Gross and Microscopic Observations on the Lingual Structure of the West Indian Manatee (*Trichechus manatus latirostris*)

Milton Jay Levin

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master's of Science
In
Veterinary Medical Sciences

Carl Pfeiffer, Chair
Thomas Caceci
Martha Moon
Bonnie Smith

August 4, 1999
Blacksburg, Virginia

Keywords: Manatee, Tongue, Papillae, Taste Bud

Gross and Microscopic Observations on the Lingual Structure of
the West Indian Manatee (*Trichechus manatus latirostris*)

Milton Jay Levin

(ABSTRACT)

The West Indian manatee tongue was examined macroscopically, light microscopically, and electron microscopically (scanning and transmission). The tongue was slender, muscular, and firmly fixed in the oral cavity. Only the cranial tip was free and mobile. Numerous filiform papillae were distributed over the dorsal surface of the rostral lingual region. Caudal to the filiform papillae, multiple raised, round papillae were distributed over the majority of the dorsum. Fungiform papillae were restricted to the lateral margins of the tongue. Caudally, the dorsal and lateral regions showed numerous open fossae and pits. Microscopic examination showed the majority of the lingual dorsum to be covered with a thick stratified squamous epithelium. The caudal dorsal and lateral open pits led to well-developed mucous salivary glands. Foliate papillae, located on the caudal region of the tongue, contained taste buds embedded in the epidermis. Glands within the foliate papillae were mostly mucous, though some seromucous glands were evident. Throughout the tongue, striated muscle was abundant below the epidermis. Blood vessels, lymph channels, and nerve fibers were freely distributed throughout the intermuscular stroma. Nerve fibers reacted positively with neuron specific enolase antibody throughout the lingual structure, including nerve bundles, muscle bundles, glands, and taste buds. Electron microscopy revealed cytoplasmic vacuoles juxtaposed to the nucleus in the stratum spinosum of the foliate papillary region.

Acknowledgements

I would like to thank the members of my committee, Dr. Carl Pfeiffer, Dr. Bonnie Smith, Dr. Thomas Caceci, and Dr. Martha Moon, for their guidance and support during this project. I would also like to thank Dr. Sentiel Rommel from the Marine Mammal Pathobiology Laboratory in St. Petersburg, FL, for collecting and shipping tissue samples to our laboratory. Special thanks to Ms. Theresa Sharp of Covance Labs, North America, for preparing all microscopic slides used in this study. I would also like to acknowledge Ms. Kathy Lowe and Ms. Virginia Viers for their training and support in electron microscopic techniques.

TABLE OF CONTENT

List of Figures

List of Tables

Objectives

The purpose of this research was to provide a more complete and high resolution morphologic description of the West Indian manatee tongue than has previously been available. Though the manatee's tongue has been described macroscopically, only limited light microscopic descriptions have been published. These existing light microscopic works do not specifically address taste buds, intrinsic musculature, or innervation. No electron microscopical, histochemical or immunohistochemical analysis has been reported.

Manatees are highly endangered marine mammals whose possible extinction may occur within our lifetime. Therefore, scientists must gather as much biological and life history data on this species as possible. These data may prove helpful in management and conservation efforts to protect this species from extinction in future generations.

The present work used gross, microscopical (light, scanning and transmission electron microscopy), and immunohistochemical techniques to elucidate further the anatomy of the West Indian Manatee tongue. Surface topology of the dorsal and lateral aspects of the tongue, qualitative assessment of the taste buds and regional distribution of taste buds were investigated.

Historical Background

Manatees

Currently, five published reports (ranging from 1868 to 1980) describe the gross and microscopic anatomy of the West Indian manatee tongue. All papers present similar gross descriptions, differing mainly in their characterization of the distribution and types of lingual papillae. No published reports deal with the ability of manatees or dugongs to taste.

The first paper, "Organ of Taste" (Owen 1868), compared the tongues of several mammalian species including humans, cetaceans, and ruminants. A paragraph is included noting lingual features on the manatee, dugong, and extinct Steller's sea cow. In particular, the author noted differences in the epithelium among the three Sirenians, describing Steller's sea cow as having the maximal development, followed by the dugong, and then the manatee. An illustration (Fig. 1) of the dugong tongue was also included.

The second paper, "On the Form and Structure of the Manatee *(Manatus americanus)*" (Murie 1872), described the anatomy of the entire manatee in remarkable detail. This paper is considered one the best accounts on overall manatee anatomy even to this day. The tongue was described as "having brush-like retroverted filiform papillae towards the tip, many irregularly and different-sized fungiform papillae, and a very numerous, closely arranged, double set of circumvallate glands situated at the root." Murie (1872) also noted that the tongue was incapable of being protruded. A plate (Fig. 2) was included to illustrate the tongue *in situ.*

The third paper, "Further Observations of the Gustatory Organs of the Mammalia" (Tuckerman, 1892), included a review of the general description of the manatee tongue. Long and delicate filiform papillae were noted on the free portion of the tongue. Tuckerman was the first to note glands beneath the epithelium, and remarked that they were probably serous. No regular arrangement of bulbs (taste buds) was observed, as
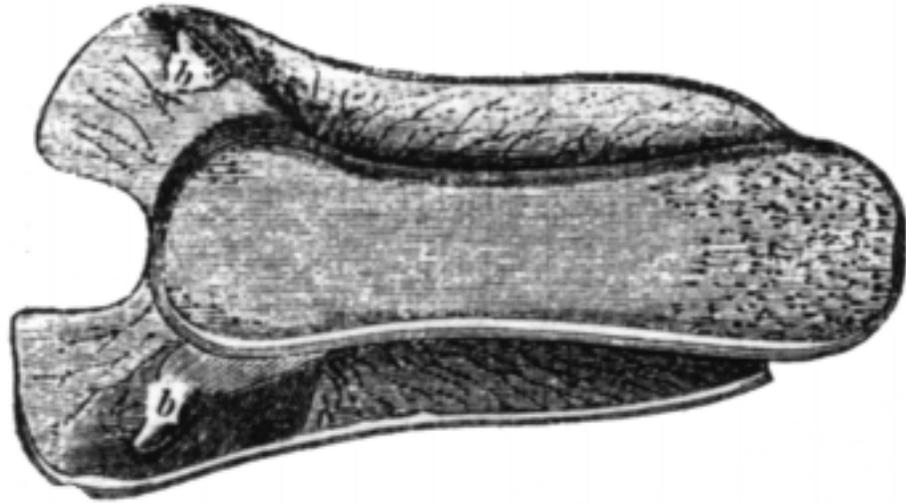
Figure 1. Owen's dugongtongue

Richard Owen's (1868) illustration of a dugong tongue (Halicore).
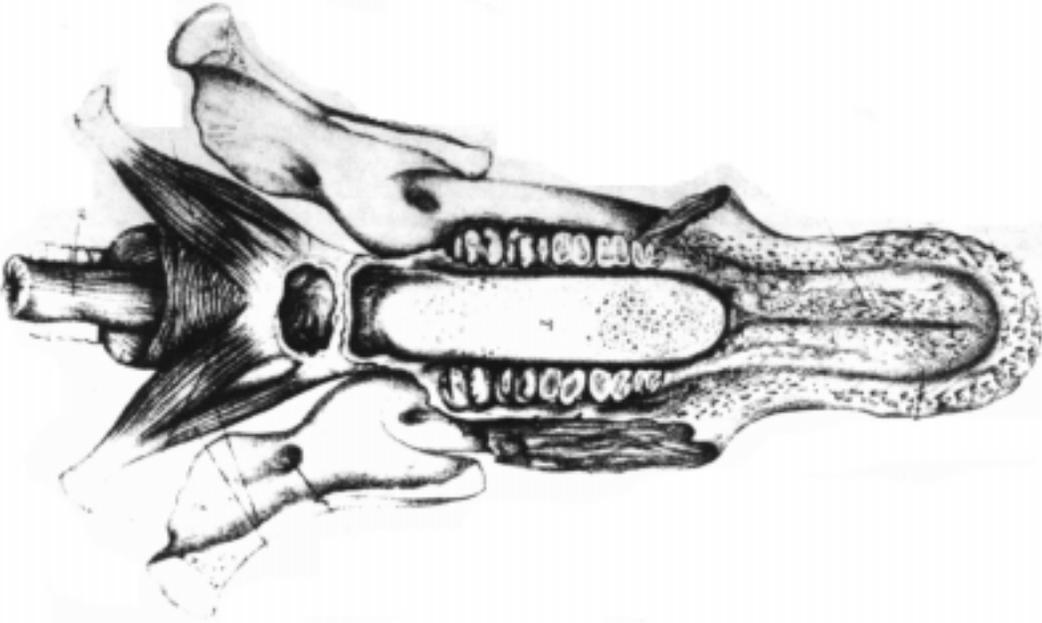
Figure 2. Murie's manatee tongue

James Murie's (1872) illustration of a manatee tongue.

they were small and located on the free surface of the papillae and lateral areas. Tuckerman recognized that fresh samples were needed to further study the gustatory structures.

The fourth paper, "The Comparative Anatomy of the Tongues of the Mammalia" (Sonntag, 1922), compared the anatomy of several mammalian orders, including cetacean and sirenian representatives. The author noted that manatee tongues differed considerably from those of cetaceans, and were more similar to the ungulate tongue. Sonntag described the manatee's tongue as muscular, firm, and not very mobile. The apex was noted as rounded and devoid of the lobules which are characteristic of the cetacean tongue. Retroverted cuticular spines were noted behind the apex. Sonntag remarked that the immobile nature of the tongue allowed it to aid in cropping of the vegetative diet. The dorsal surface was noted as plain and smooth rostrally, and as having many folds towards the pharyngeal area. Well-developed lateral structures were also noted, appearing as large cushions with numerous fissures. The halicore (Steller's sea cow) was noted as having clusters of pits, analogous the vallate papillae in *Manatus*. Illustrations of the tongue (Fig. 3) were provided for *Manatus* and the halicore.

The fifth publication, "A Comparative Morphological Study on the Tongues of Manatee and Dugong (Sirenia)" (Yamasaki and Komatsu, 1980), is both the most recent and complete work to date. This paper compared the lingual morphology of two manatee species, the African manatee, *Trichechus senegalensis* and the West Indian manatee, *Trichechus manatus*. These were then compared to the dugong, *Dugong dugong*. The authors noted, as did Murie (Murie, 1872), that the tongue was fixed within the oral cavity and incapable of being protruded. Neither circumvallate glands, papillae, a sulcus terminalis nor a foramen caecum were seen. Due to the lack of these features typically seen in terrestrial mammals, the tongue could not be divided into a body and root. Filiform papillae were again noted at the tip for both manatees and dugongs, though they extended further posteriorly in the dugong. Papillae on the dorsal and lateral surfaces of the manatee's tongue exhibited variations of the vallate and foliate papillae. Abundant adipose tissue was seen in the caudal portion of the tongue. The authors wrote, "the most
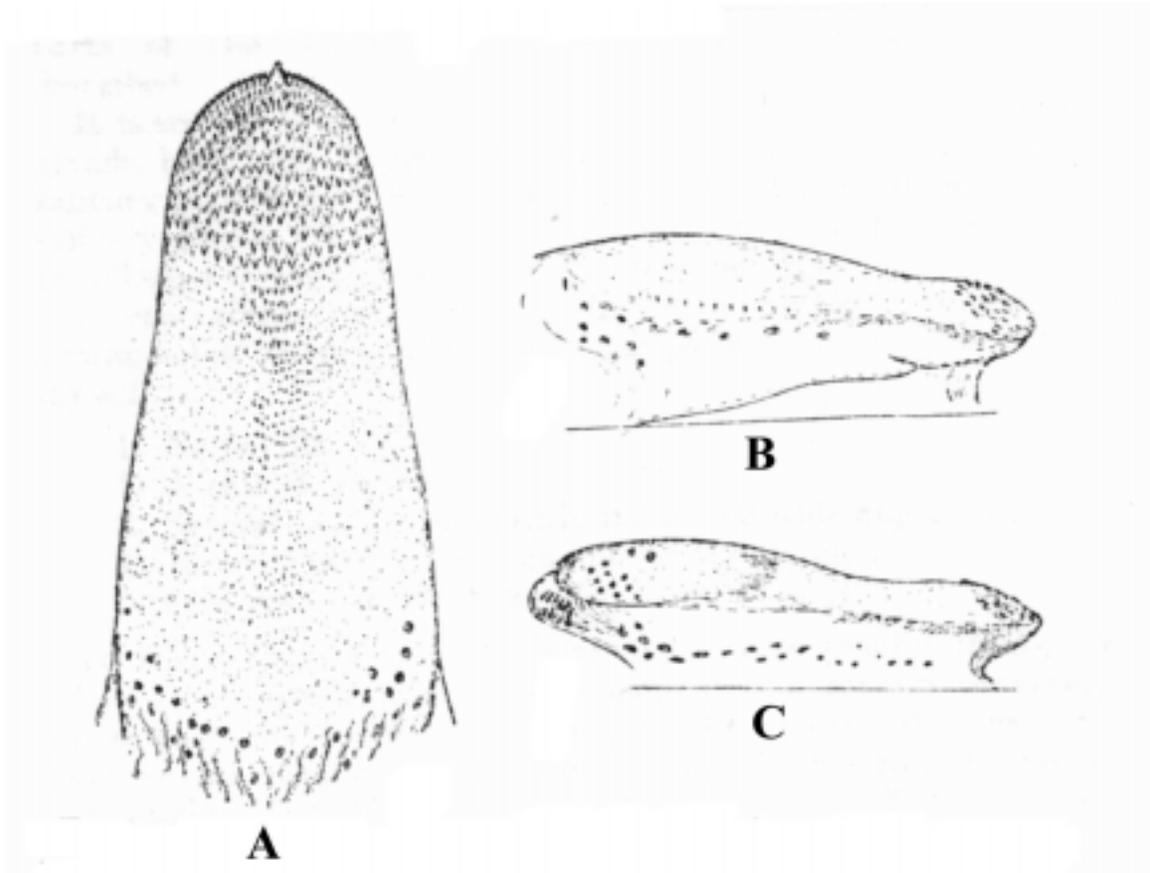
Figure 3. Sonntag's manatee and dugong tongues

Charles Sonntag's (1922) illustration of a dugong (Halicore) tongue (A and B) manatee (*Manatus americanus*) tongue (C).

characteristic feature of the sirenian tongue is the presence of peculiar gustatory organs, a multifossulate swelling in the manatee and pits in the dugong, with notable differences in the organs being marked between the two families." Within these organs, taste buds were located in the thin epithelium of the caudal fossae in the manatee, and in the epithelium of the pits in the dugong. The tongues of both manatees and dugongs had well-developed mucous glands, and occasional mixed seromucous glands. Fungiform papillae on the lateral margin were thought to act as a tactile organ. For the first time, gross photographs and light micrographs were presented for the manatee and dugong. The gross photographs showed the entire tongue from a dorsal and lateral view. Higher magnifications revealed the pits, lateral patches, and fossulae for both species. Micrographs presented transverse sections of the fossulae, lateral swellings, pits, mucous, and taste buds. A table was provided to compare the anatomical differences between the manatee and dugong tongues in reference to the apex, spines, dorsum, gustatory organ, and glands of the gustatory organ.

In previous papers describing the lingual anatomy of the West Indian manatee, some authors had included notes on the tongue of the dugong, also a member of the Order Sirenia. Additional papers have been published dealing solely with the dugong. Other papers compared the West Indian manatee tongue to other species within the order Sirenia. One paper specifically described the tongue of the Amazonian manatee, *Manatus inunguis*. These various publications are discussed immediately below. In addition, despite noteworthy differences in gross form, habitat, and diet the elephant is a close modern relative of Sirenian species, the following discussion of Sirenian lingual anatomy will also include remarks on the Probosidean tongue.

Dugongs

The first report on dugong anatomy, "Particulars Respecting the Anatomy of the Dugong, Intended as a Supplement to Sir T.S. Raffle's Account of that Animal" (Home, 1820) included remarks on lingual anatomy. The tongue was described as having two nipple-like processes at its base, one on each side. The tongue was noted to be, "only loose for half an inch at the point (apex), which is covered with long villi, showing that the sense

of taste is very delicate." The "long villi" most likely referred to filiform papillae, which in fact, contain no taste buds. An illustrated plate (Fig. 4) was included in Home's report

In the second report, "The Red Sea Dugong" (Gohar, 1957), a detailed account of gross lingual anatomy was made for the first time. The paper began by noting the small size of the tongue compared to body size. The tongue was described as fixed between the mandibular rami and incapable of reaching the rostral region of the mouth. The lingual shape was described as narrow at the rostral tip and broader near the caudal portion, with the middle region strongly bowed dorsally. The rostral tip of the tongue was described as truncated not pointed or bifid as reported in earlier works. Bristles, most likely filiform papillae, were described on the tip of the tongue. These "bristles" decreased in length and number posteriorly. The bristles had well-developed dermal papillar extensions. The tip of the tongue was noted as thin, hard, cornified, and nail-like. The remaining dorsum had a variety of soft papillae, some being stiff while others were soft. On the middle third of the tongue, the author noted for the first time a new type of papilla, the calici-penicilliformes, a modified circumvallate papilla. These were similar to circumvallate papillae as seen in other mammals, but lacked a raised ridge or vallus. These were scattered in low numbers with ordinary digitiform papillae. No typical circumvallate papillae were described. On the lateral surfaces, fungiform papillae and pin-like openings leading to glands were noted. The epithelium was described as thickly stratified with numerous smaller papillae over the entire surface. Deep to the epithelium, lobulated glandular tissue was noted. Most of the glands were mucous in nature, though some serous lobules were also present. Ducts from these glands led to the dorsal surface of the tongue. The remaining lingual substance was composed of striated muscle bundles running in various directions. The author noted, "the musculature of the tongue is comparatively weak, as is expected in view of its weak mobility." Finally, the author stated that more research was needed to investigate and elucidate, "the function of the different types of papillae and their innervation, as well as the structure, distribution, and role of the glands." An illustration (Fig. 5) was provided.
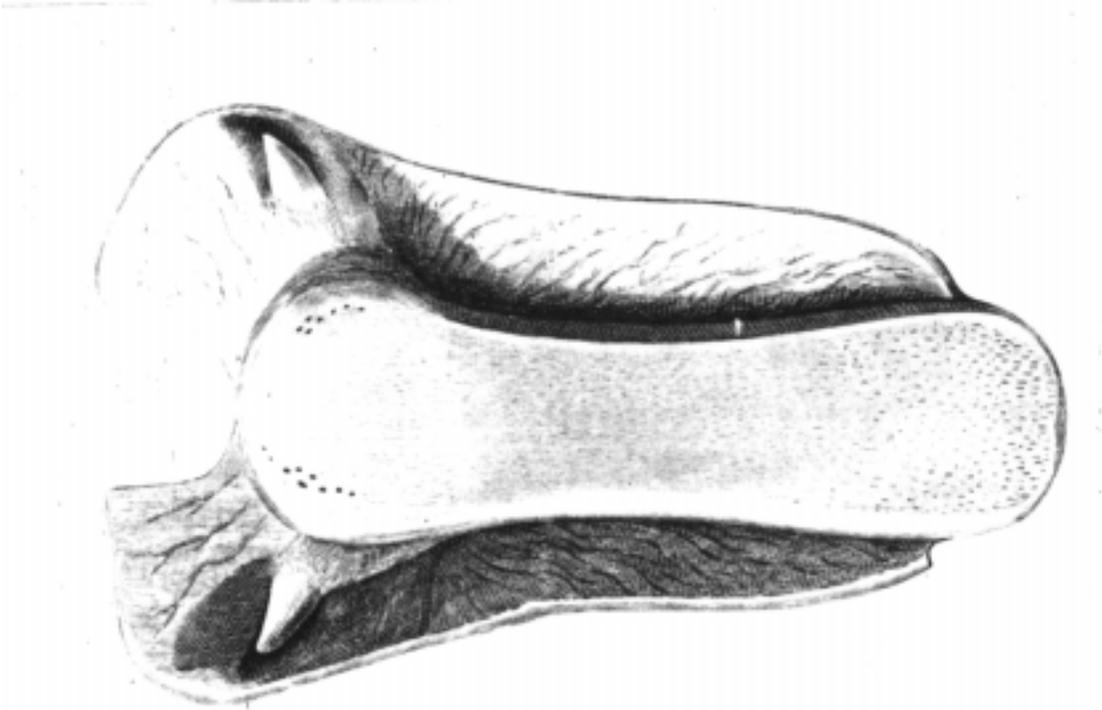
Figure 4. Home's dugong tongue

Edward Home's (1820) illustration of a dugong tongue.
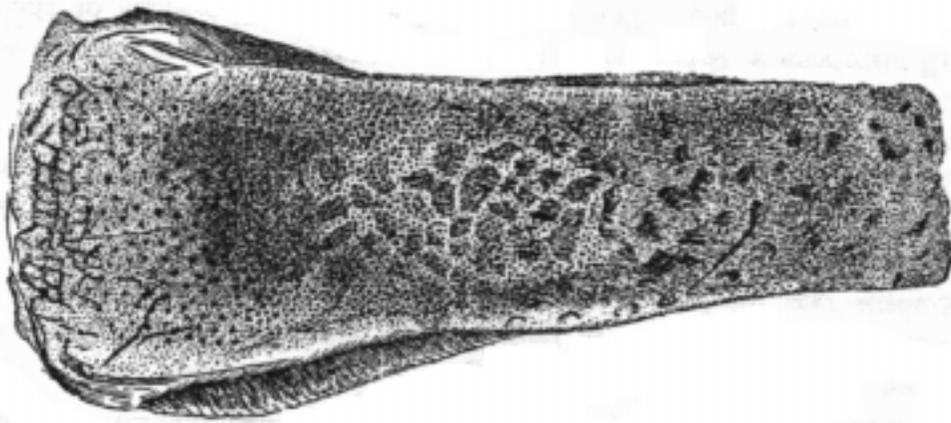
Figure 5. Gohar's dugong tongue

H.A.F. Gohar's (1957) illustration of dugong tongue.

The anatomy of an Amazonian manatee was described in the paper, "Notes upon the Anatomy of a Manatee (Manatus inunguis) lately living in the Society's Garden" (Beddard, 1897). Very little was mentioned on the tongue, noting only that it was similar to the American species, *Manatus latirostris*. The author noted the appearance of circumvallate papillae, also known as Mayer's organ in older literature. Two illustrated views (Fig. 6) were included.

Most recently, Yamasaki, et al., followed their earlier paper on the tongues of manatees and dugongs with a more complete description of the dugong tongue, "An anatomical note on the tongue of the Dugong, *Dugong dugong*" (Yamasaki et al., 1981). The report compared the tongue of the dugong and the African manatee, *Trichechus senegalensis*. The paper reported findings similar to those of their previous paper (1980). The authors remarked that the greatest difference between the two species included the presence of two groups of pits in the dugong, and the multifossulate swellings in the manatee. They also emphasized the well-developed serous glands associated with the dorsal and lateral pits. The authors suggested that the enzymes contained in the serous fluid might convert the polysaccharides of seagrasses and algae into smaller molecules, which might stimulate the taste buds. The product of the glands was also suggested as possibly rinsing the taste buds. Lastly, mucous glands at the root of the tongue were noted as much better developed than those in the manatee. The authors suggested, "it would be of interest to determine whether or not morphological differences between dugong and manatee tongues have any relationship to food habits."

