	Number of sections	Result
1	Sh.V#I (1)-(16)	Apex linguae	16	58	Positive on fungiform papillae
2	Sh.V#II (1)-(25)	" "	25	70	" "
3	Sh.V#III (1)-(16)	" "	16	38	" "
4	Sh.V#IV (1)-(25)	Epiglottis, esophagus, and pharynx	25	81	6 sections were positive
5	Sh.V#V (1)-(25)	Pharynx and radix linguae	25	99	Negative
6	Sh.V#VI (1)-(22)	Group of fungiform papillae on corpus linguae	22	87	"
7	Sh.V#VII (1)-(25)	Circumvallate region	25	120	Positive
8	Sh.V#VIII (1)-(16)	Palatum molle	16	49	Negative

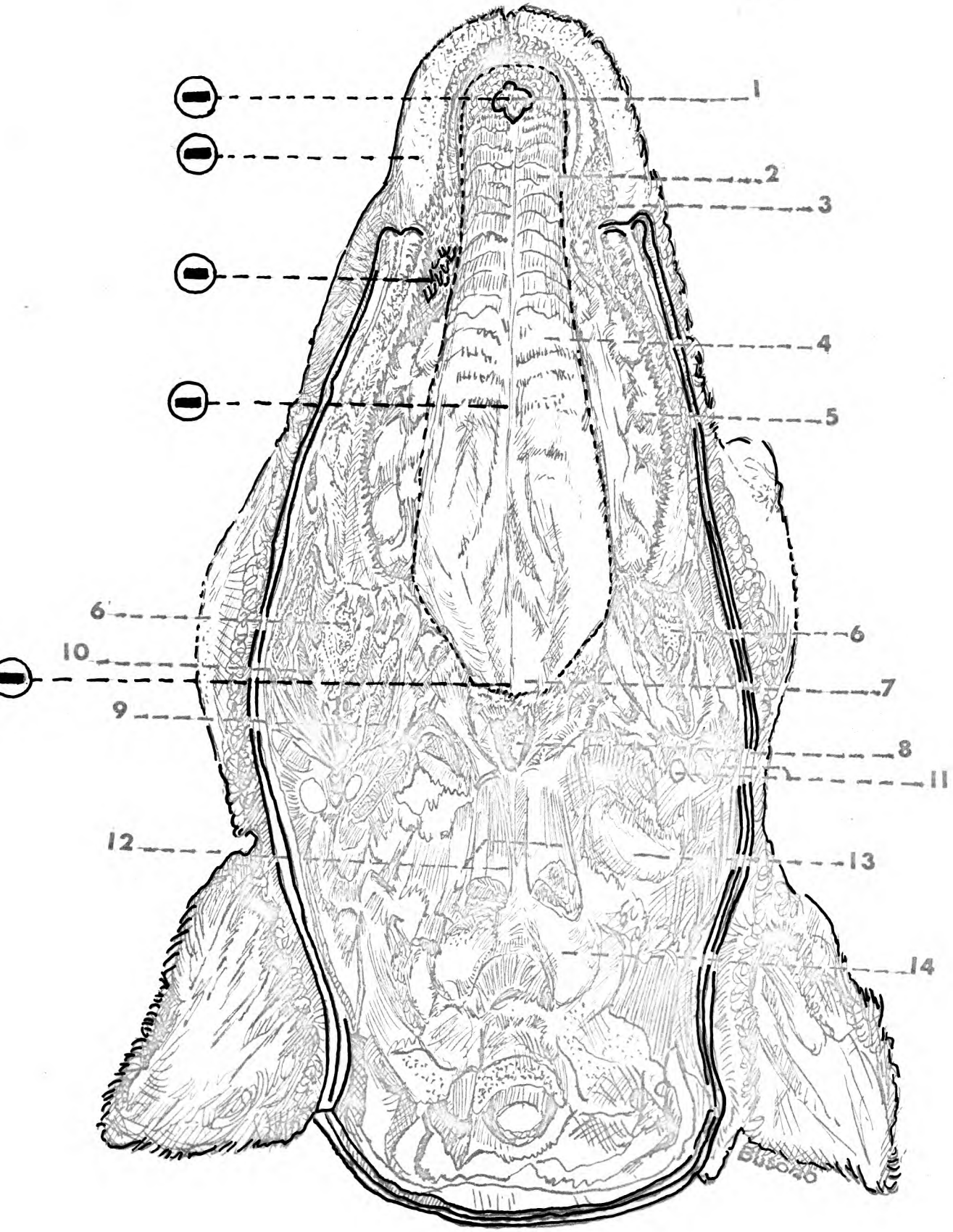
EXPLANATION OF PLATE I

A ventral view of a hard palate of a sheep fetus.
Two times actual size.
Mandible, tongue, and part of the muscles have been removed.

1. Papilla incisiva.
2. Ruga palatina.
3. Conical papillae.
4. Palatum durum.
5. Teeth Anlagen.
6. Part of the mandible.
7. Posterior part of hard palate (transition to soft palate).
8. Choana and vomer.
9. Pterygoid muscle.
10. Masseter muscle.
11. External maxillary artery and vein.
12. Rectus capitis ventralis major muscle.
13. Temporal condyle.
14. Occipital condyle.

⊖ = taste buds negative.