Elephant

Limited information on the elephant's tongue anatomy has been published. However, a paper entitled, *On the Anatomy of the African Elephant (Elephas africanus, Blum)* (Forbes, 1879) described and illustrated (Fig. 7) the tongue of a single female African elephant. Several similarities to the sirenian tongue were present. The tongue was described as small for the size of the body with a short free tip, both features
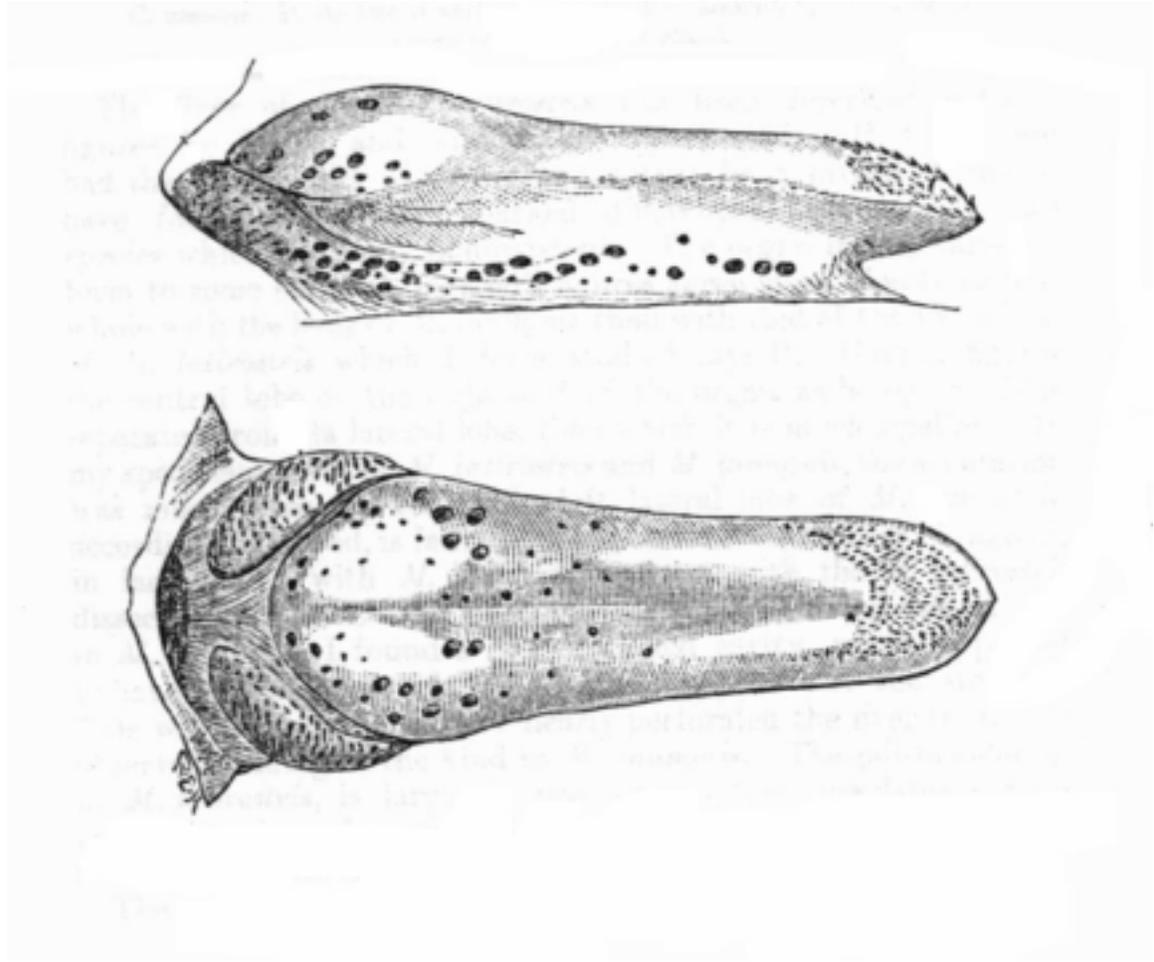
Figure 6. Beddard's manatee tongue

Frank Beddard's (1897) illustration of a manatee tongue (*Manatus inunguis*).
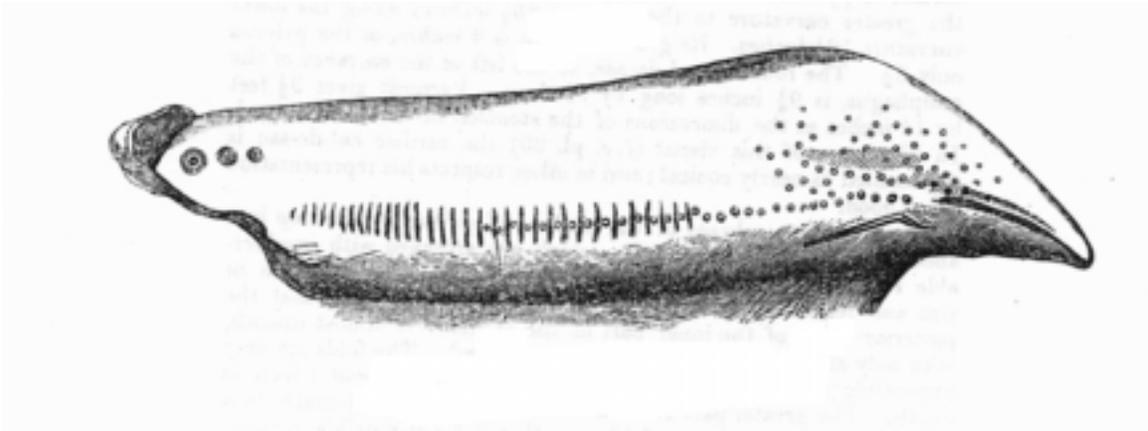
Figure 7. Forbe's elephant tongue

W. A. Forbes' (1879) illustration of an African elephant tongue (*Elephas africanus*).

characteristic of manatee tongues. Small, fine filiform papillae were present, giving the tongue a velvety texture. A small number of fungiform papillae were present along the lateral walls from the rostral portion to about the mid-region of the tongue. Small glands in the lingual substance were also seen in this area.   Extending beyond the fungiform papillae were a number of vertical slits referred to as Mayer's organ, each of which was provided with a pair of glandular papillae connected to mucous glands. Three to four circumvallate papillae were situated near the base of the tongue. These small papillae were approximately 3/8 inch in diameter.

Manatees: General Aspects

Manatee anatomy

The most complete reports on general manatee anatomy were published by James Murie (Murie, 1885; Murie, 1872). More current papers report findings on the eyes (Cohen et al., 1982; Griebel and Schmid, 1997; Griebel and Schmid, 1996), kidneys (Hill and Reynolds, 1989; Maluf, 1995; Maluf, 1989), brain (Reep et al., 1989; Reep and O'Shea, 1990), gastrointestinal tract (Reynolds and Rommel, 1996; Reynolds and Krause, 1982), and facial bristles (Reep et al., 1998). Only five brief reports have been published on the gross anatomy of the tongue, as outlined earlier.

Taxonomic Classification

The Order Sirenia, is comprised of two families, Trichechidae (manatees) and Dugongidae (dugongs). Four species presently grace the marine world; (1) the West Indian manatee (*Trichechus manatus*), (2) the West African manatee (*Trichechus senegalensis*), (3) the Amazonian manatee (*Trichechus inunguis*), and (4) the dugong (*Dugong dugong*). The Steller's sea cow (*Hydrodamalis gigas*) was driven to extinction by human activity in 1768. The Steller' sea cow was the only sirenian that lived in non-tropical waters, inhabiting the waters of the Bering Sea. The West Indian manatee has been divided into two sub-species, the Florida manatee (*Trichechus manatus latirostris*) and the Antillean manatee (*Trichechus manatus manatus*). Fossil records and biochemical analysis (Kleinschmidt et al., 1986) have shown that elephants, aardvarks, and hyraxes are the closest living relatives to the sirenians. The Florida manatee was the focus of the present thesis.

Distribution

The Florida manatee inhabits the coastal and inland waters of Florida. Manatees are highly endangered marine mammals, found in several countries. Currently 1900 animals are estimated to remain in the wild. Individuals may range as far west as Texas and as far north as Virginia. During the summer months, manatees move freely around the state in

search of food. During the winter months, when the temperature falls below 68°F, manatees must move to warmer waters, such as natural hot springs, or power plant discharge canals.

Physical characteristics

Adults may reach lengths up to 13 feet and weigh up to 2500 pounds, with females tending to be larger than males. Gestation requires 13 months with a single calf produced in each pregnancy.   One mammary teat is located underneath each pectoral flipper. Manatees possess only six cervical vertebrae rather than the seven of most other mammals. Hind limbs are vestigial and the tail is paddle-shaped. The ribs are dense and lack marrow cavities. Formed elements of the blood are produced in the vertebral column. The lungs are located along the dorsum separated from the abdominal cavity by a hemi-diaphragm.

Senses

Senses such as hearing, eyesight, smell, and taste are poorly understood. Manatees have large auditory ossicles within the middle ear. The ear is adapted for detecting low frequencies (3-5 kHz). The eyes are well developed and possess both rods and cones, but color vision has been untested in manatees. The small internal nasal bones have olfactory tissue, but the smelling capability is still unknown. Manatees lack a vomeronasal organ. Though this and other papers report that manatees have taste buds, their ability to taste is also poorly understood.

Eating habits

Manatees are the only exclusively herbivorous marine mammals. A variety of seagrasses comprises their diet, including turtle grass, shoal grass, mangrove leaves, and manatee grass. Manatees avoid eating specific plants containing natural toxins, such as spatterdock and waterpennywort. Manatees consume about 4-9% of the their body weight in wet vegetation per day. Feeding takes place at all levels in the water column, including submerged, emergent, floating, and shoreline vegetation. Manatees use their forelimbs and flexible lips to manipulate food into the oral cavity. Vegetation is broken

into small pieces by the action of the mandible working the plants against horny, ridged pads of the hard palate. Subsequently, molars help grind the food prior to swallowing.

Materials and Methods

Sample collection

Due to the endangered status of the animal, specimens were obtained only from animals found dead. Accessible manatee carcasses were retrieved, and a complete necropsy was performed by the Florida Department for Environmental Protection, Florida Marine Research Institute, Marine Mammal Pathobiology Laboratory, St. Petersburg, Florida. Necropsies were performed as soon as possible after retrieval. For gross and light microscopic study, tongues were removed (Fig. 8), fixed, and stored in 10% neutral buffered formalin (NBF). For electron microscopy, individual sections, approximately 3 mm^3, were fixed in 4.4% paraformaldehyde/5% glutaraldehyde/2.75% picric acid, in a 0.05M sodium cacodylate buffer. The samples were then shipped to our laboratory.

In our laboratory, tongues were examined grossly, their dimensions determined, and a dissecting microscope used to describe surface features such as lingual papillae. Lateral fungiform-like papillae were quantified. Photographs were prepared of whole tongues using a Nikon 6006 and Medical Nikor lens. A Nikon SMZ-U camera mounted on a dissecting microscope was used for high resolution subgross photographs; micrographs were digitally captured using an Olympus Vanox microscope.

A total of nine tongues representing both sexes and various ages was received over the course of the study (Table 1). Due to the limited number of samples, no attempt was made to demonstrate sex- and age-related changes in tongue morphology.

Light Microscopy

To initiate this study, a single tongue, MNE 9818, was selected and nine areas (Fig. 9) were sampled for light microscopy. Cross sections were made from the right half of the tongue to include areas representing all surface papillae. Sections were placed in labeled biopsy cassettes, stored in 10% NBF, and shipped to COVANCE Labs North America (Vienna, VA) for routine processing. Histological samples were paraffin-embedded and sectioned at 5.0 μm. Sections were stained with hematoxylin and eosin (H&E) to

Figure 8. Manatee necropsy

Removal of tongue using a mid-sagittal cut with a band saw.
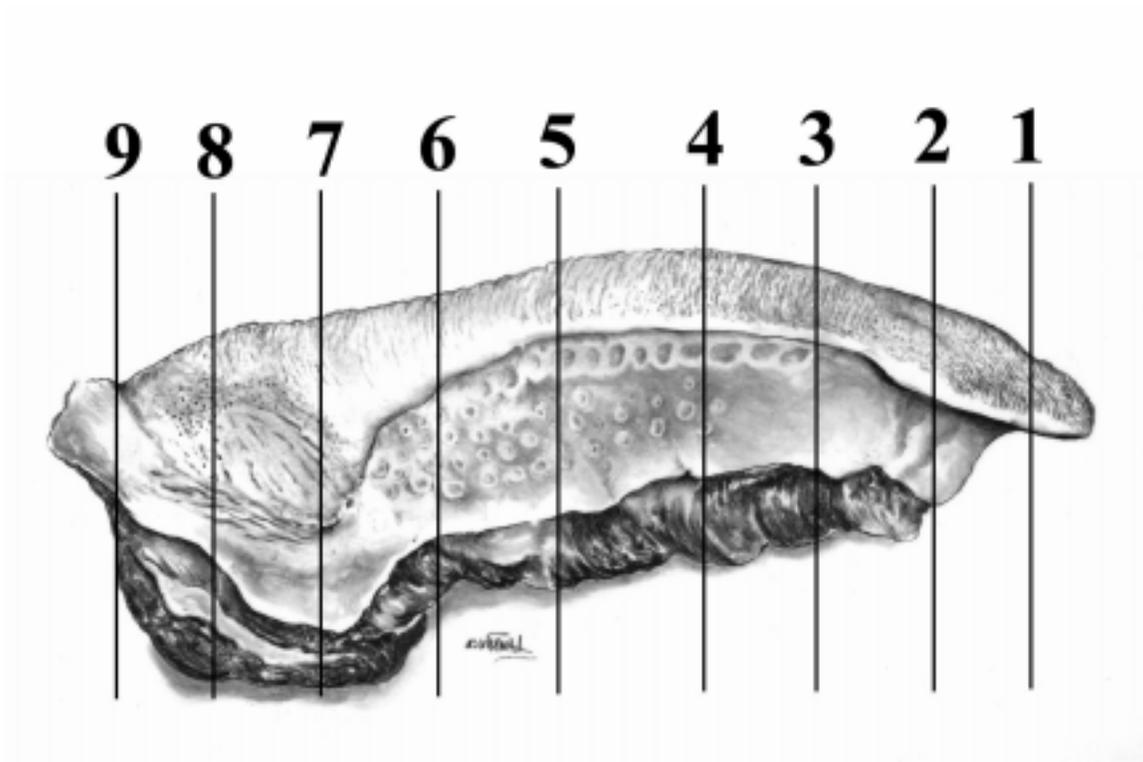
Figure 9. Sites of collection

Sites of cross sections (1-9) including dorsal and right lateral wall, taken for light microscopy (rostral tip to right).

examine general cellular structure, with Gomori's trichrome to examine connective tissue and keratin, and with periodic Acid-Schiff (PAS) to examine carbohydrates.

Transmission Electron Microscopy (TEM)

Fresh lingual samples from the same nine regions sampled for light microscopy were dissected and post-fixed in 4.4% paraformaldehyde/5% glutaraldehyde/2.75% picric acid, in a 0.05M sodium cacodylate buffer, for a minimum of 24 hours. Samples then underwent three 15-minute washes with 0.1 M sodium cacodylate buffer (pH 7.4). Samples were post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 hour, and washed in buffer again three times (15 minutes each). Samples were then dehydrated in an alcohol (Flex®) series (50%, 70%, 90%, 95%, and 100%) for 10 minutes each. Samples were embedded in Maraglas plastic (Caceci, 1984) in preparation for microtomy. Semithin sections (1.0 μm) were cut and tri-stained (Humphrey and Pittman, 1974) for orientation and preliminary study by light microscopy. Thin sections (80 Å) were subsequently cut and doubly stained with lead citrate and uranyl acetate and studied with a JEOL 100 CX-II transmission electron microscope operating at 80 kV.

Scanning Electron Microscopy (SEM)

Additional samples representing all types of papilla types were fixed in a similar manner as those for TEM. Following dehydration with alcohol, these samples were critical point dried (LADD CPD), mounted and coated with gold (approximately 1500 Å) in a SPI-Module sputter coater (West Chester, PA) and examined in a Cambridge Stereoscan 90 (Cambridge, England) scanning electron microscope operating at 25 kV.

Immunohistochemistry

Mouse anti-neuron specific enolase (Zymed, San Francisco) was chosen as the antibody to demonstrate neural elements in the tongue. Sections previously embedded in paraffin from all regions of the tongue were stained. Tissue blocks were sectioned at 5 μm, placed on positively charged slides, and processed. Slides were deparaffinized in xylene (twice, 10 minutes each), rinsed in Flex® 100% (twice, 3 minutes each), rinsed in Flex®

95% (once, 3 minutes), rinsed in Flex® 80% (once, 3 minutes), washed for 5 minutes with tap water, and finally washed for 3 minutes with distilled water. Slides were then placed in a preheated 95°C waterbath in a solution of DAKO® Target Retrieval solution (1:10 dilution in distilled water) for 30 minutes. Slides were removed, allowed to cool, and then rinsed in distilled water for 5 and then 3 minutes. Slides were then placed in the Optimax Plus Automated Cell Stainer (San Ramon, CA) programmed for the antibody used. Slides were counterstained with hematoxylin and coverslipped.

Results

<u>Macroscopic Observations</u>

The tongue was situated in and restricted to the caudal region of the head (Fig. 10). The tongue was small, muscular, and firmly fixed in the oral cavity, with only a small part of the rostral portion of the tongue free and mobile.

The extirpated tongue (Figs. 11 and 12) again demonstrated the restricted nature of the short free tip. Lateral patch-like, fungiform papillae and open pits could be seen from the lateral view (Fig. 11). The epidermis and intrinsic musculature were demonstrated in the mid-sagittal view. The base of the tongue was firmly attached to the hyoid bone (Fig. 12).

Papillae (Fig. 13) were distributed over the majority of the lingual surface, with various types located in specific areas.

At higher magnification (Figs. 14, 15, and 16) the short, free tip possessed numerous hair-like filiform papillae, approximately 2-5 mm in height. The numbers of papillae were greatest at the free tip and gradually disappeared caudally.

The dorsal surface (Figs. 17 and 18) demonstrated fungiform papillae with rounded free surfaces. These papillae ranged from 1-5 mm in diameter.

Fungiform papillae, multiple patch-like areas, were located on the lateral surfaces of the tongue (Figs. 19 and 20). The number of papillae was approximately equal on both lateral surfaces (Table 2) and their diameter ranged from 2 to 5 mm.

Numerous open pits (Fig. 21), less than 1 mm in diameter, along the base and intermixed with the fungiform papillae, were located on the lateral margins of the tongue. Numerous open pits leading down to the esophagus were observed caudal and dorsal to the foliate papillae.

Foliate papillae with multi-fossulate openings (Fig. 22) were located on each caudal dorsal margin of the tongue. Older literature refers to these as circumvallate, but upon gross examination, they appeared as foliate papillae.
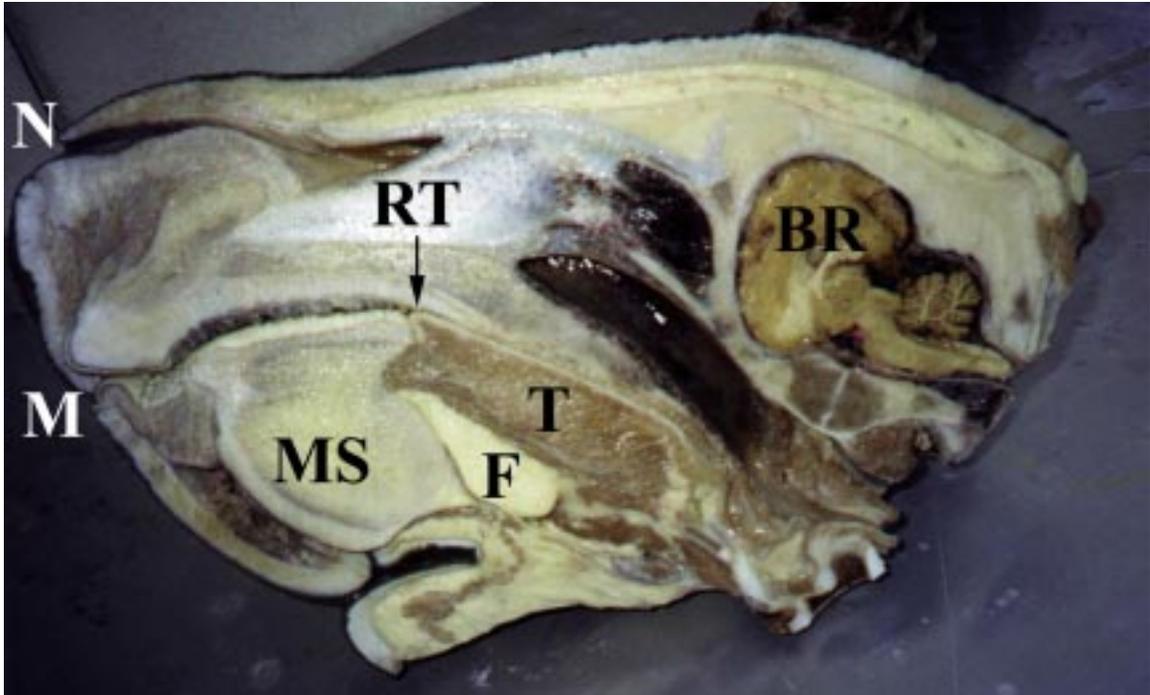
Figure 10. Mid-sagittal view of head

Mid-sagittal view of an adult manatee head showing the tongue (T), brain (BR), external opening of nares (N), external opening of mouth (M), mandibular symphsis (MS), rostral free tip of tongue (RT), and sublingual fat pad (F).

Figure 11. Lateral view of tongue

Lateral view of tongue showing the rostral free tip (RT) and laterally placed fungiform papilla (FU).

Figure 12. Mid-sagittal view of tongue

Mid-sagittal view of tongue showing the rostral free tip (RT), hyoid bone (H), intrinsic muscles (MU), and sublingual fat pad (F).
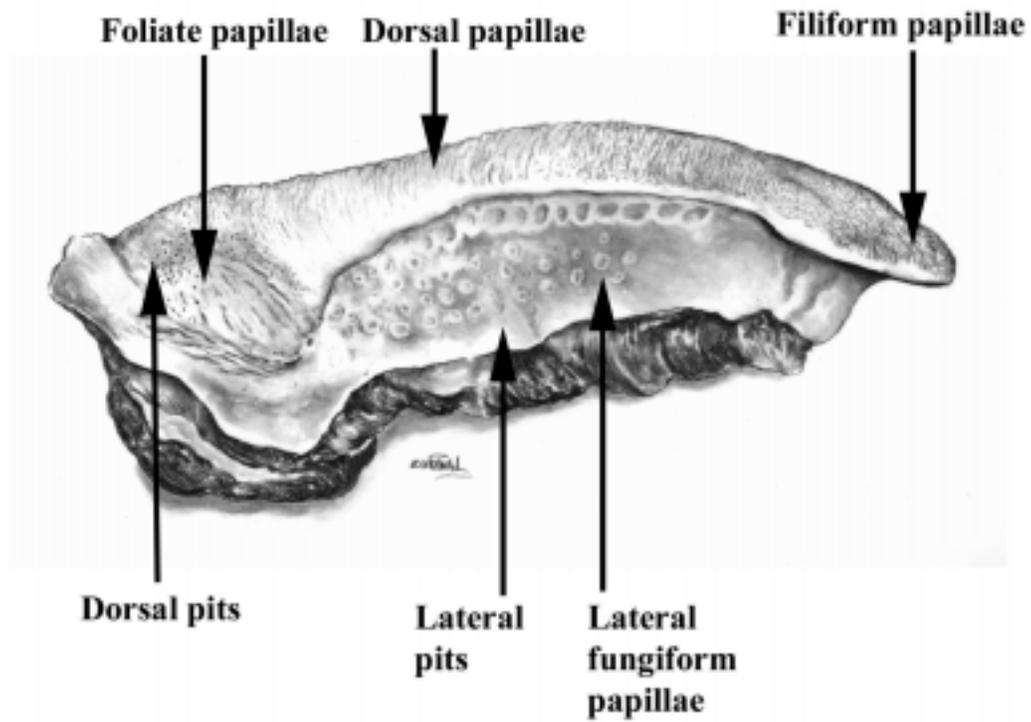
Figure 13. Tongue surface features

Major papillary features of the manatee tongue.
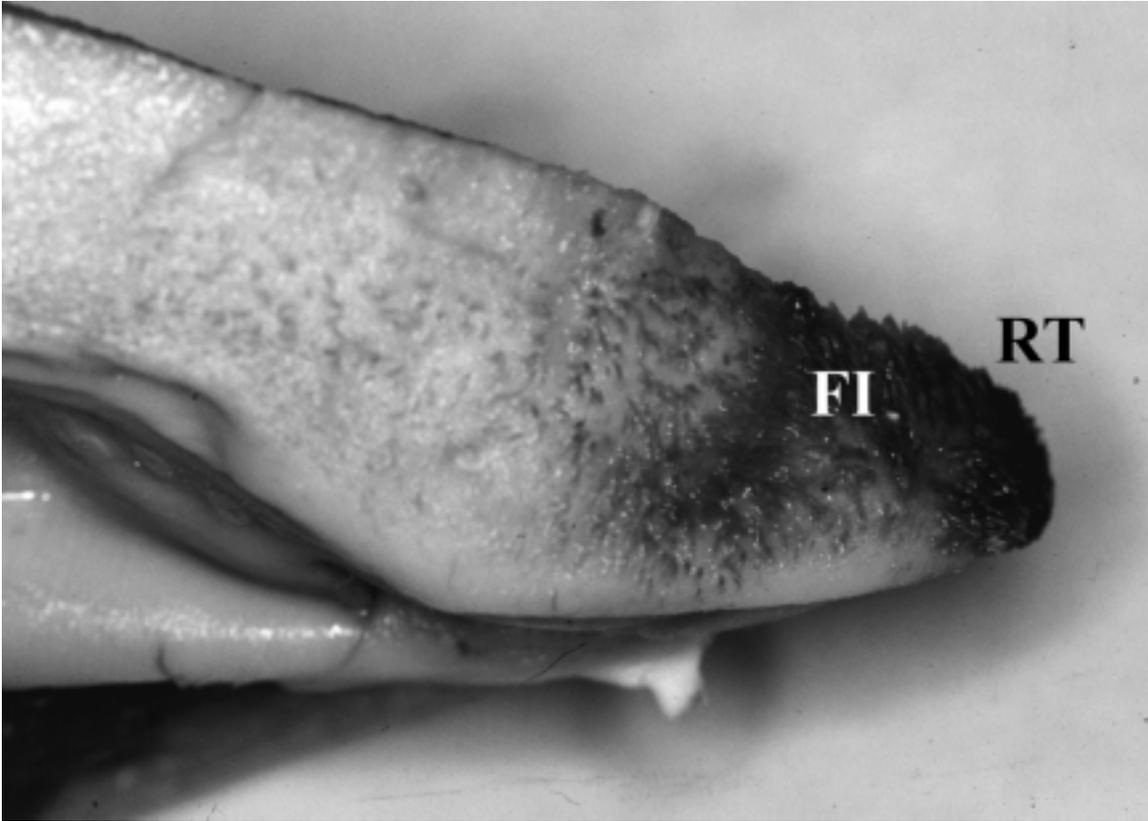
Figure 14. Rostral tip of tongue

Filiform papillae (FI) at rostral tip (RT).
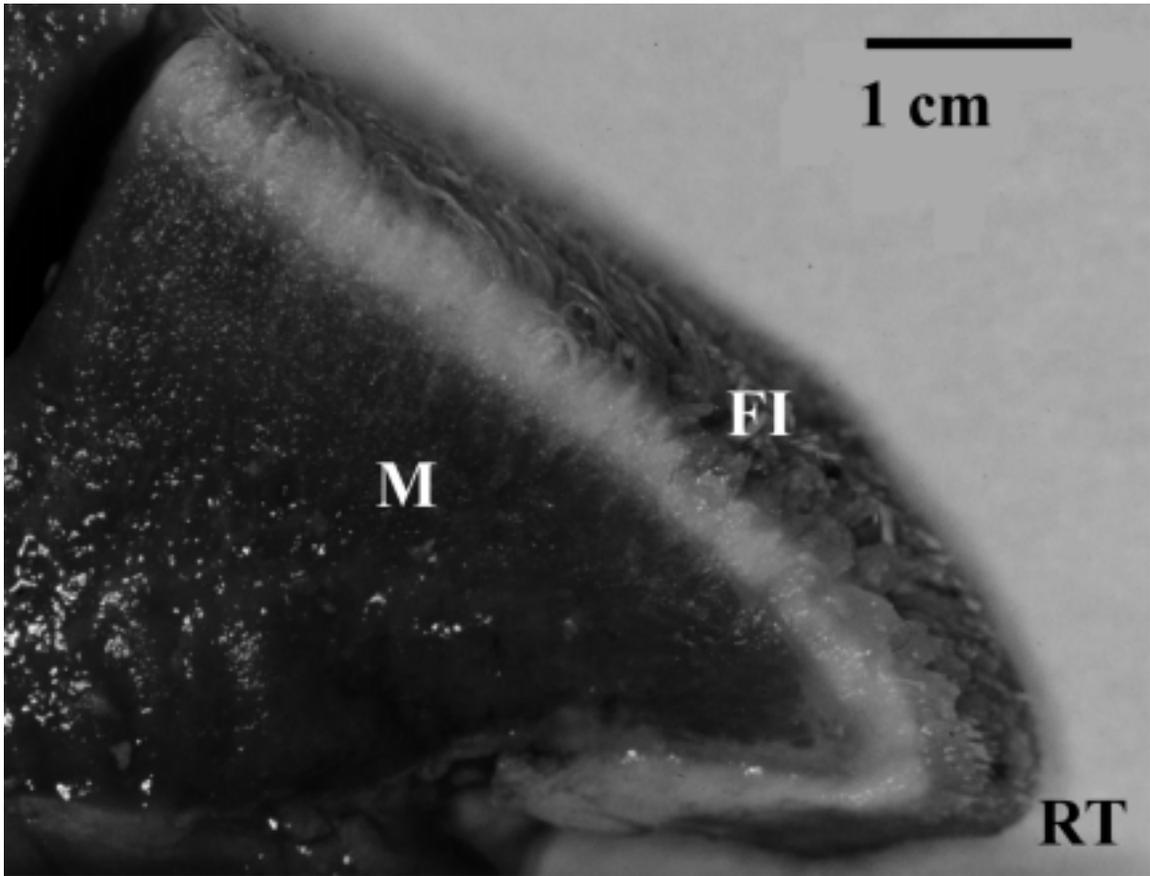
Figure 15. Filiform papillae

Mid-sagittal view through rostral free tip (RT) showing filiform papillae (FI) and intrinsic muscles (M).
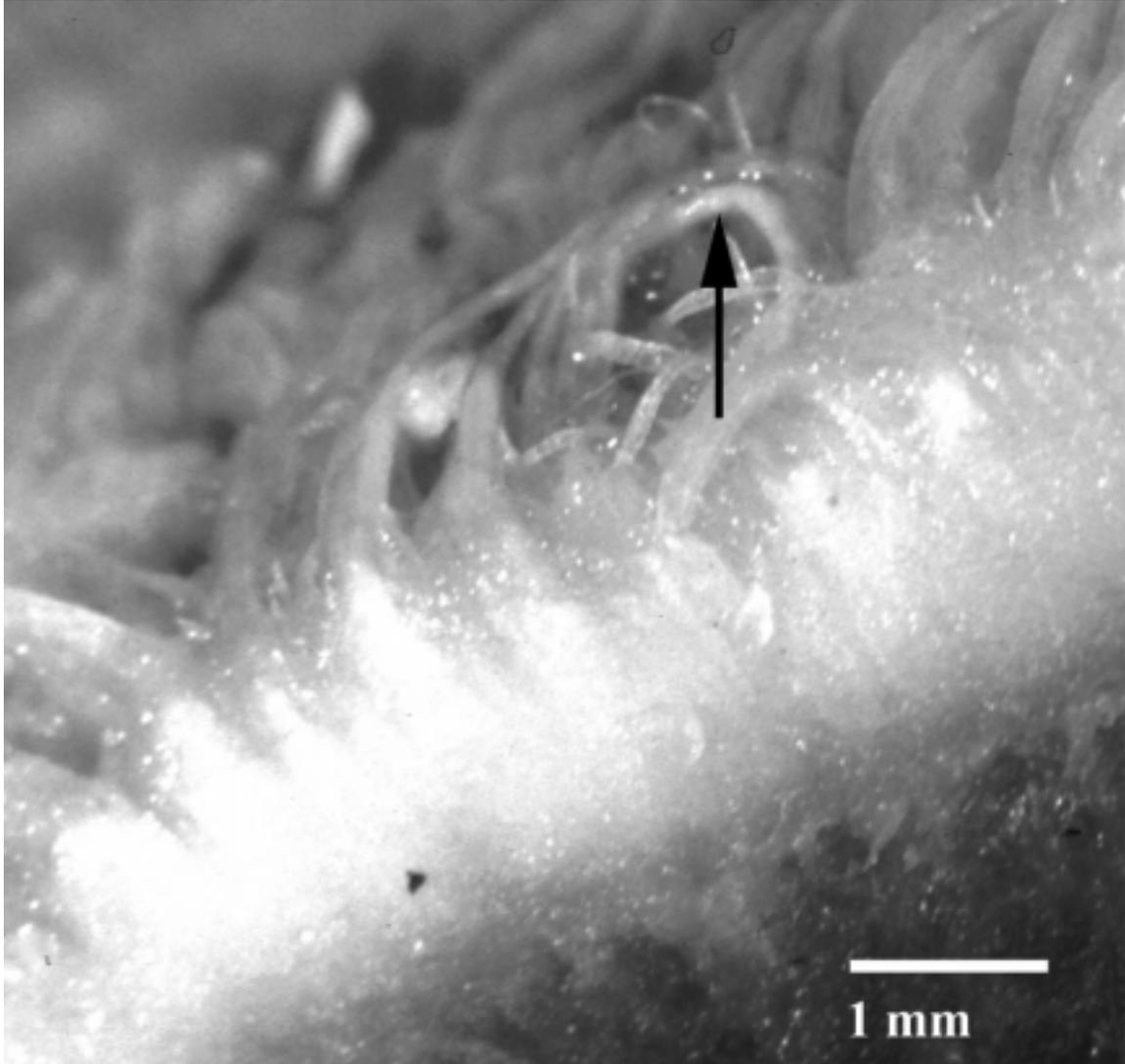
Figure 16. Filiform papillae

High magnification of filiform papillae (arrow).
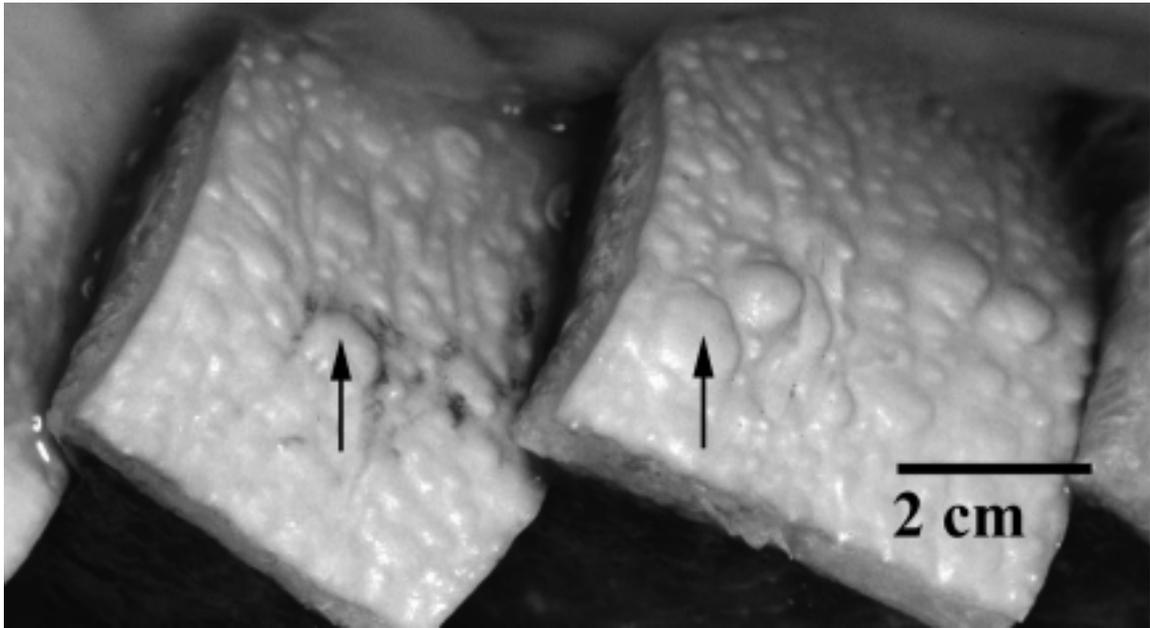
Figure 17. Dorsal papillae

Sections of dorsal lingual surface showing numerous fungiform papillae (arrows).
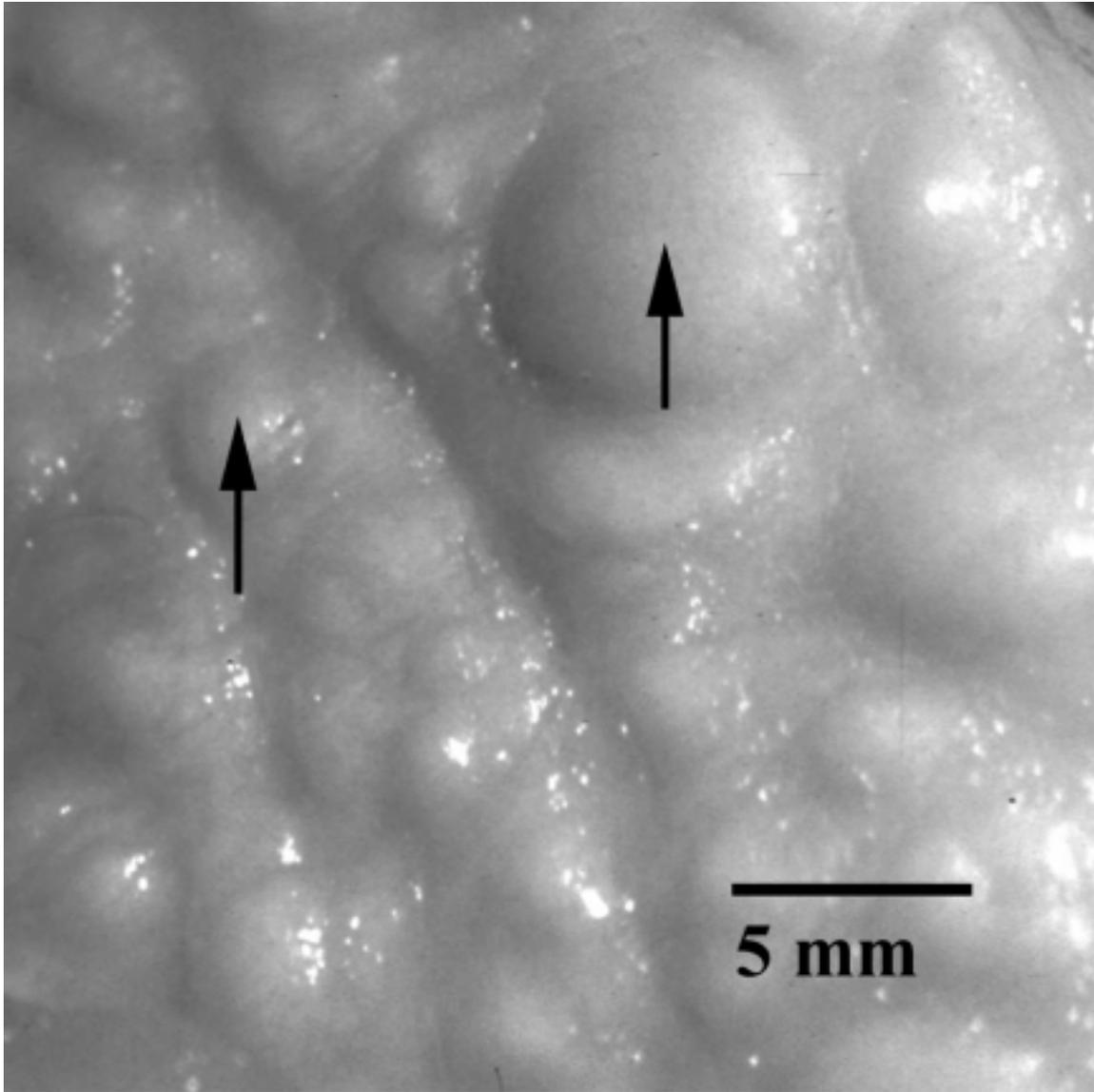
Figure 18. Dorsal papillae

High magnification of dorsal papillae (arrows).
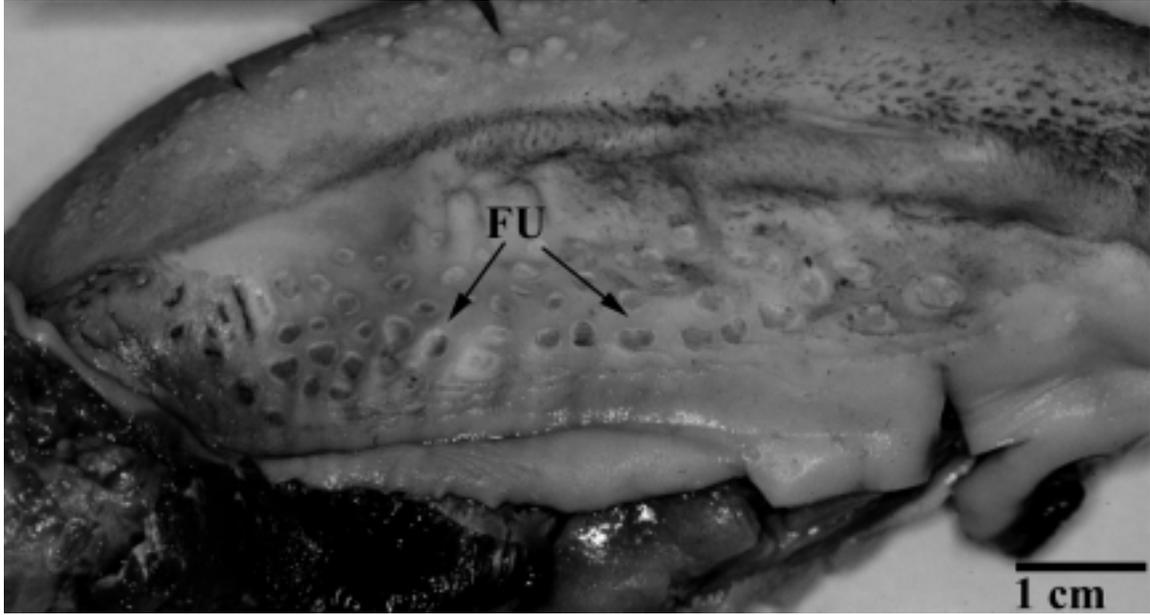
Figure 19. Fungiform papillae

Right lateral wall showing numerous fungiform papillae (FU) (rostral tip to right).
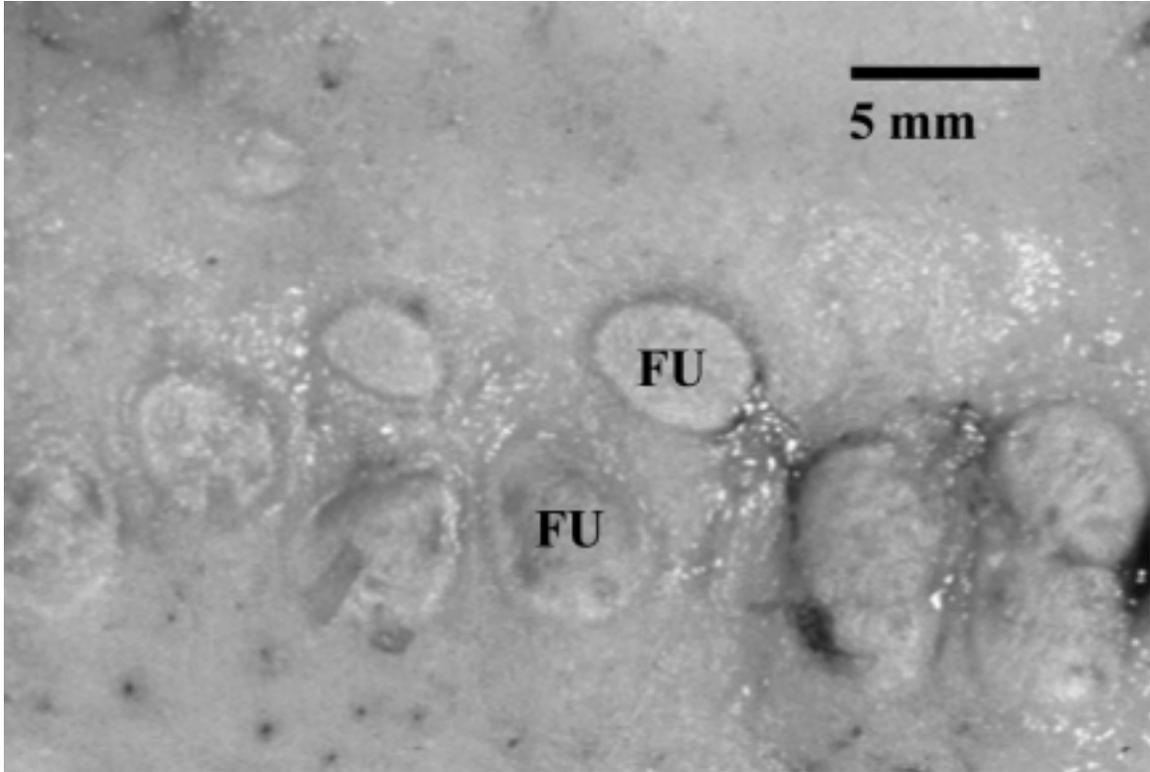
Figure 20. Fungiform papillae

High magnification of fungiform papillae (FU).
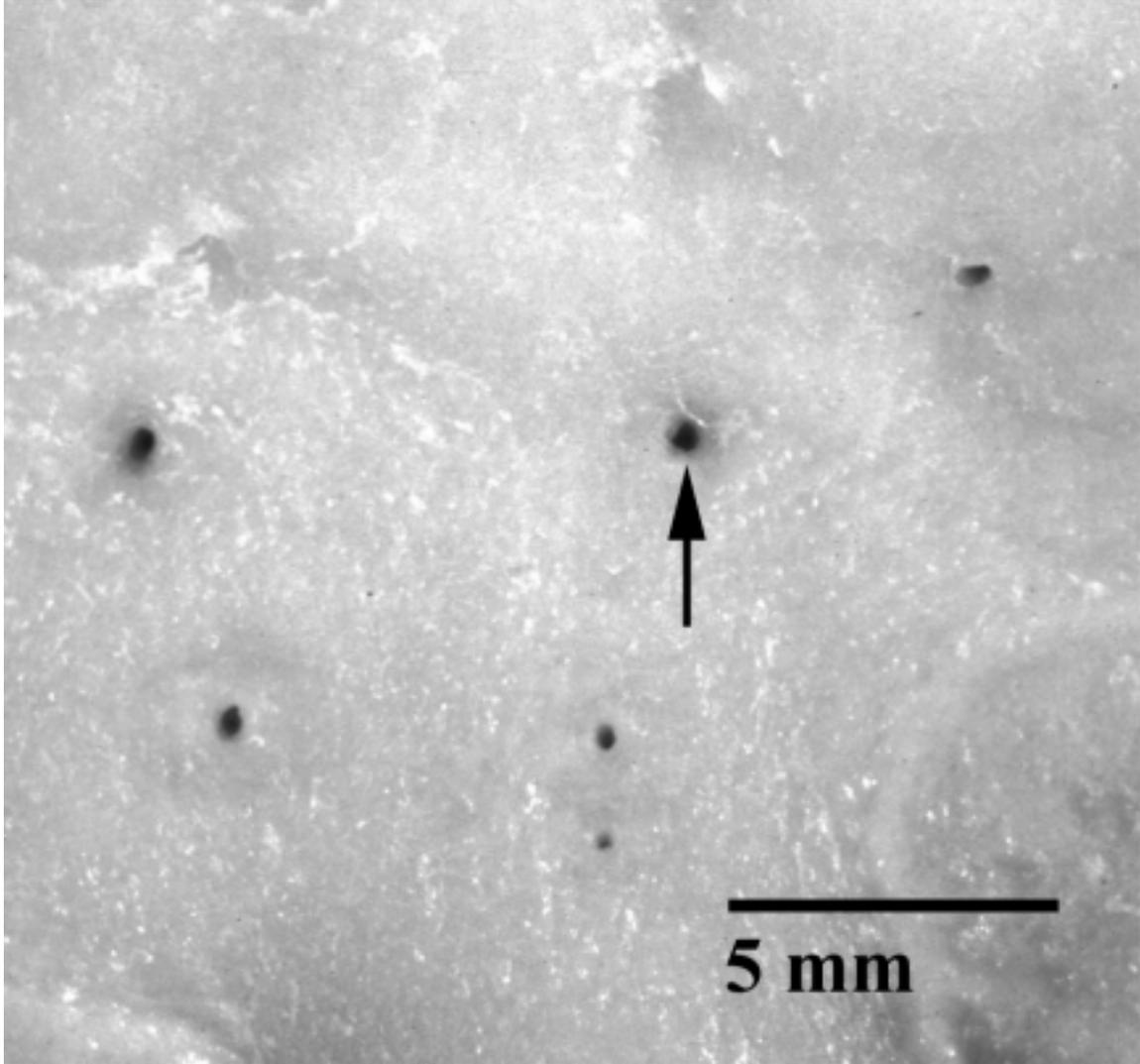
Figure 21. Lateral pits

High magnification of lateral pits (arrow).
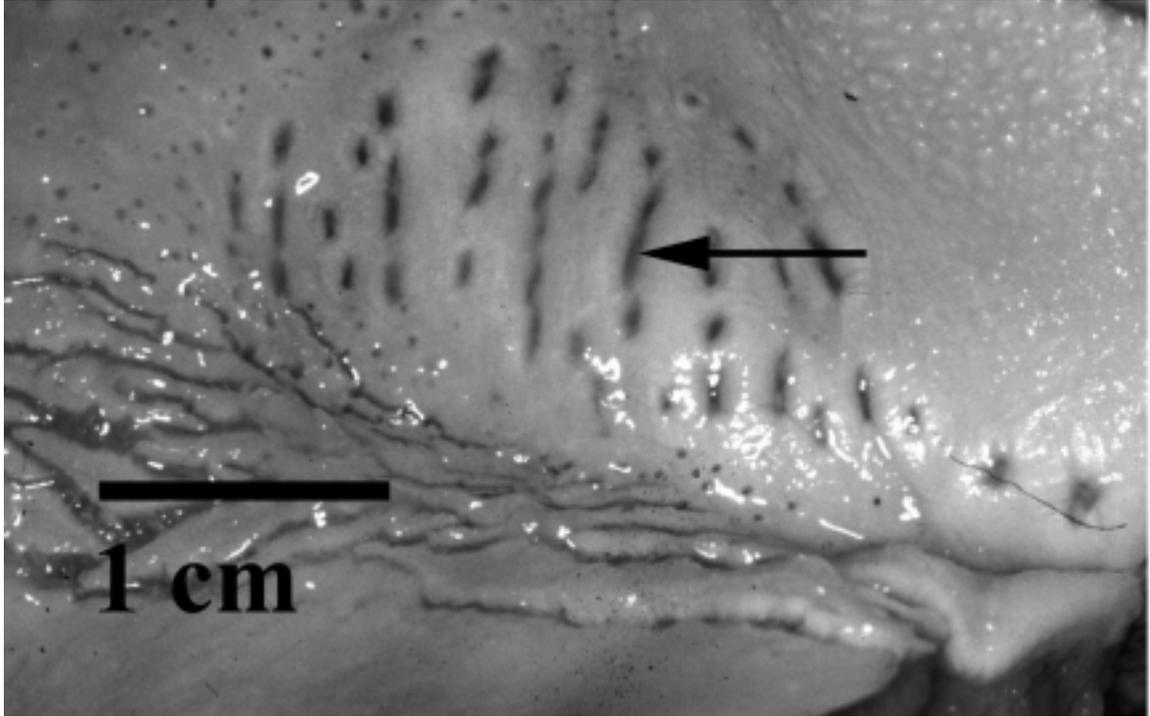
Figure 22. Foliate papillae

Foliate papillae on the right caudal margin showing opening into crypt (arrow).