PLATE I



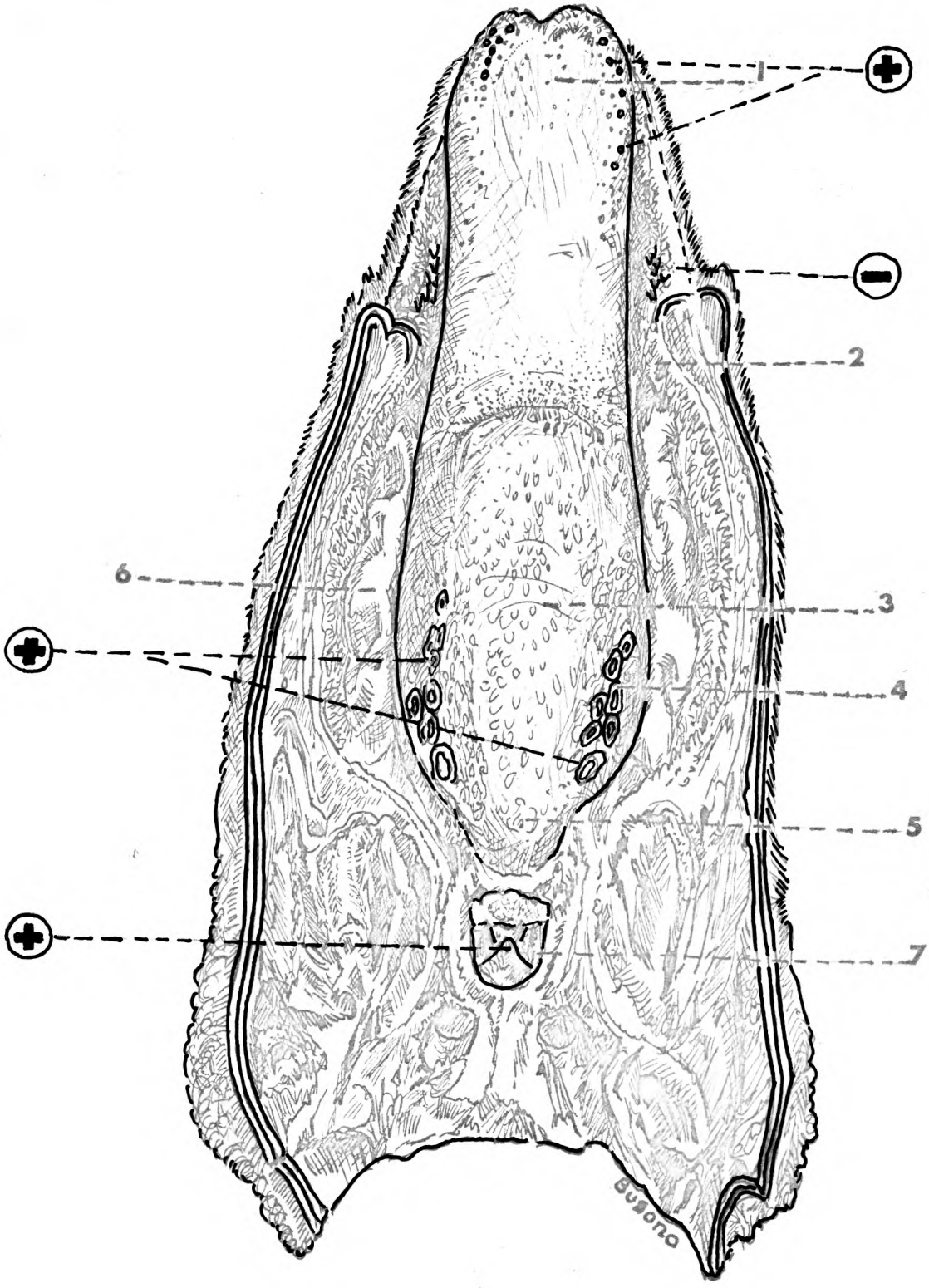
EXPLANATION OF PLATE II

Dorsal view of lower jaw and tongue of a fetus. Two times actual size.

1. Apex linguae.
2. Conical papillae.
3. Dorsum linguae.
4. Circumvallate papillae region.
5. Radix linguae.
6. Teeth Anlagen.
7. Pharynx and larynx.

⊕ = taste buds positive.
⊖ = taste buds negative.