Microscopic Observations

Rostral Lingual Tip

The filiform papillae (Figs. 23 and 24) characteristic of the dorsal rostral lingual region were structured with a wide base that narrowed to a point at the distal tip. These papillae were approximately 2-5 mm in height, and largely composed of keratin. The ventral lingual surface lacked papillae of any kind and the stratum corneum was not well developed. Striated muscle fibers were evident in this region, as well as nerve fibers and blood vessels in the dermis. The ventral rostral surface lacked filiform papillae (Fig. 25) and appeared smooth. Keratin was not as pronounced as on the dorsal free surface.

Dorsal Surface

The majority of the dorsal lingual surface was covered by a stratified squamous, keratinized epithelium (Figs. 26 and 27). The epidermis was thickest (2-3 mm) in the rostral region and decreased caudally (1-2 mm). The dermis subjacent to the epidermis was composed of dense irregular collagen fibers, supporting nerve fibers, arteries, and veins. This layer was thickest at the mid-region of the tongue.

Numerous fungiform-like papillae (Fig. 28), caudal to the filiform papillae, covered the majority of the dorsum. Previous literature has described the surface as smooth, but upon careful examination, this was not found to be the case. The papillae ranged from 1-3 mm in diameter, and were uniformly less than 1 mm in height. The epidermis covering the papillae was thinner than that of the adjacent epidermis, being reduced by approximately one-fourth. Dermal ridges projected upward into the papillae. The stratum corneum (the keratinized layer covering the papillae) was more pronounced than on the adjacent epithelium.

Multifossulate openings of the foliate papillae (Fig. 29) were observed on the left and right caudal margins. Taste buds were restricted solely to the foliate papillae. The taste buds were embedded in the epidermis (Figs. 30 and 31), and demonstrated an oval shape and appearance typical of other mammals. In one fold of the papillae, 8-12 taste buds were present.
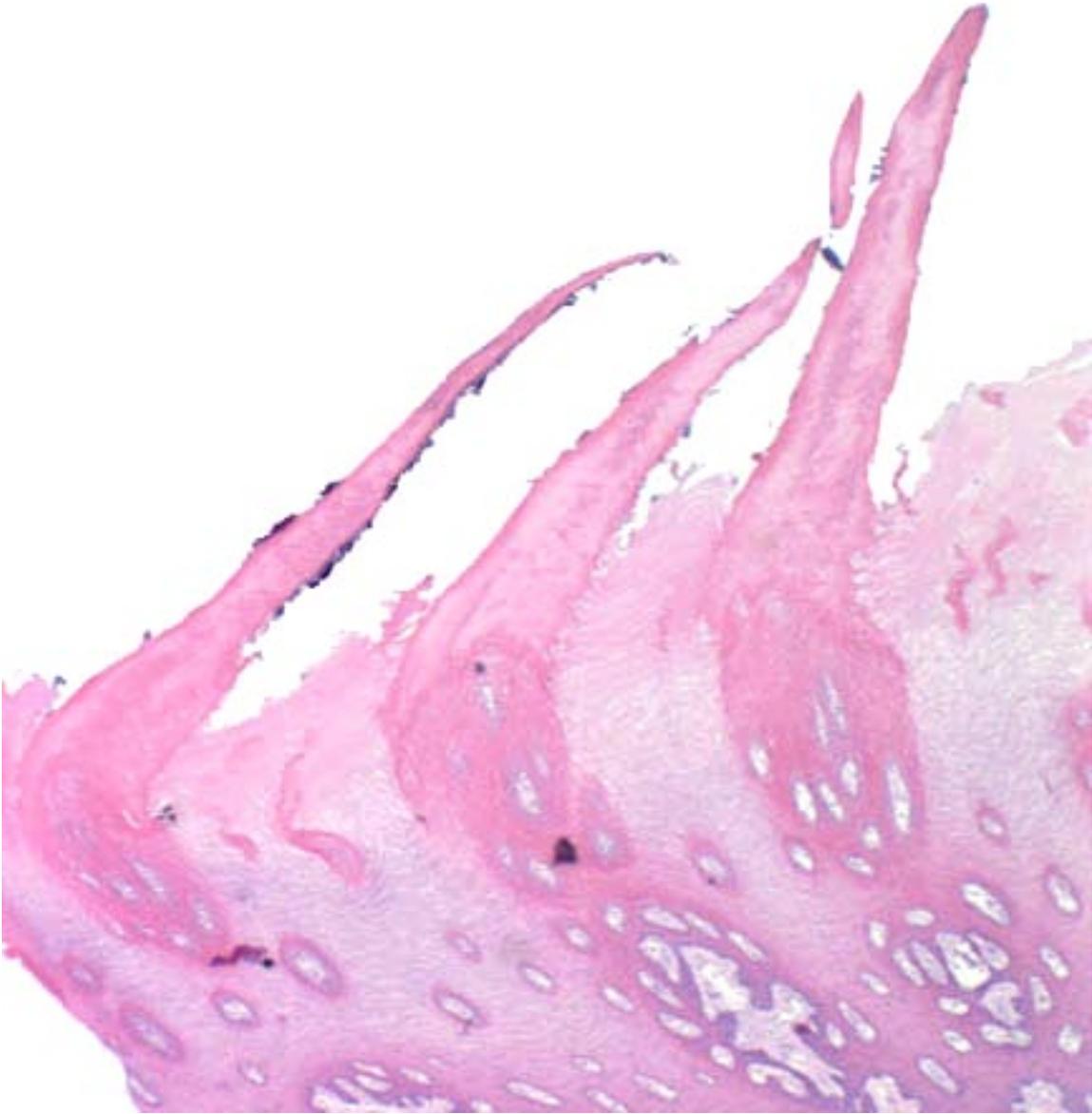
Figure 23. Filiform papillae

Filiform papillae at rostral tip. x40 (H&E).
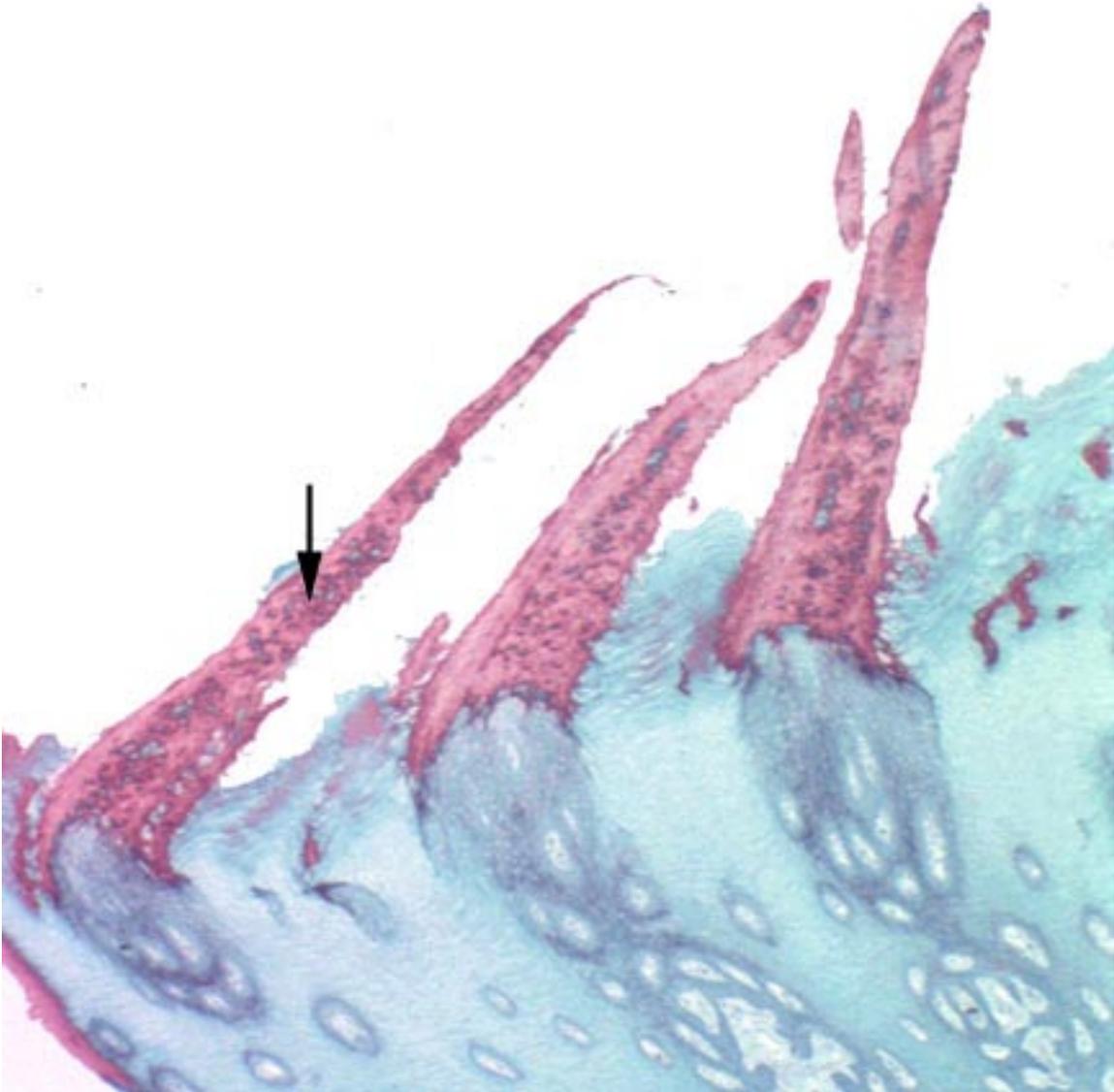
Figure 24. Filiform papillae

Filiform papillae at rostral tip showing keratinized free portion (arrow). x40 (Trichrome).
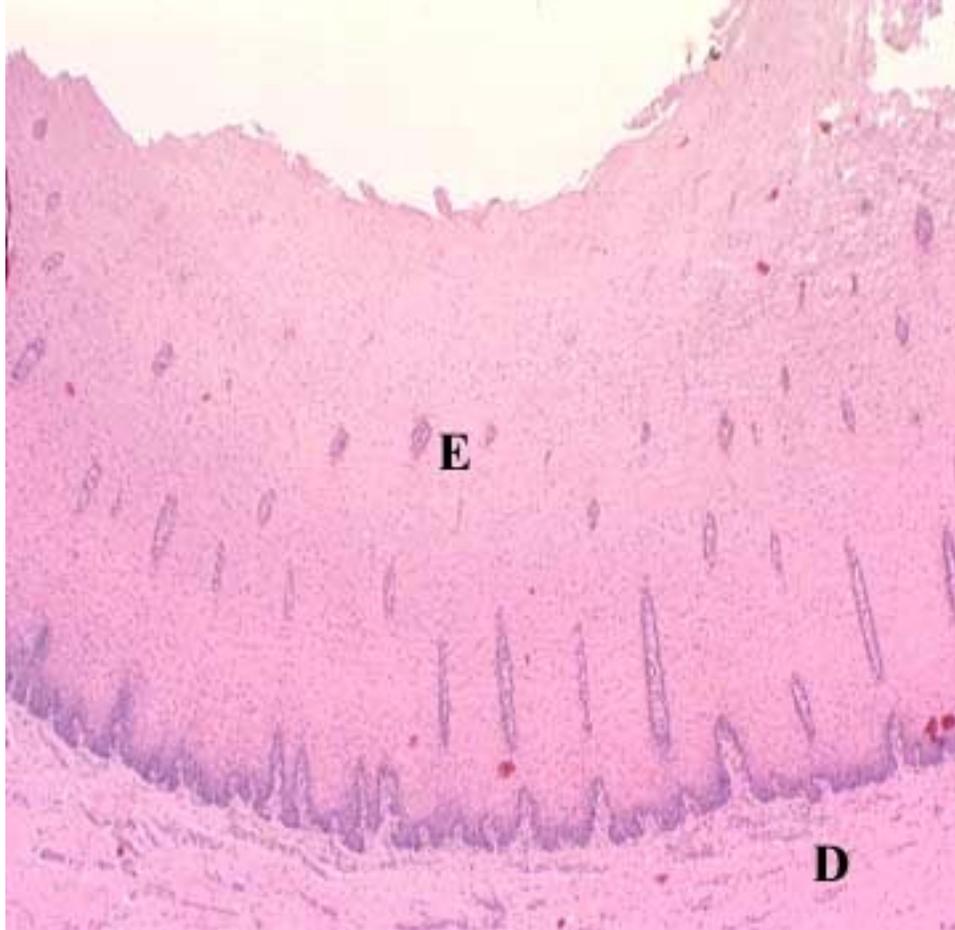
Figure 25. Ventral epithelium

Ventral rostral surface showing papillary-free epidermis (E) and dermis (D). x450
(H&E).
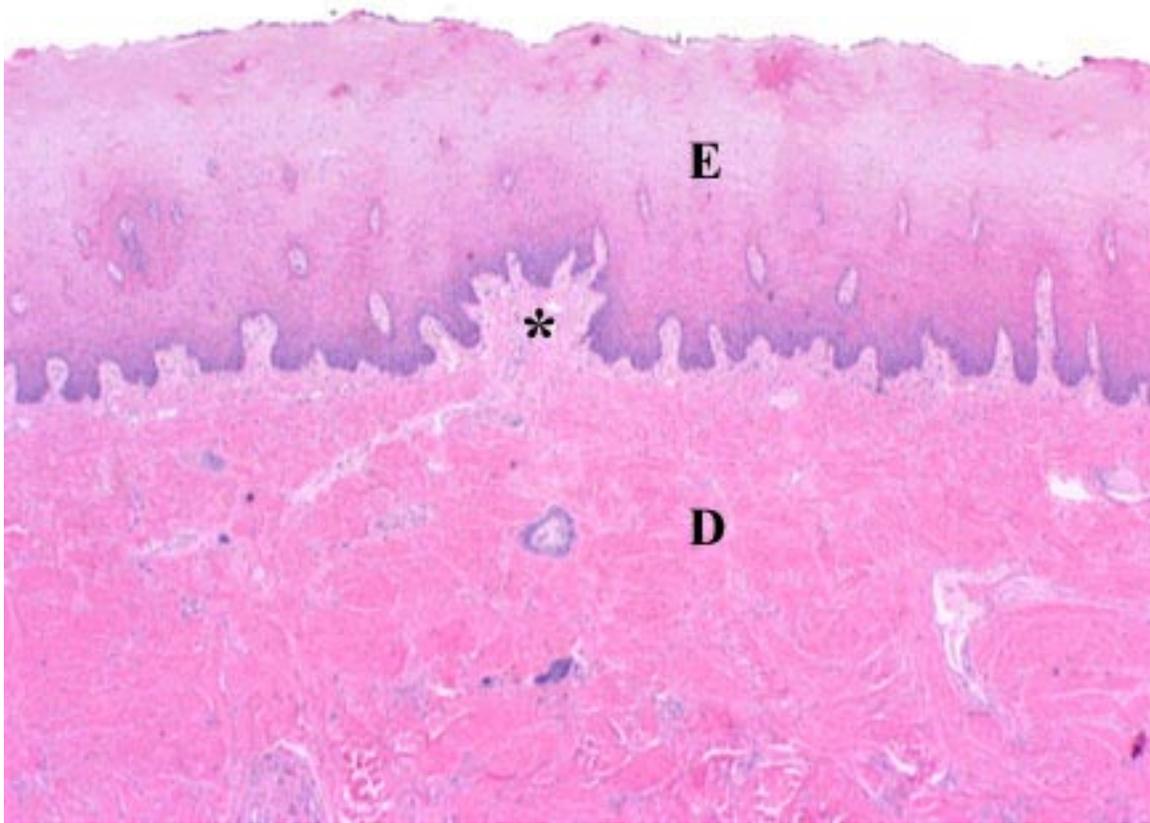
Figure 26. Surface epithelium

Dorsal lingual surface noting epidermis (E), dermis (D), and dermal ridge (asterisk). x40 (H&E).
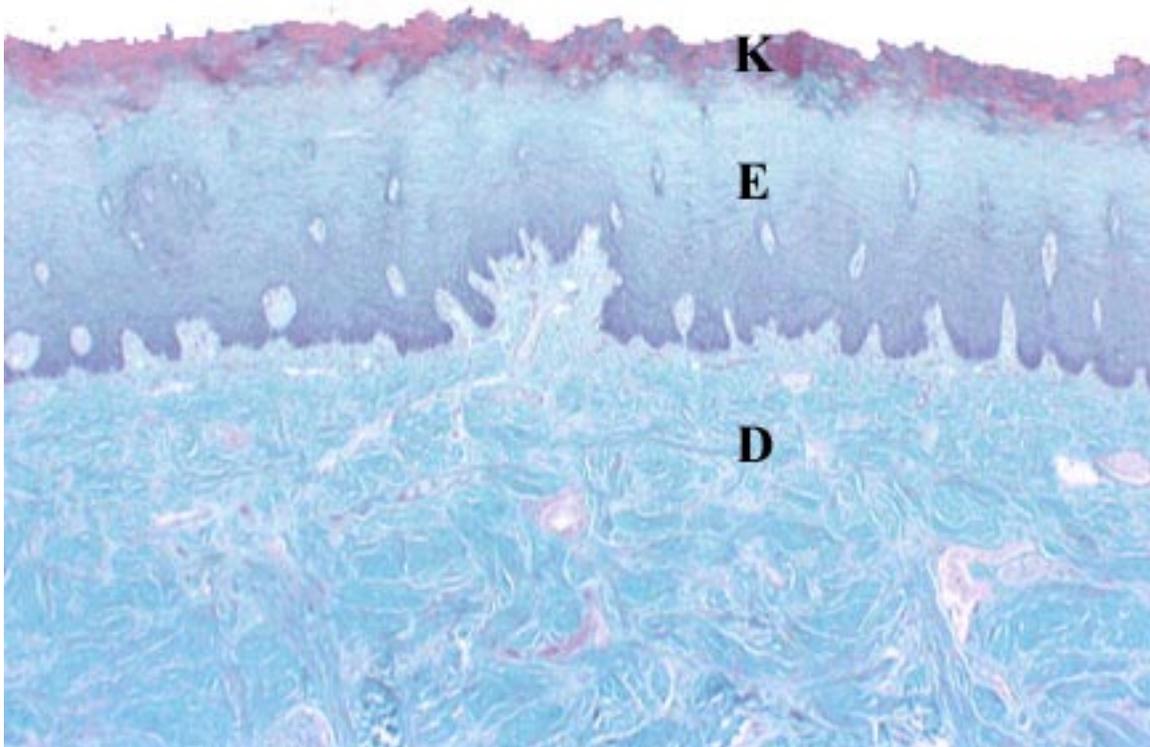
Figure 27. Surface epithelium

Dorsal lingual surface noting surface keratin (K), epidermis (E), and dermis (D). x40 (Trichrome).
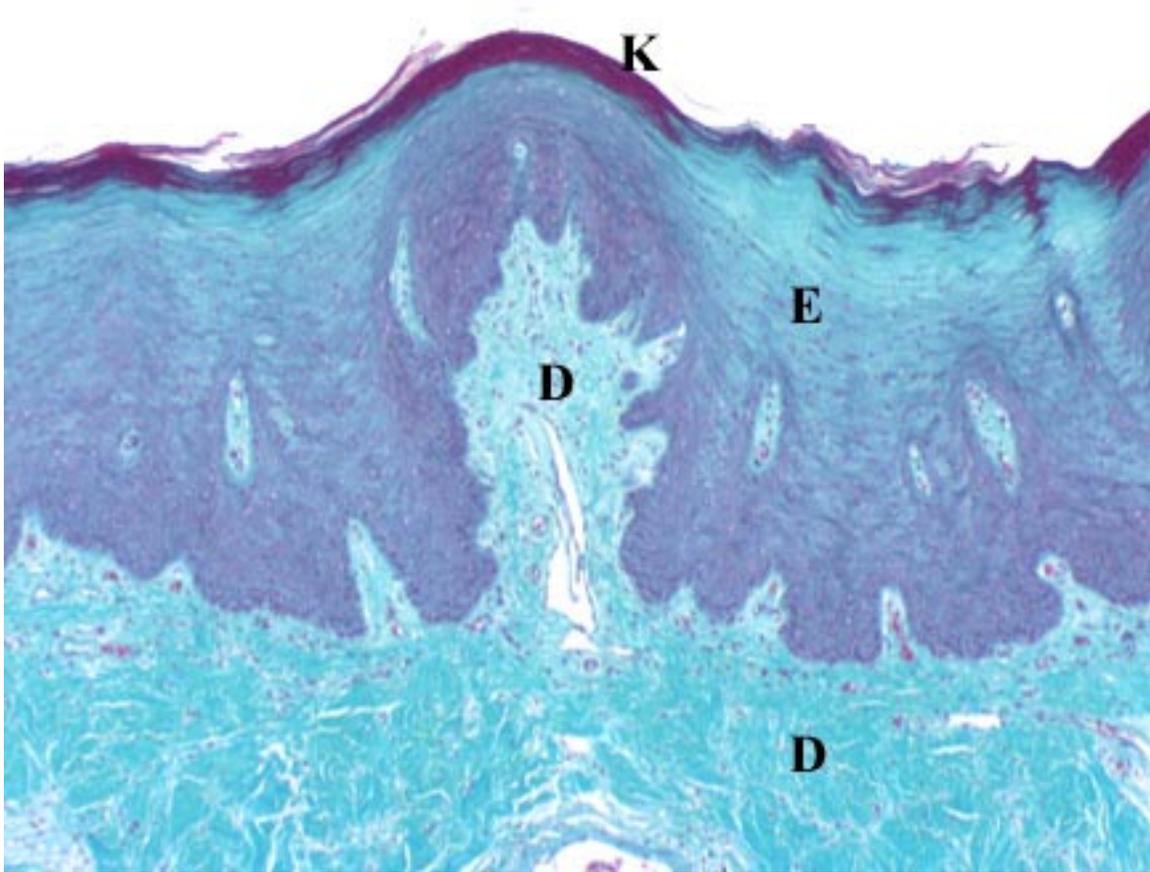
Figure 28. Dorsal papillae

Dorsal fungiform papilla noting surface keratin (K) and reduced epidermis (E) over core
of dermis (D). x40 (Trichrome).
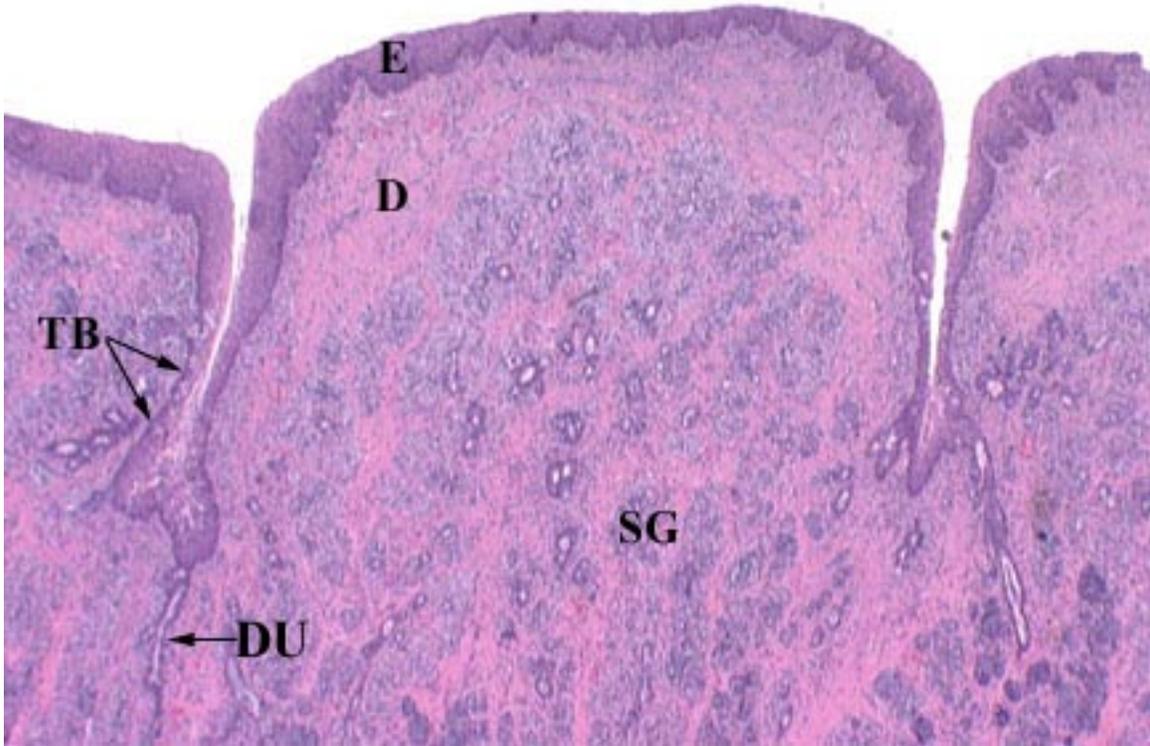
Figure 29. Foliate papillae

Foliate papillae noting epidermis (E), dermis (D), lingual salivary glands (SG), taste buds
(TB) embedded in the epidermis, and ducts (DU) leading to crypts. x40 (H&E).
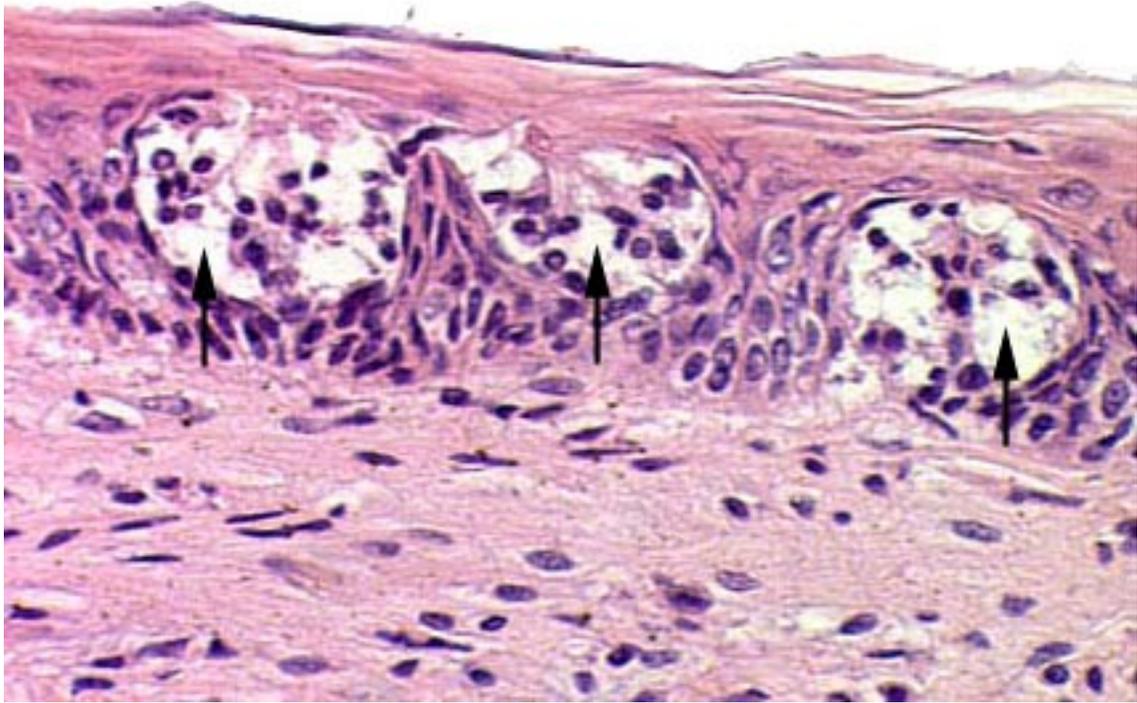
Figure 30. Taste buds

Three taste buds (arrows) in epidermis of foliate papilla. x2250 (H&E).
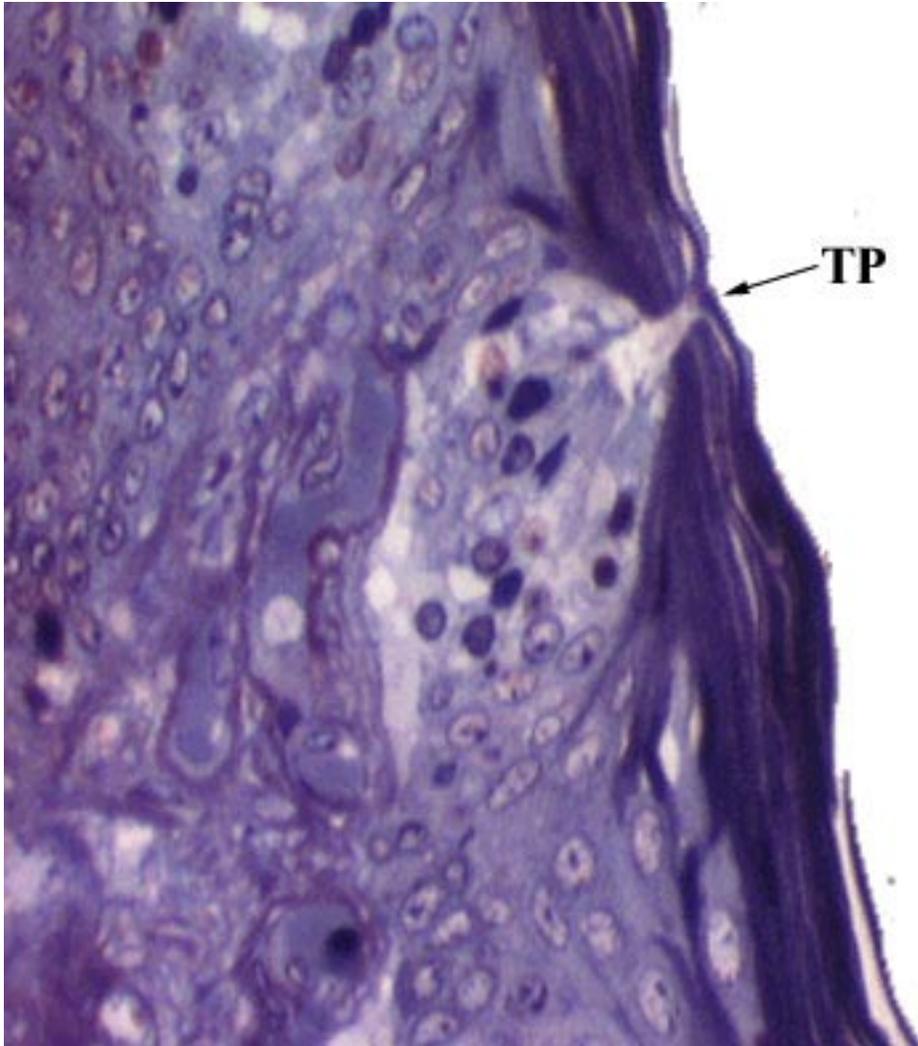
Figure 31. Taste bud

Single taste bud, noting taste pore (TP), in foliate papillae. x2250 (Tri-stain).

Numerous open pits, leading to mainly mucous salivary glands in the dermis (Fig. 32), were observed in the dorsal caudal region of the tongue, proximal to the esophagus. The epidermis was thinnest in this region.

Lateral surface

The lateral surface was also covered by a stratified, keratinized squamous epithelium (Fig. 33). Epidermal pegs and dermal ridges were more extensive in the dorsal wall regions. The epidermis was also more keratinized in this same area. The thickness of the epithelium gradually reduced ventrally. Numerous fungiform papillae (see Table 2) projected from the lateral walls. Unlike the dorsal fungiform papillae, which were small and convex in shape, these papillae were larger in diameter and appeared flat (Fig. 34). Similar to the dorsal surface papillae, the covering epithelium was reduced compared to the adjacent areas; the stratum corneum was also thicker when compared to the adjacent areas.

Numerous open pits (Fig 35) were observed intermixed with and ventral to the fungiform papillae. Ducts from these pits led to extensive mucous lingual salivary glands.

Lingual salivary glands

Multiple salivary glands were distributed throughout the lingual substance, being both mucous and seromucous in nature. Glands were classified at compound tubulo-acinar in shape (Fig. 36 and 37). Periodic acid-Schiff staining demonstrated that the majority of the glands were mucous in nature (Fig 38 and 39). Seromucous glands were limited to the foliate papillae and presented as serous demilumes (Fig. 40). Ducts from seromucous glands could be seen opening into the crypts of the foliate papillae.
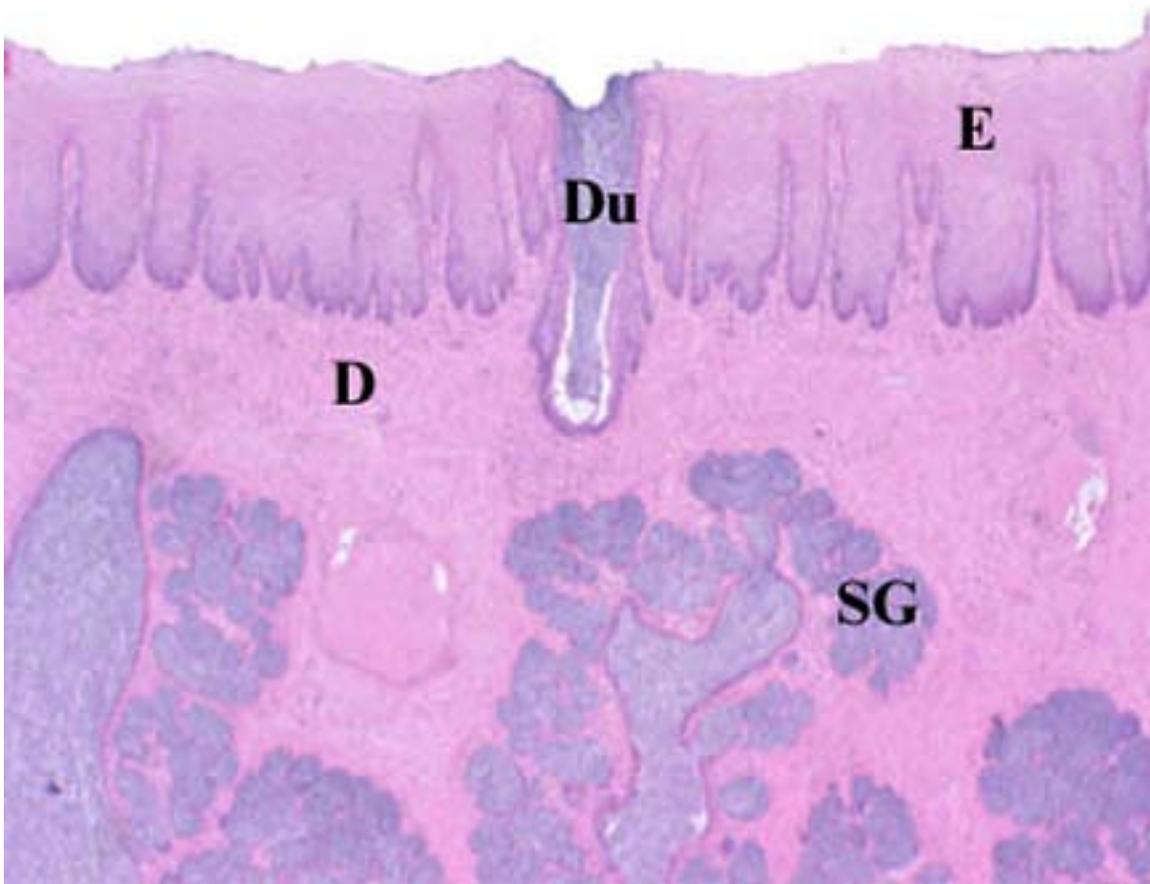
Figure 32. Dorsal pit

Caudal dorsal pit area showing duct (Du) leading to surface. Also showing are epidermis
(E), dermis (D), and lingual salivary glands (SG). x40 (H&E).
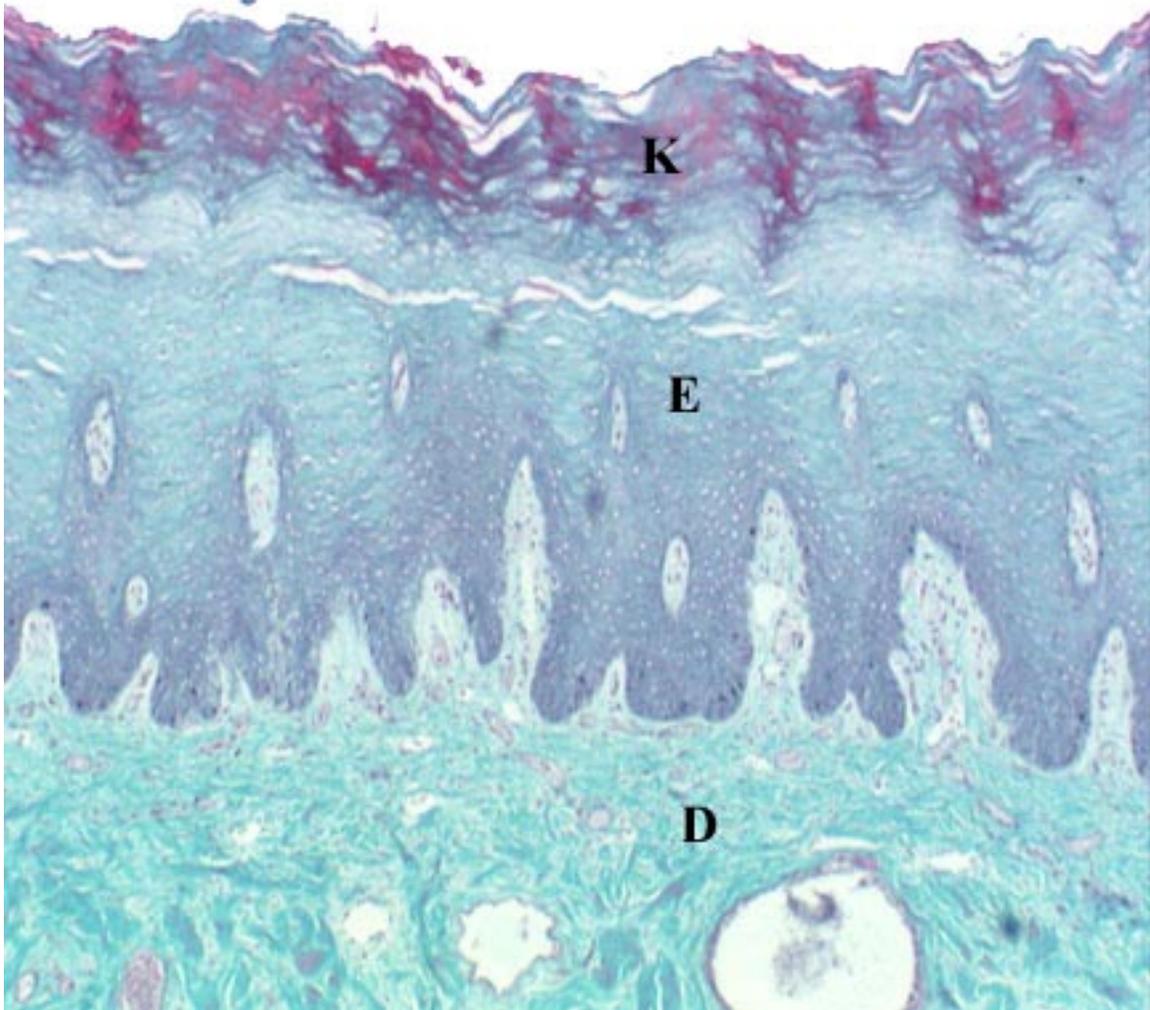
Figure 33. Lateral epithelium

Lateral lingual surface noting surface keratin (K), epidermis (E), and dermis (D). x900 (Trichrome).

Figure 34. Fungiform papillae

Single lateral fungiform papilla showing epidermis (E), dermal core (D), and muscle (M). x40 (H&E).
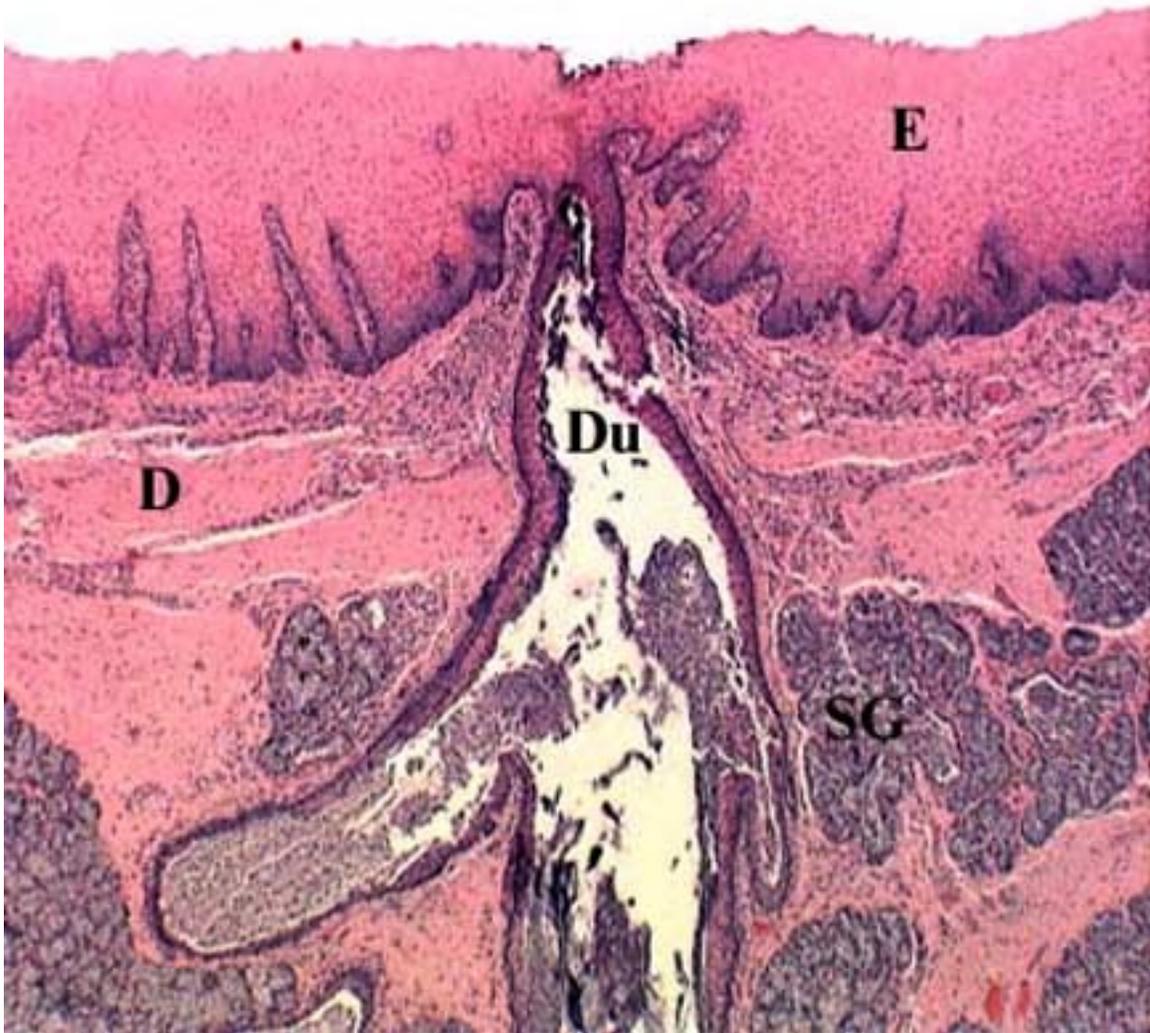
Figure 35. Lateral pit