PLATE II



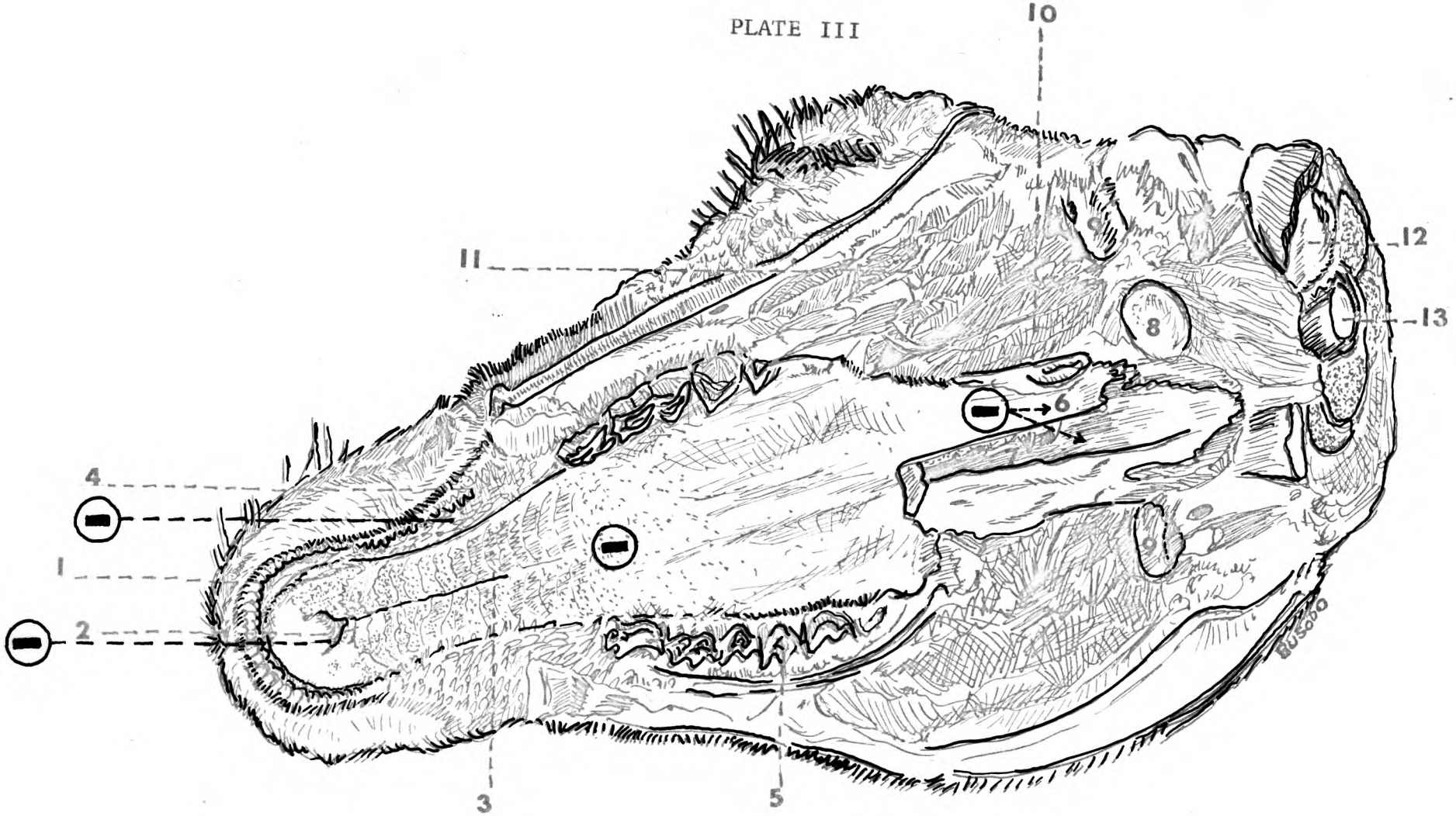
EXPLANATION OF PLATE III

Ventral view of a hard palate of an adult sheep.
Actual size.

1. Upper lip.
2. Papilla incisiva.
3. Rugae palatini.
4. Conical papillae.
5. Upper premolars and molars.
6. Soft palate, oral surface.
7. Choana.
8. Suprapharyngeal lymph gland.
9. Temporal condyle.
10. Pterygoid muscle.
11. Masseter muscle.
12. Occipital condyle.
13. Spinal cord.

⊖ = taste buds negative.

PLATE III



EXPLANATION OF PLATE IV

Dorsal and lateral view of a sheep tongue including larynx. Right half of larynx and radix linguae removed. Actual size.