Lateral pit showing mucous lingual glands (SG) and duct (Du) leading to surface, epidermis (E) and dermis (D). x40 (H&E).
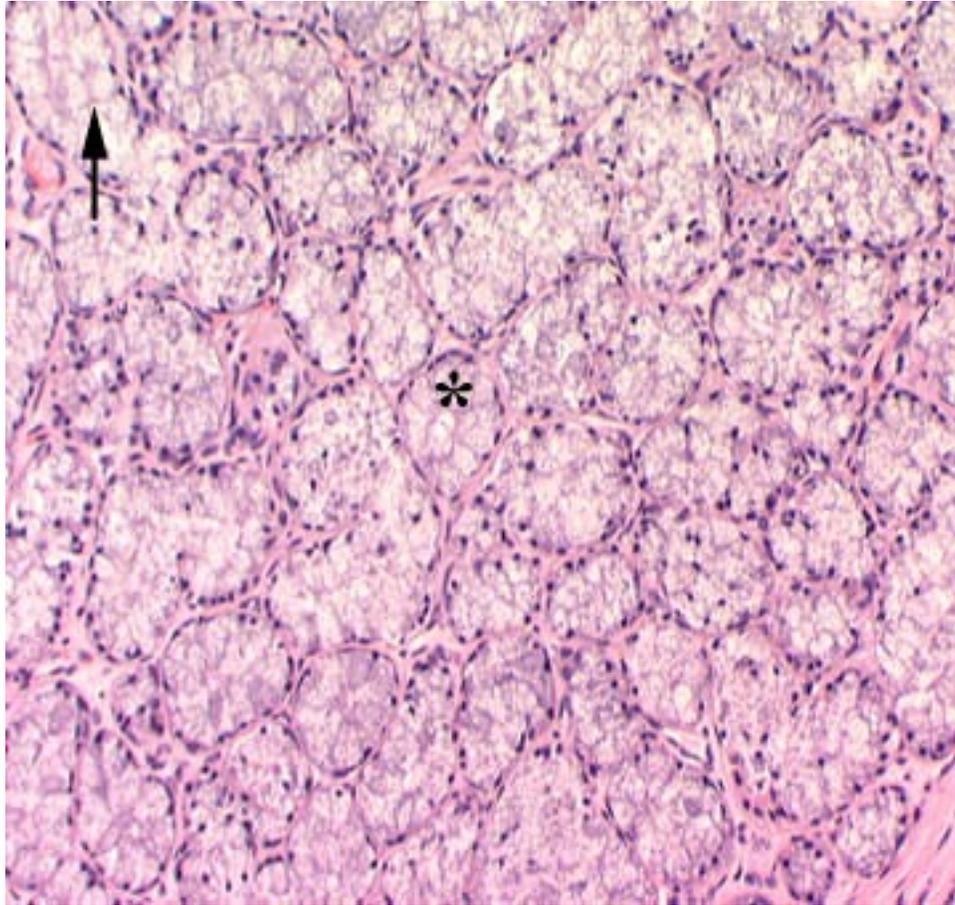
Figure 36. Salivary glands

Mucous lingual glands showing both acinus (asterisk) and tubular (arrow) portions. x160
(H&E).
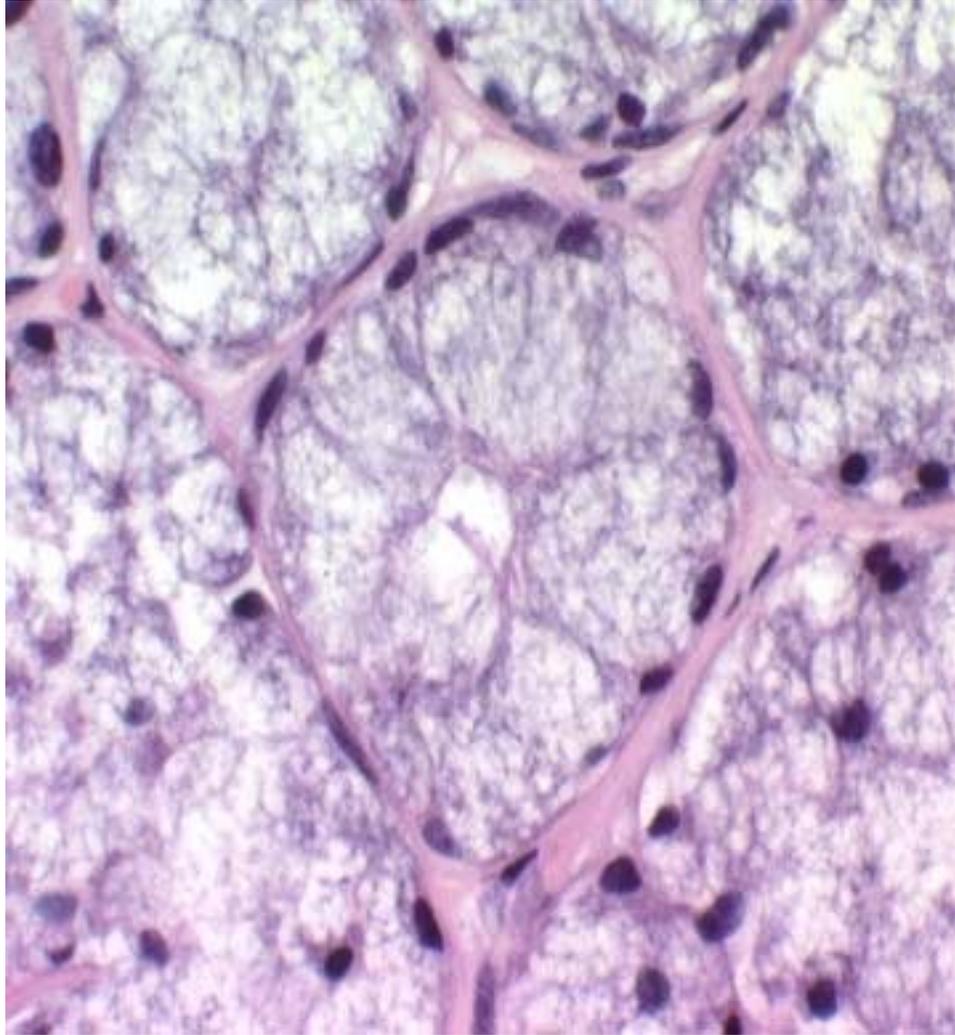
Figure 37. Salivary gland

Single mucous acinus. x600 (H&E).
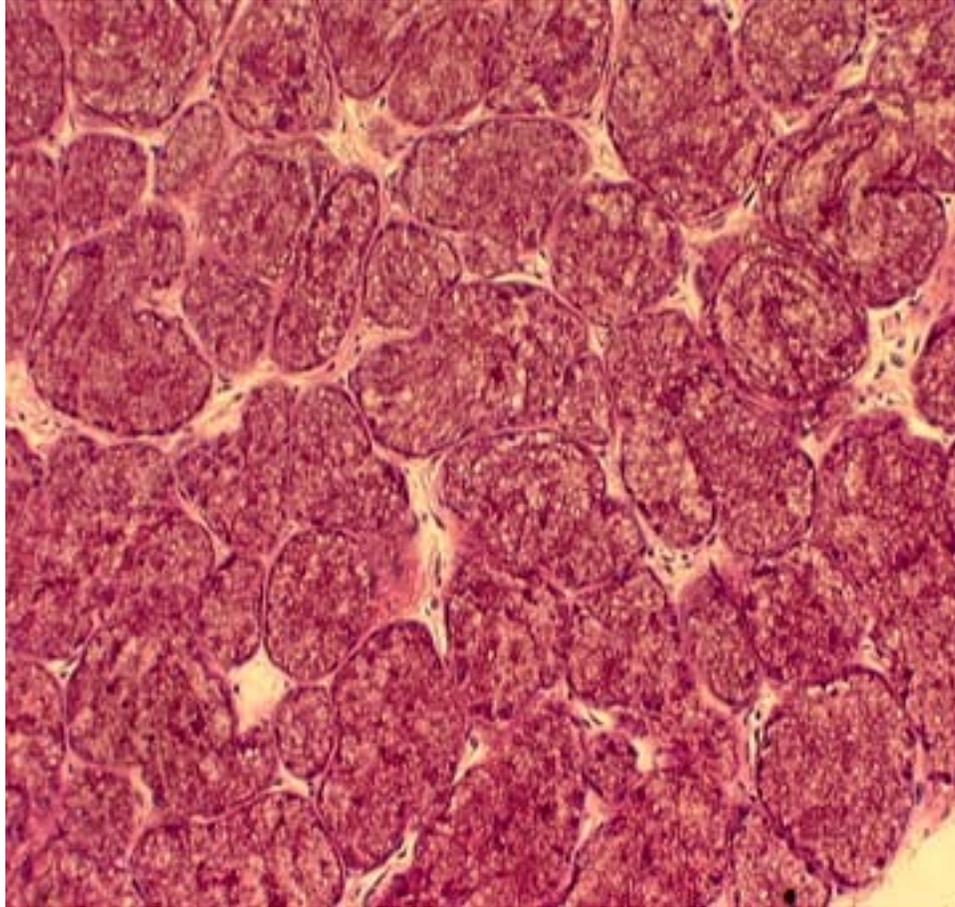
Figure 38. Salivary glands

Histochemistry revealing carbohydrate nature of mucous salivary glands. x200 (PAS).
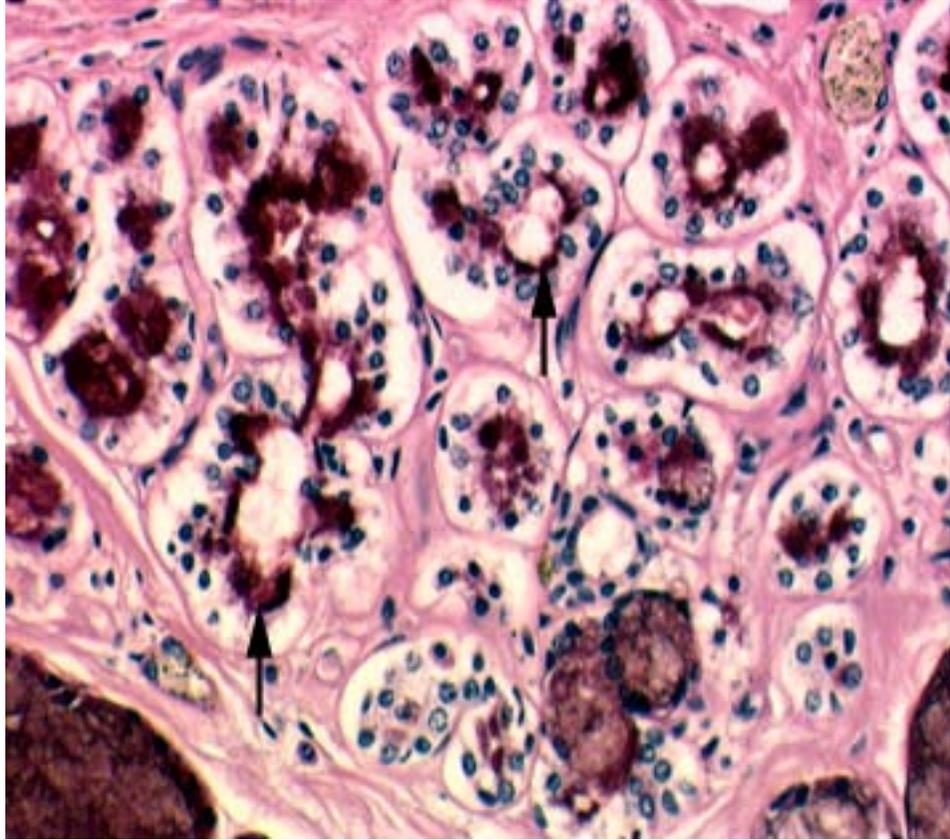
Figure 39. Salivary ducts

Small ducts showing mucous (arrows) in the lumen. x330 (PAS).
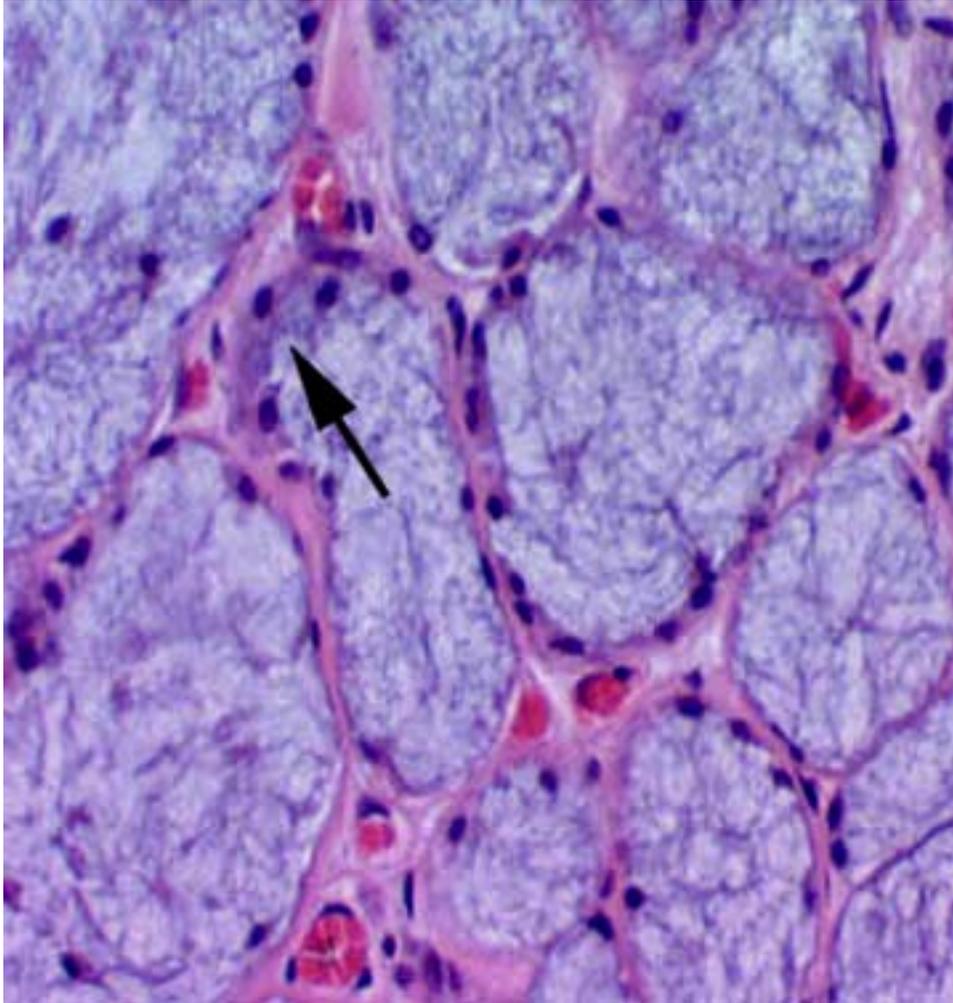
Figure 40. Mixed salivary gland

Seromucous gland in foliate papillae showing serous demilume (arrow). x650 (H&E).

Connective tissue

The dermis and hypodermis were comprised of dense, irregular connective tissue (Fig. 41). The connective tissue supported numerous blood vessels, nerve fibers, adipose tissue, and muscle.

Intrinsic musculature

The majority of the lingual substance was composed of striated skeletal muscle (Figs. 42 and 43). Muscle bundles were distributed in multiple planes, as demonstrated by muscle bundles sectioned transversely, longitudinally, and obliquely.

Adipose Tissue

The lingual structure was well-supplied with adipose tissue, with a higher concentration of fatty tissue along the caudal mid-sagittal region (Fig. 44). Apidocytes could be seen between bundles of muscle fibers and connective tissue.

Vasculature and innervation

The tongue was well-supplied with blood vessels and nerve fibers. Vessels and fibers were freely distributed in the lingual substance, supported by connective tissue. In one section from the base of the tongue, a large nerve bundle was observed adjacent to an artery, encircled by several venules (Fig. 45).
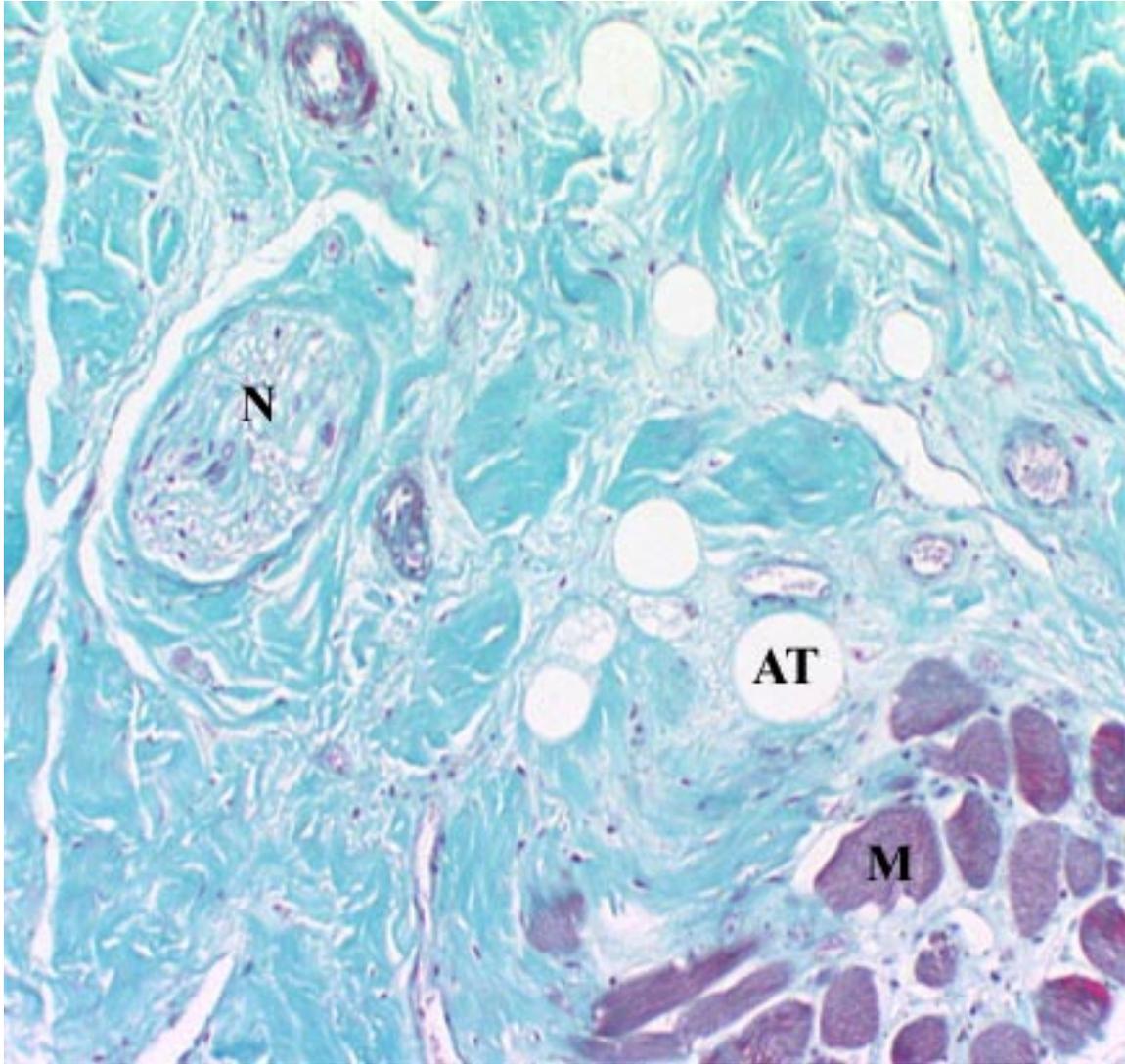
Figure 41. Connective tissue

Dermal connective tissue showing nerve fiber (N), adipose tissue (AT), and muscle (M). x450 (Trichrome).
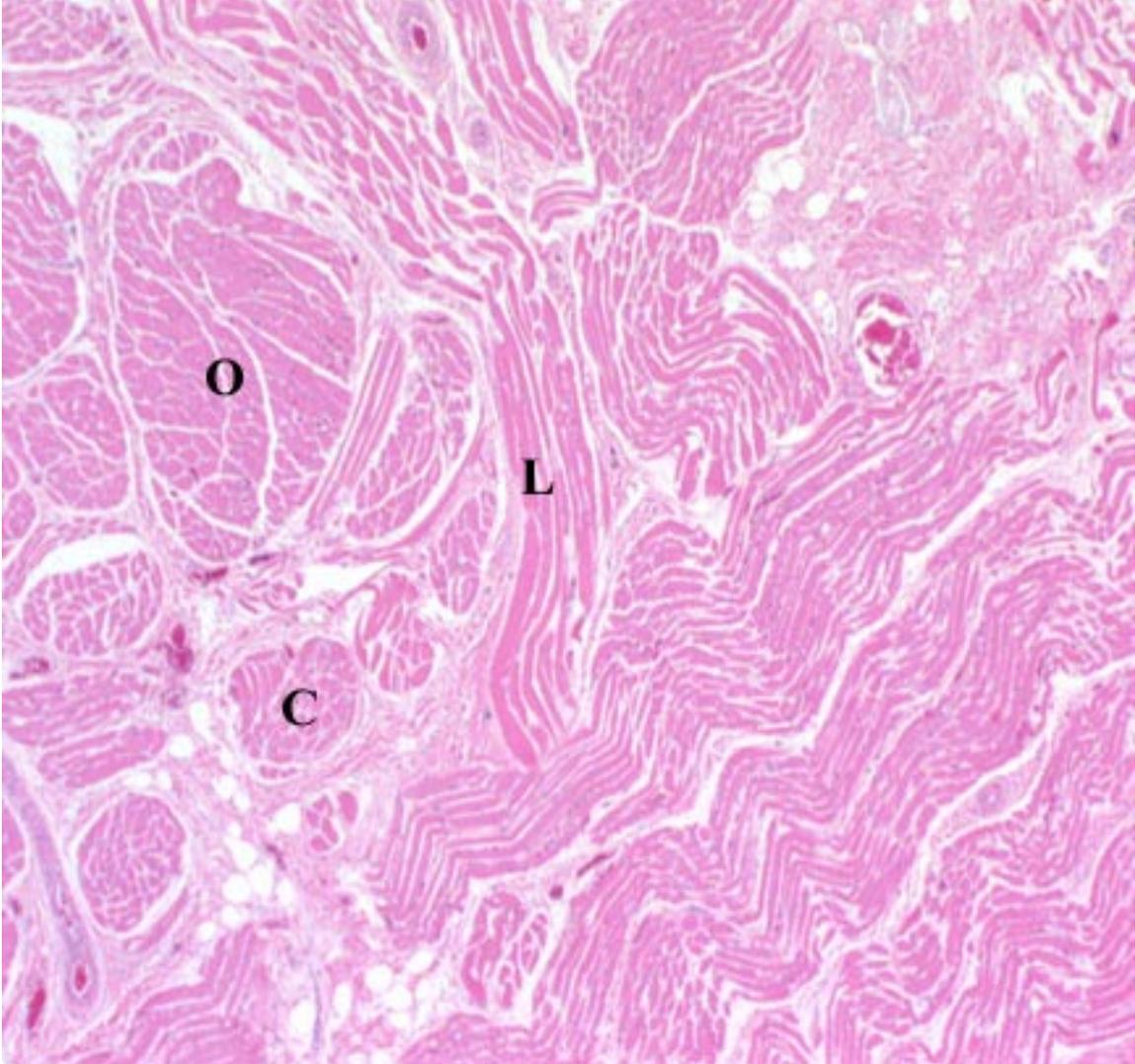
Figure 42. Intrinsic lingual musculature

Striated skeletal muscle in all planes of section, longitudinal (L), cross (C), and oblique
(O). x450 (H&E).
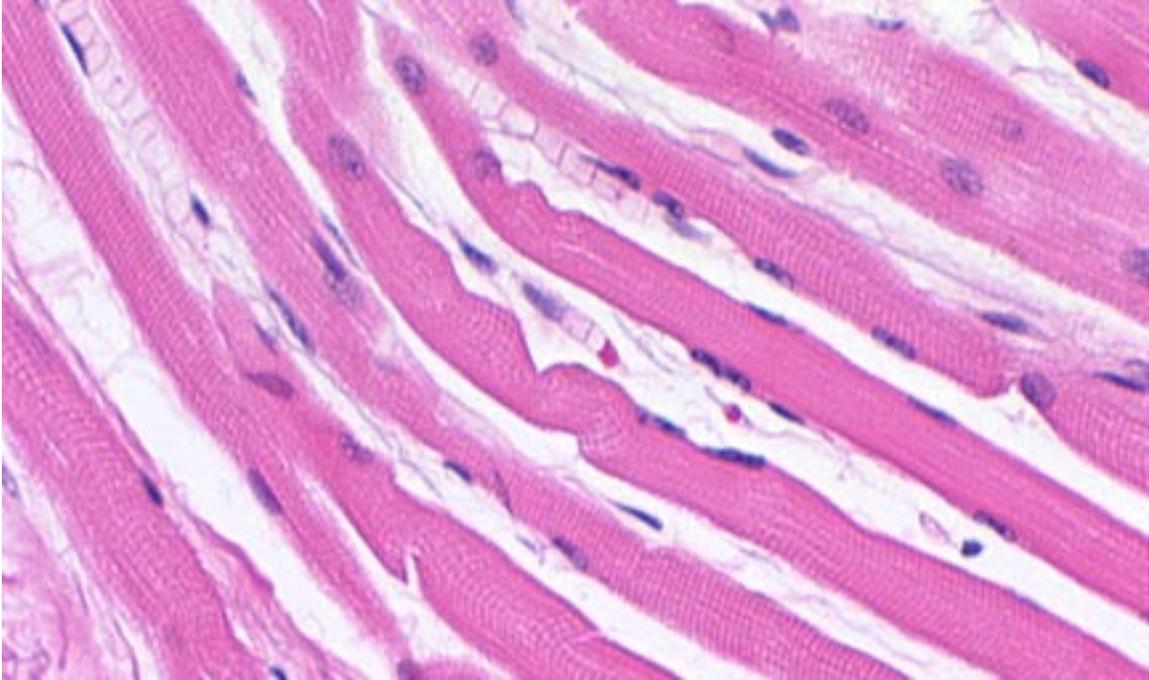
Figure 43. Intrinsic lingual musculature

Striated skeletal muscle fibrils in longitudinal section. x2050 (H&E).
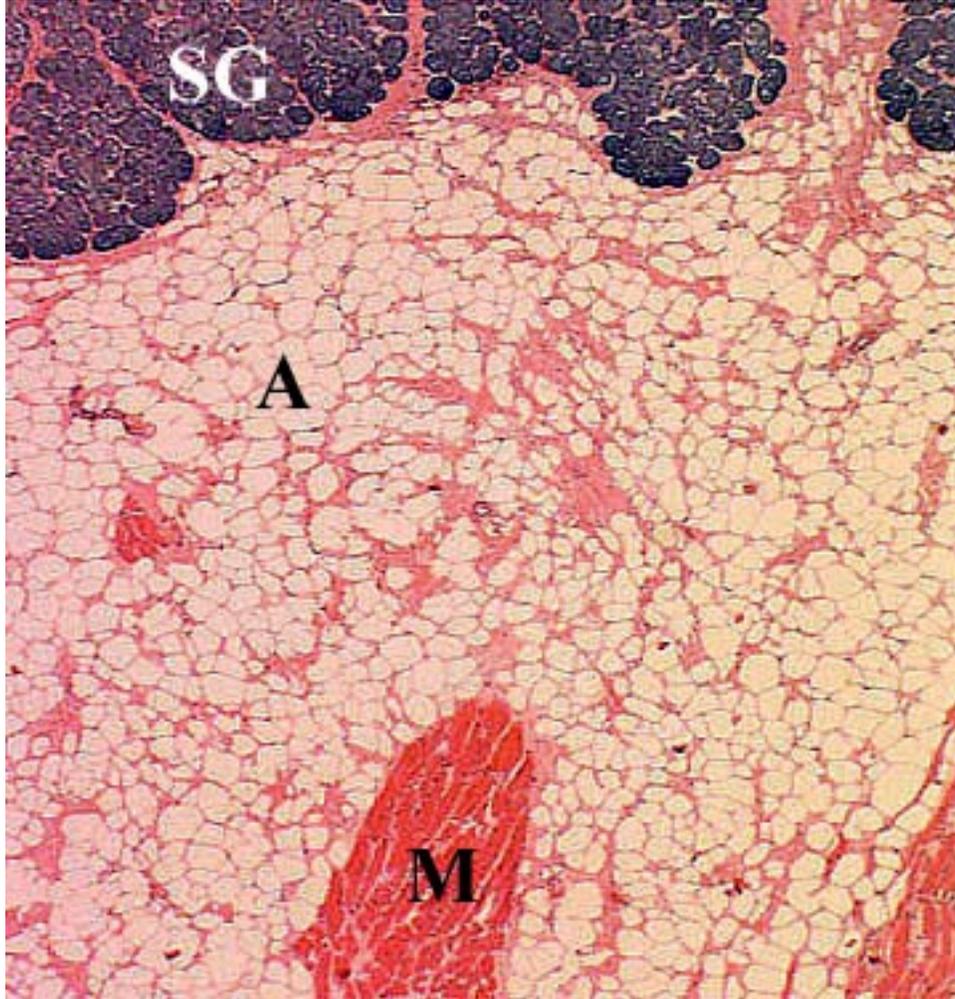
Figure 44. Adipose tissue

Adipose tissue (A), salivary glands (SG) and muscle (M) in mid-caudal tongue. x450 (H&E).
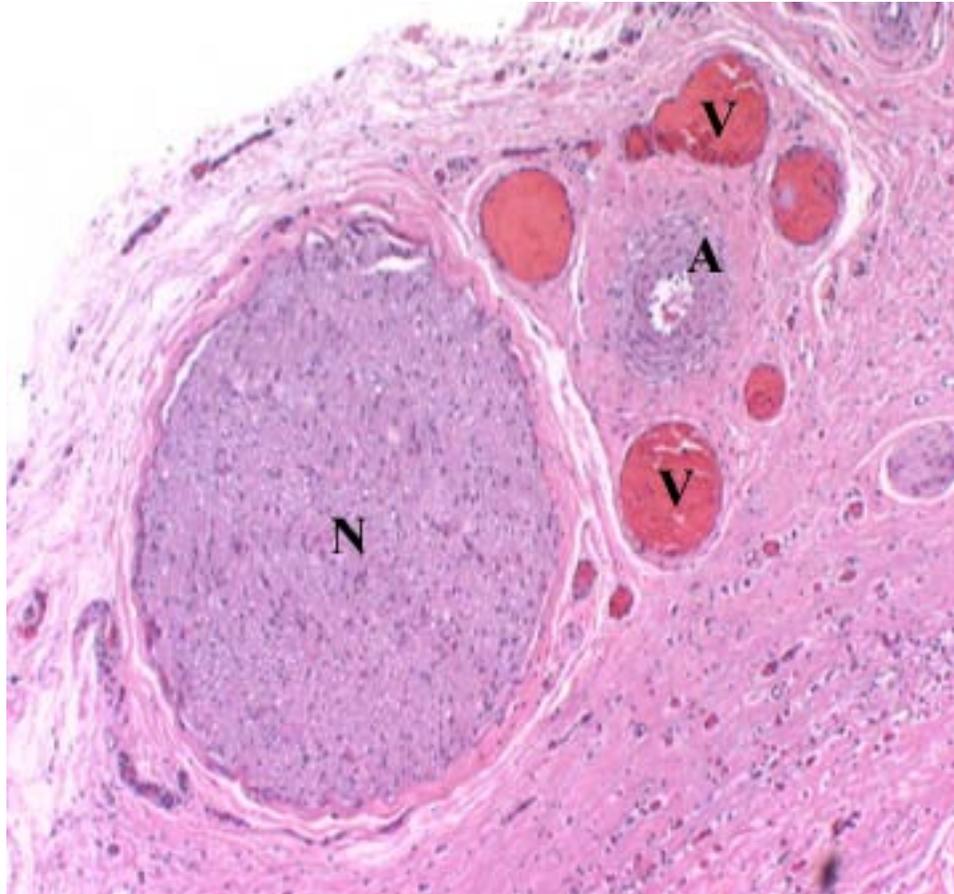
Figure 45. Nerve and vessels

Caudal margin region of tongue showing a nerve (N), artery (A), and surrounding
venules (V). x450 (H&E).

Epithelial layers

The epidermis consisted of three layers including the stratum basale, stratum spinosum, and stratum corneum. The stratum basale was the deepest layer, separated from the dermal layer by a basement membrane (Fig. 46) and was comprised of a single layer of cubiodal-shaped cells. Superficial to the stratum basale was the stratum spinosum with multiple (5-20) cell layers. Intercellular bridges, appearing as spines, connecting adjacent cells could be seen (Fig. 47). Granular vacuoles, clear to opaque, were seen adjacent to the nuclei in the stratum spinosum (Fig 48) in the epithelium of the foliate papillae. The most superficial layer of the epidermis was the stratum corneum, which was formed of multiple, flattened, amorphic squamous cells (Fig. 49). Desquamating layers of keratin could be seen in the most superficial region of the stratum corneum.
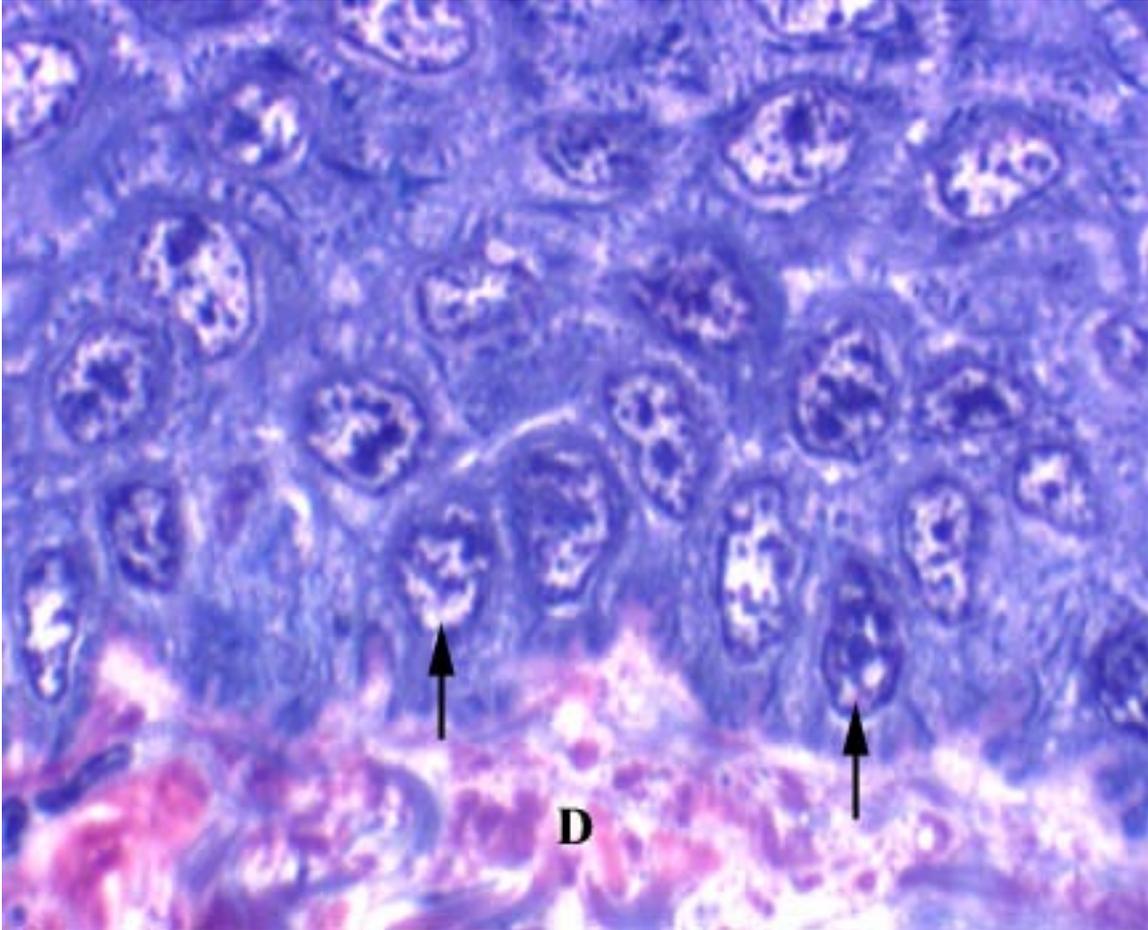
Figure 46. Stratum basale

Cubiodal shaped cells in the single layer stratum basale. Note dermal (D) layer. x2050
(Tri-stain).
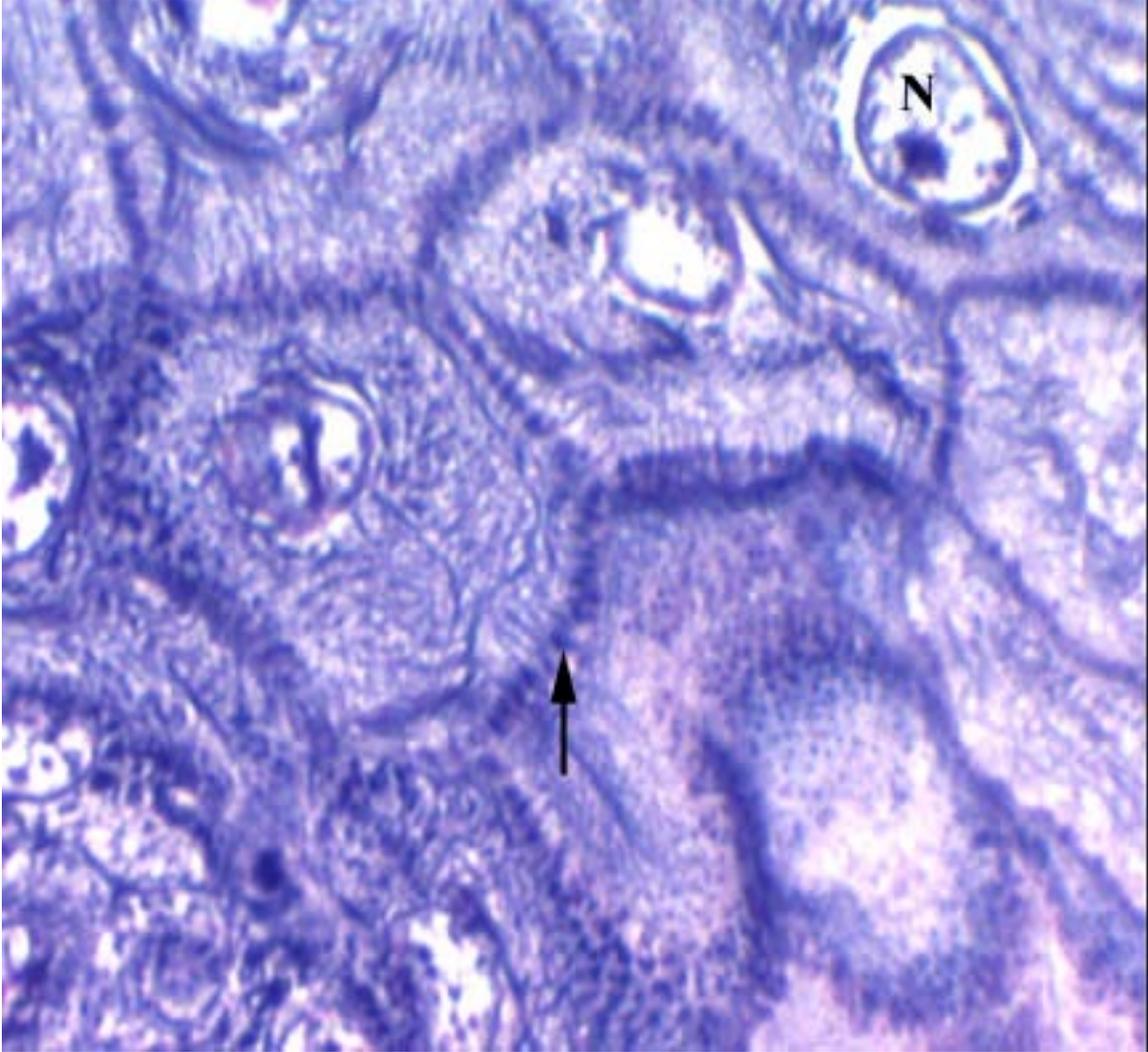
Figure 47. Stratum spinosum

Cells in the stratum spinosum showing a nucleus (N) and intercellular bridges (arrow). x2050 (Tri-stain).
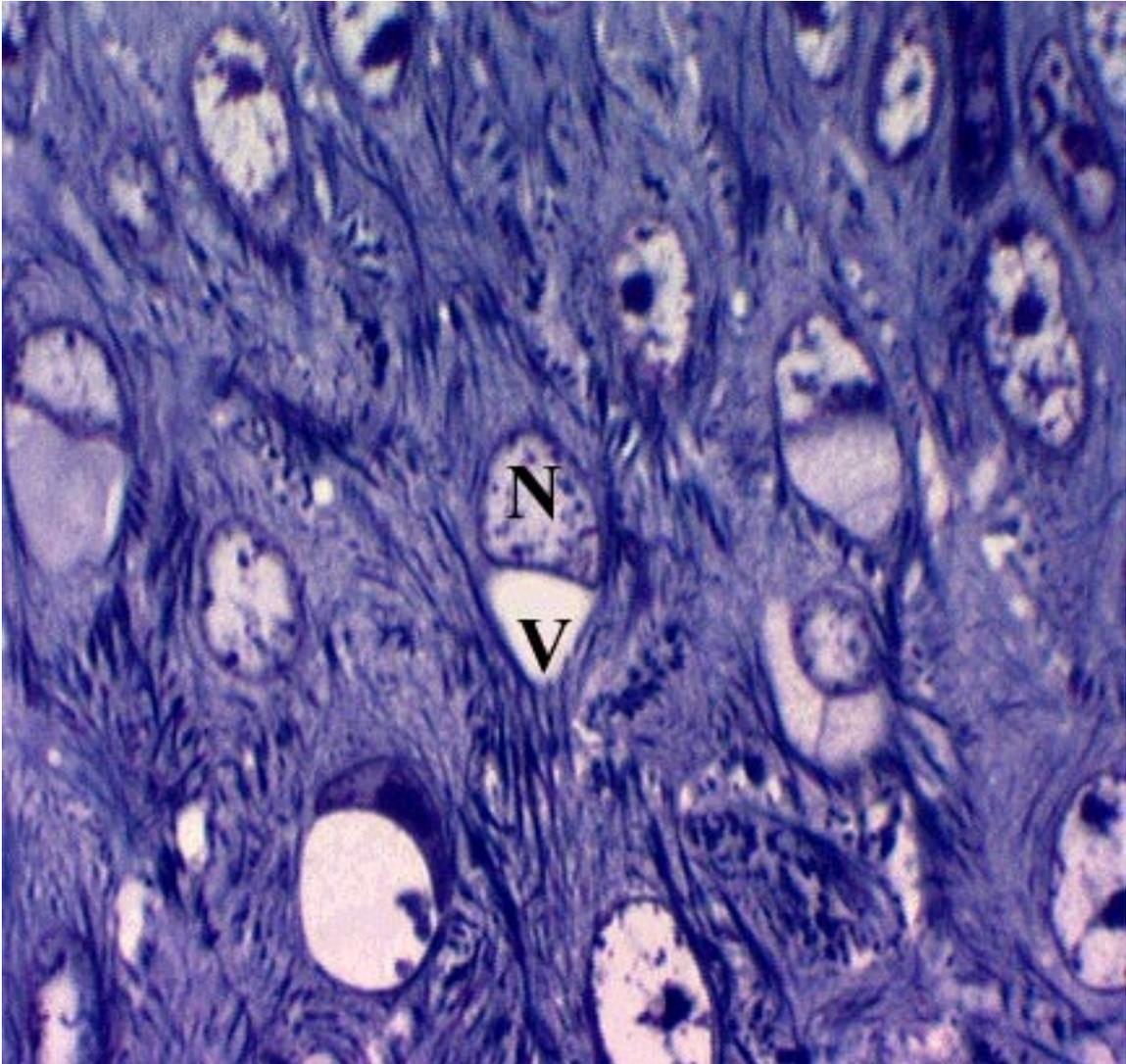
Figure 48. Stratum spinosum

Nuclei with adjacent vacuoles in the foliate papillary stratum spinosum. x2050 (Tri-stain).
.
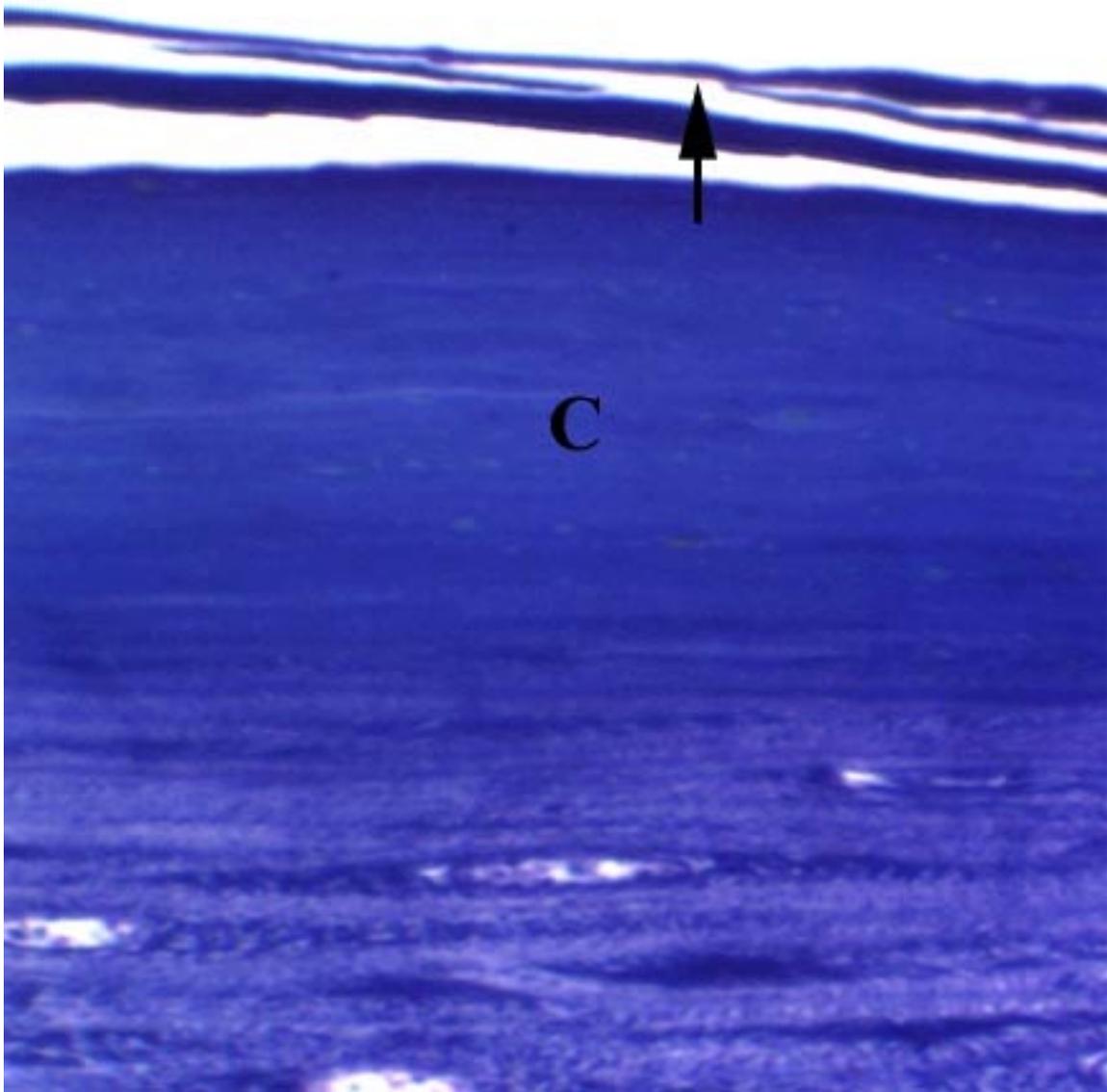
Figure 49. Stratum corneum

Outer stratum corneum layer (C) showing desquamating layers of keratin (arrow). x2050 (Tri-stain).

Immunohistochemistry

Tissue sections were stained with mouse anti-neuron specific enolase to demonstrate innervation within the lingual substance. Positive reaction between antibody and enolase was demonstrated as brown to dark brown areas by light microscopy.