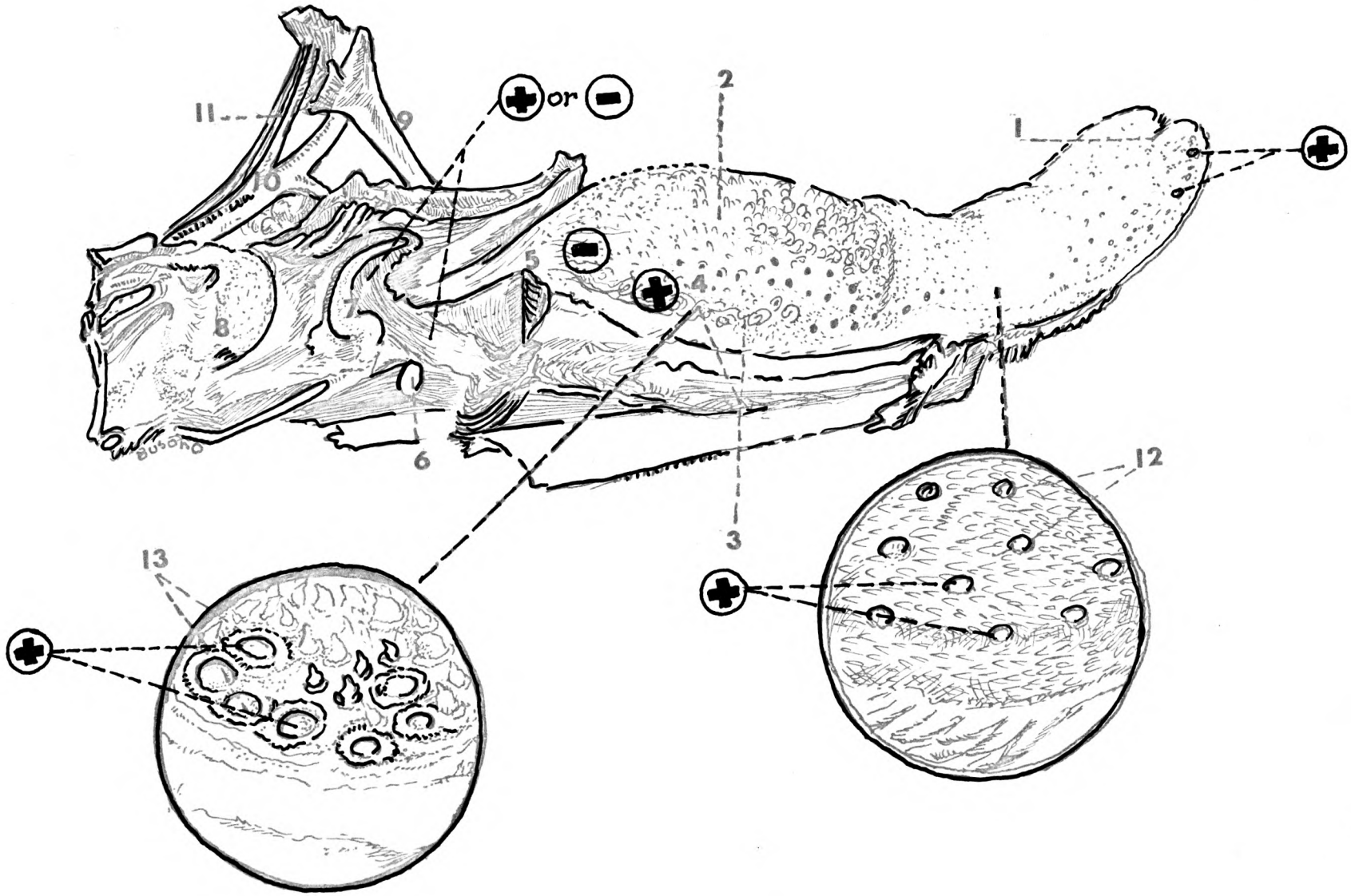
1. Apex linguae with fungiform papillae.
2. Dorsum linguae.
3. Circumvallate papillae region.
4. Dorsum linguae with fungiform papillae.
5. Radix linguae.
6. Corpus ossis hyoideus.
7. Epiglottis.
8. Arytenoid cartilage.
9. Os hyoideus.
10. Common carotid artery.
11. Vagesympathetic nerve.
12. Fungiform papillae.
13. Circumvallate papillae.

⊕ = taste buds positive.

⊖ = taste buds negative.

⊕ or ⊖ = taste buds positive or negative, according to age.

PLATE IV



EXPLANATION OF PLATE V

Fig. 1. Hematoxylin-eosin staining.
Ocular 10 times; objective 20 times.
Sh.I#III(4).
Circumvallate region.

a. Circumvallate papilla.
b. Trench.
c. Taste bud.

Fig. 2. Cresyl violet staining.
Ocular 10 times; objective 20 times.
Sh.IV#IV(21).
Circumvallate papilla.

a. Circumvallate papilla.
b. Trench.
c. Taste bud.

PLATE V

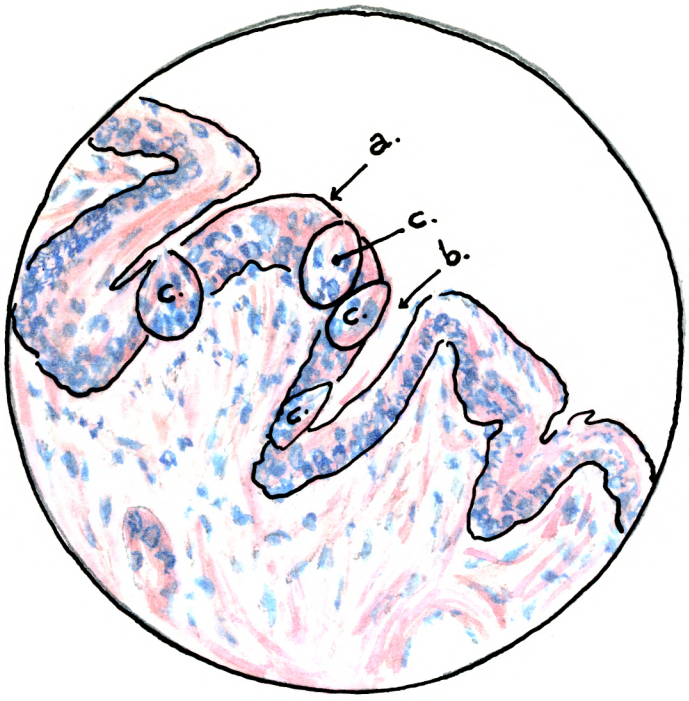


Fig 1.

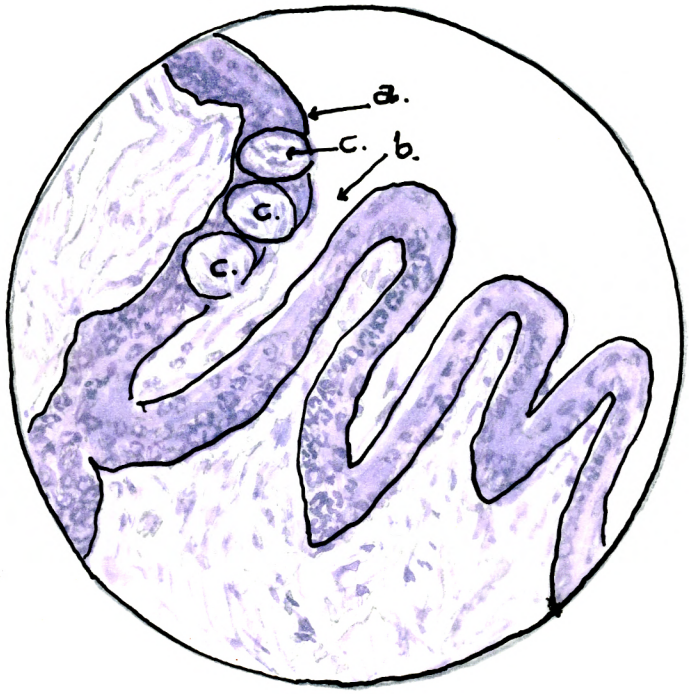


Fig 2.

EXPLANATION OF PLATE VI

Fig. 1. Cresyl violet staining.
Ocular 10 times; objective 20 times.
Sh.IV#III(24).
Apex linguae.

a. Fungiform papilla.
b. Taste bud.

Fig. 1a. Magnification of b. (taste bud).

c. Pit of taste bud.
d. Sustentacular cells.

Fig. 2. Cresyl violet staining.
Ocular 10 times; objective 20 times.
Sh.IV#I(10).
Larynx.

a. Taste bud.
b. Cartilage (embryonal).

Fig. 2a. Magnification of a. (taste bud).

c. Pit of taste bud.
d. Neuro-epithelial cell.
e. Sustentacular cell.
f. Surface cell.

PLATE VI

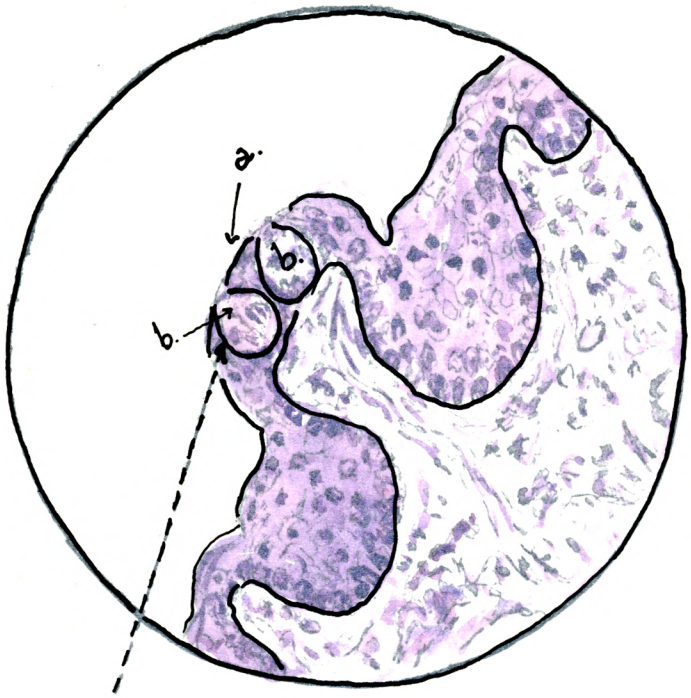


Fig 1.

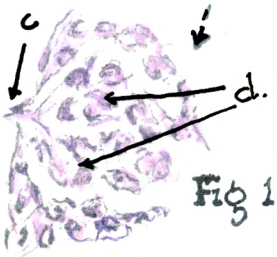


Fig 1a.

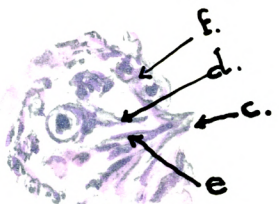


Fig 2a.

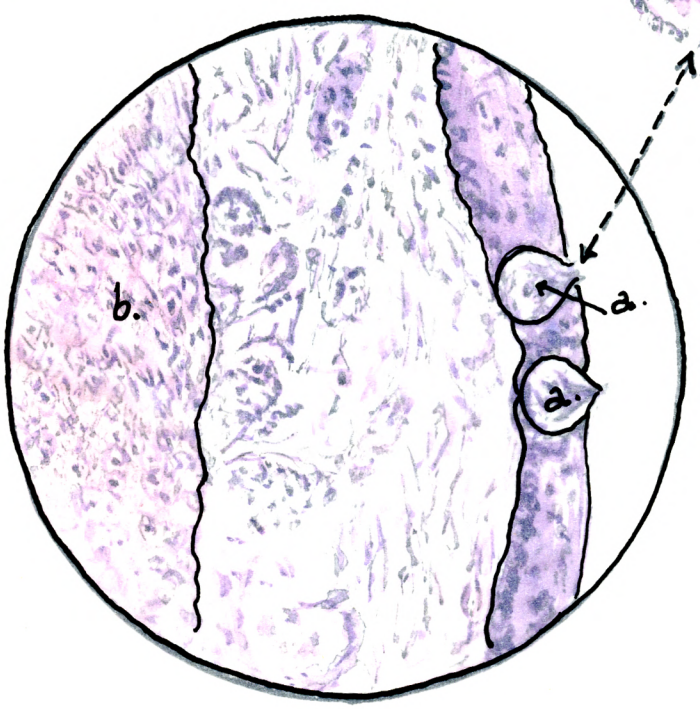


Fig. 2.

THE DISTRIBUTION OF GEMMA GUSTATORIA IN SHEEP

by

BUSONO

D.V.M., Gadjah Mada University, Indonesia, 1958

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Anatomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

Studies related to the distribution of taste buds (gemmae gustatoriae, calyculi gustatorii) of sheep were presented. For this purpose 1396 slides representing 2780 sections of the tongue, soft palate, pharynx, larynx, spiglottis, anterior part of the esophagus, hard palate including the papilla incisiva, lips, cheek, and conical papillae of the sheep were made.

A staining method was developed using 0.25% cresyl violet solution (in distilled water) as a progressive instead of a regressive stain. Cresyl violet was originally a stain for demonstrating Nissl substances in neurons. Previous to paraffin embedding, dehydration and clearing was accomplished with acetone and benzene in order to accelerate the entire process.

The results of mapping of these taste buds on 3 feti and 2 adult sheep were as follows:

The circumvallate papillae always bear taste buds on their side walls in an adult sheep as well as on the surface facing the cavum oris in a fetus or newborn.

Taste buds have been found in the fungiform papillae but not in all of them. The apex linguae's fungiform papillae have more gemmae gustatoriae than those papillae on the dorsum linguae.

The pharynx and its neighboring structures: the epiglottis, anterior region of the esophagus, and the aditus laryngis are found to have scattered taste buds during fetal life up to a few months after birth. In an adult, these buds atrophy and vanish.

The soft palate was devoid of taste buds. This finding differed with that of Lalonde and Eglitis (1961), who found taste

buds on the posterior and lateral edge of the uvula of a newborn human.

The hard palate, papilla incisiva, lips, cheek, and conical papillae were examined for taste buds, but none were detected.