Nerve fibers distributed throughout the dermal and hypodermal layers were readily stained with the antibody (Fig. 50). Nerve fibers were observed both independently as well as associated with lingual salivary glands and ducts (Fig. 51 and 52). Staining was also noted in the walls of arteries (Fig. 53) and veins. Taste buds also reacted positively with the antibody (Figs. 54, 55, and 56).
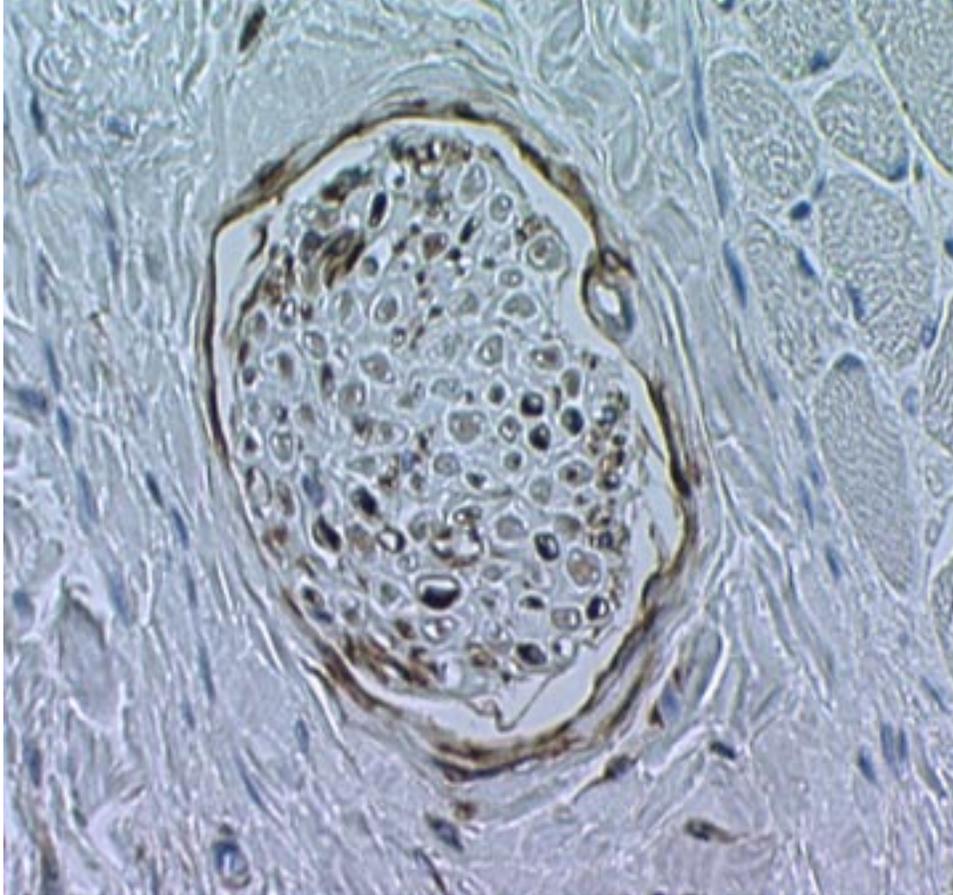
Figure 50. Peripheral nerve

Nerve showing enolase/antibody reactivity. x2250 (Antibody counterstained with hematoxylin).
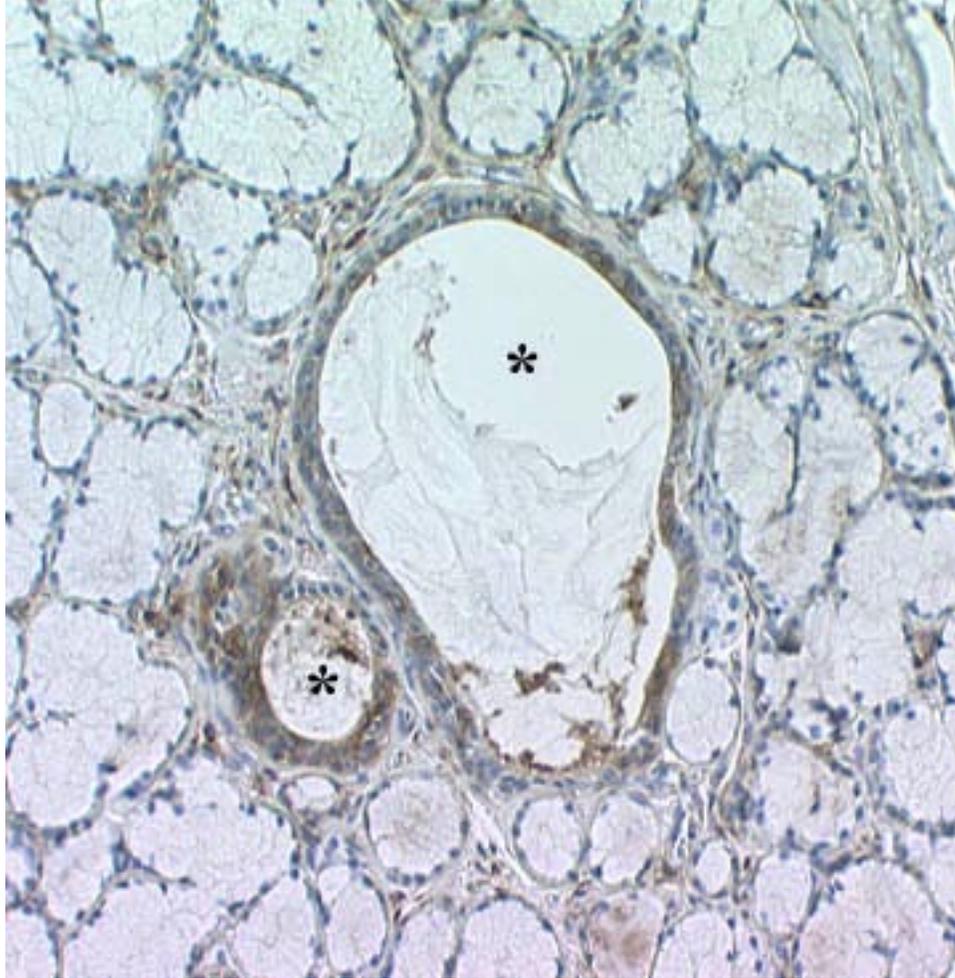
Figure 51. Striated salivary ducts

Two salivary ducts (asterisks) showing enolase/antibody reactivity. x900 (Antibody counterstained with hematoxylin).
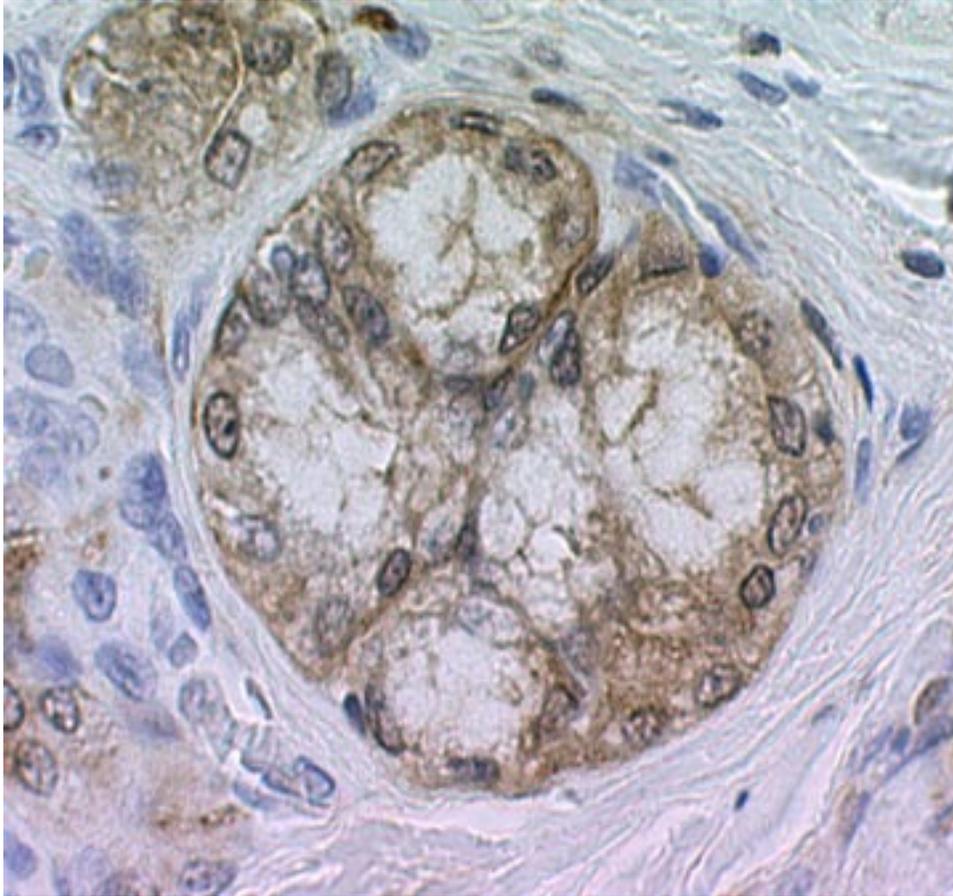
Figure 52. Mucous acinus

Mucous acinus showing enolase/antibody reactivity. x2250 (Antibody counterstained with hematoxylin).
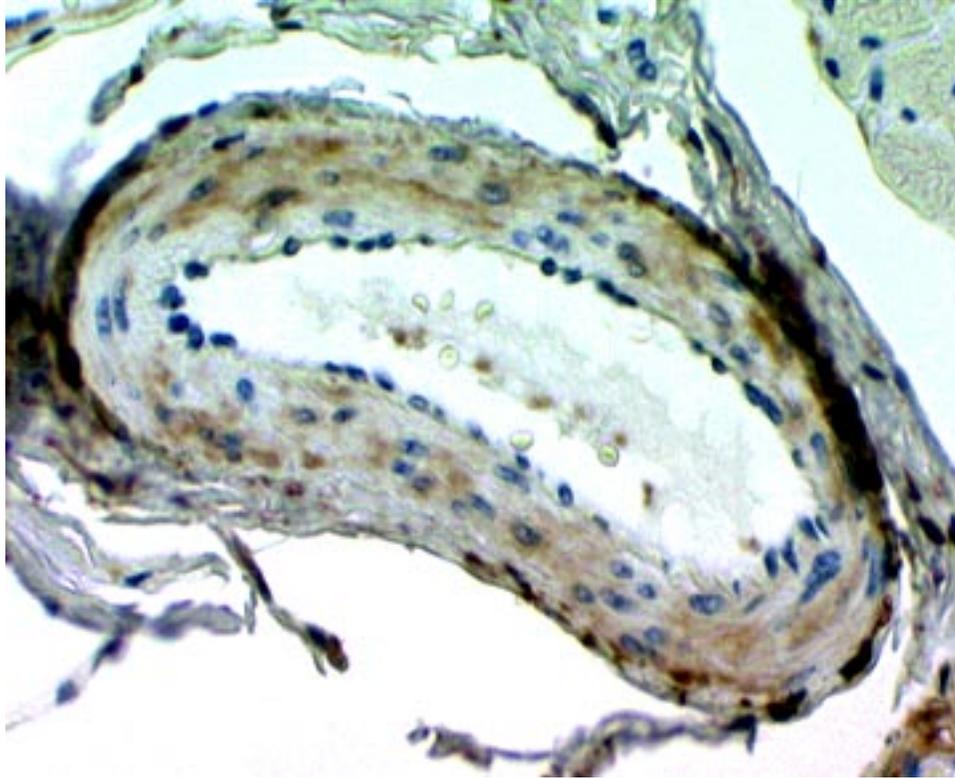
Figure 53. Artery

Artery showing reactivity with enolase antibody. x650 (Antibody counterstained with hematoxylin).
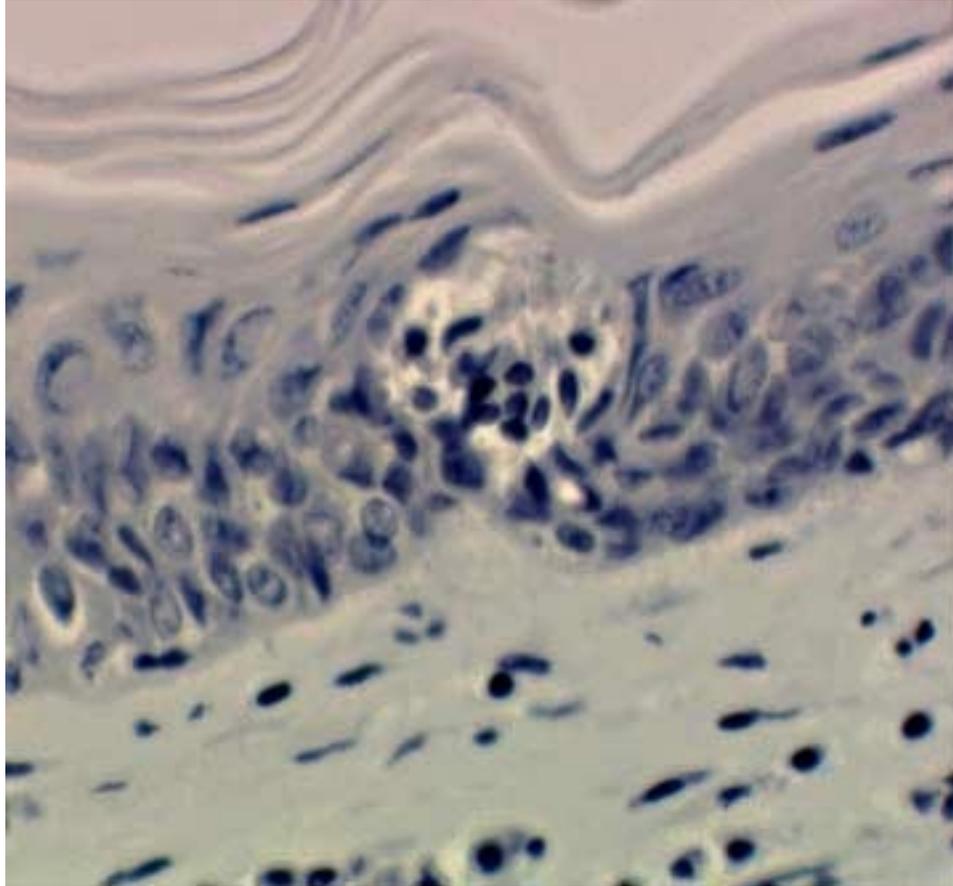
Figure 54. Taste bud

Single taste bud embedded in epidermis of foliate papillae not stained for nerve fibers.
x650 (Hematoxylin).
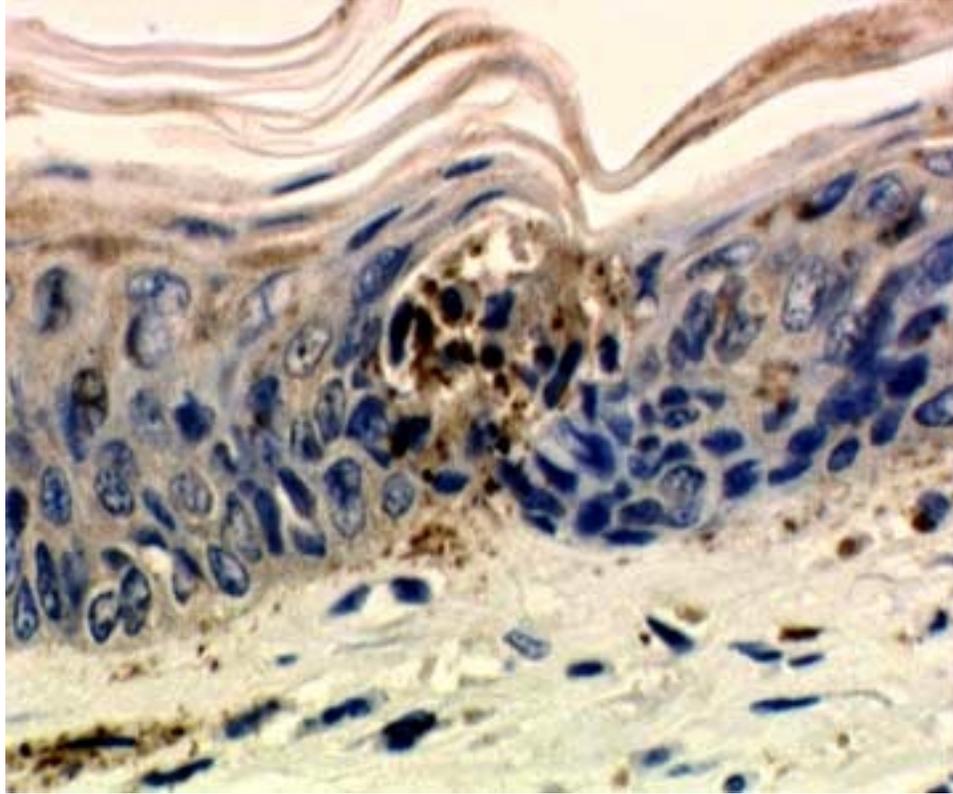
Figure 55. Taste bud

Single taste bud embedded in epidermis of foliate papilla showing enolase/antibody reactivity. x650 (Antibody counterstained with hematoxylin).
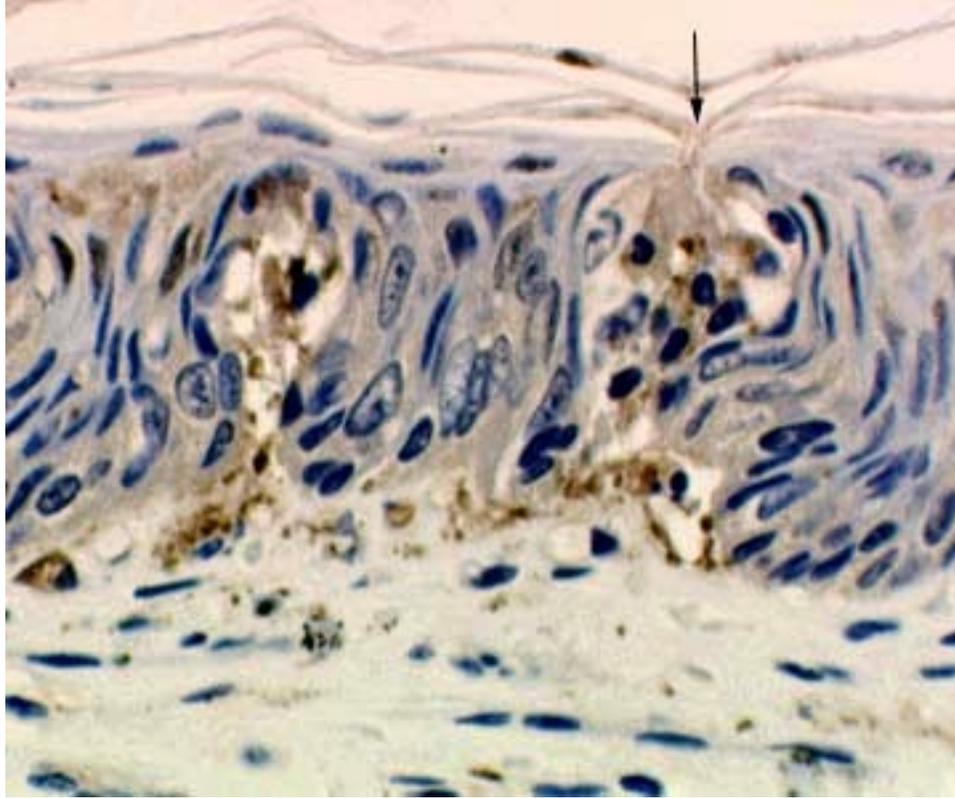
Figure 56. Taste buds

Two taste buds embedded in epidermis of foliate papillae showing enolase/antibody reactivity. Note taste pore (arrow) of one taste bud. x650 (Antibody counterstained with hematoxylin).

Rostral tip and papillae

Filiform papillae on the dorsal surface of the free tip appeared as hair-like extensions projecting from the epidermis. The papillae ranged from 1-5 mm in height. At their rostral tips, the papillae were long and broad (Fig. 57) with both pointed and blunted ends. Caudally, the papillae were shorter and more conical in shape (Figs. 58 and 59) with pointed distal ends. At high magnification, flakes of keratin could be seen on the outer shaft of the papillae (Fig. 60). The ventral surface of the free rostral tip possessed a stratified, keratinized epithelium (Fig. 61) with no papillae.

Dorsal surface and papillae

The dorsal surface of the tongue was a covered by stratified keratinized epithelium. Desquamated keratin flakes could be seen adjacent to dorsal fungiform-like papillae (Fig. 62). Papillae of different sizes were visualized (Fig. 63). At high magnification, individual squamous cells with microfolds could be delineated (Fig. 64).

Lateral surface and papillae

The lateral surface of the tongue also possessed a stratified squamous keratinized epithelium. Desquamation of surface epithelium on the dorsal surface was evident (Fig. 65). Large patch-like fungiform papillae were present (Fig. 66). The thickened keratinized layer covering the fungiform papillae was evident in section (Fig. 67). The opening of lateral pits leading to salivary glands were also seen (Fig. 68).

Fungiform papillae

The multi-fossulate openings of the foliate papillae (Fig. 69) were present on the dorsal caudal margins of the tongue. Deep crypts between the foliate papillae were present (Fig. 70). The epithelium surrounding the folds of the papillae appeared smooth (Fig. 71).
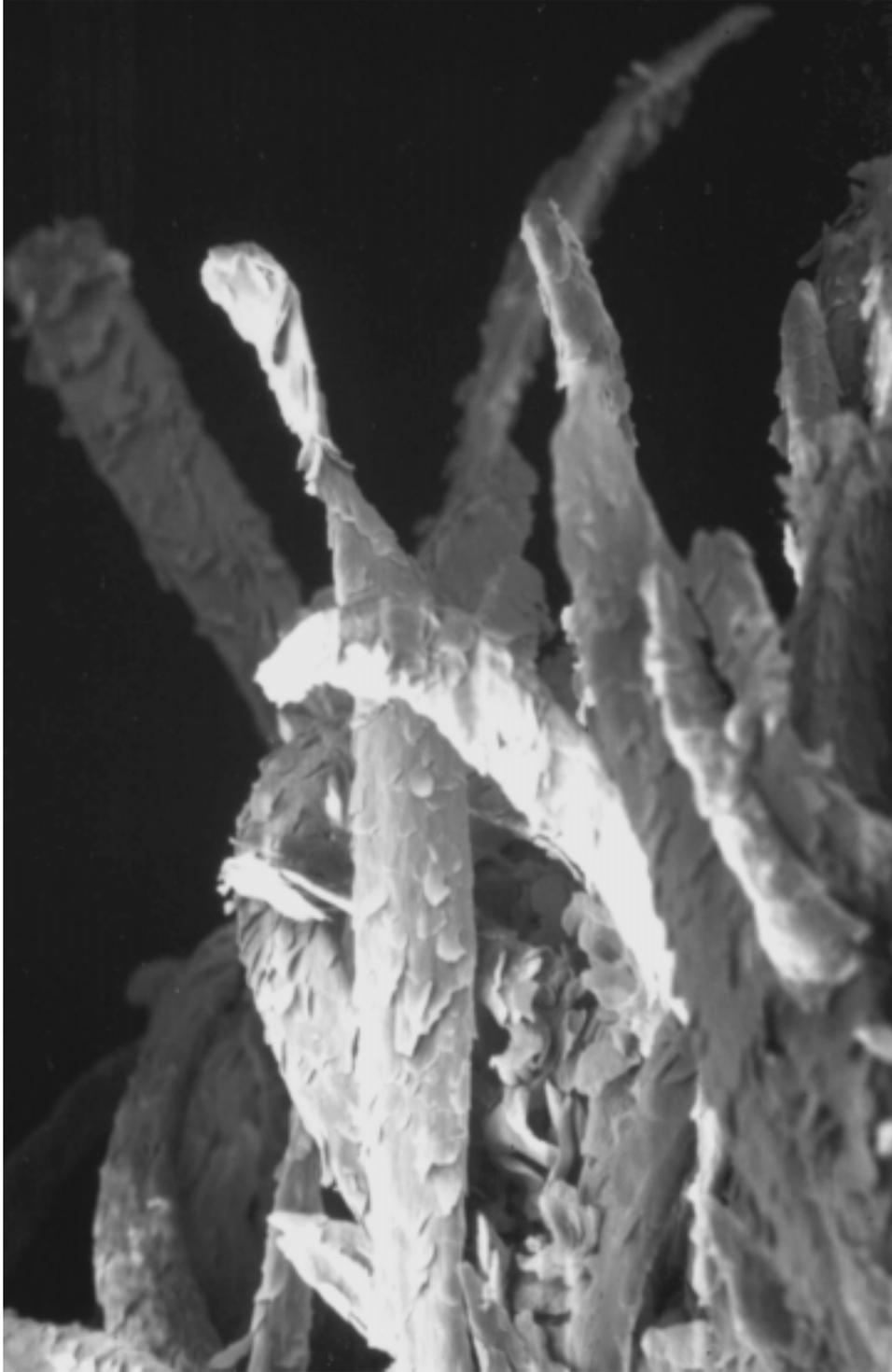
Figure 57. Filiform papillae

Filiform papillae at rostral-most surface showing long, broad extensions.
x145 (SEM).
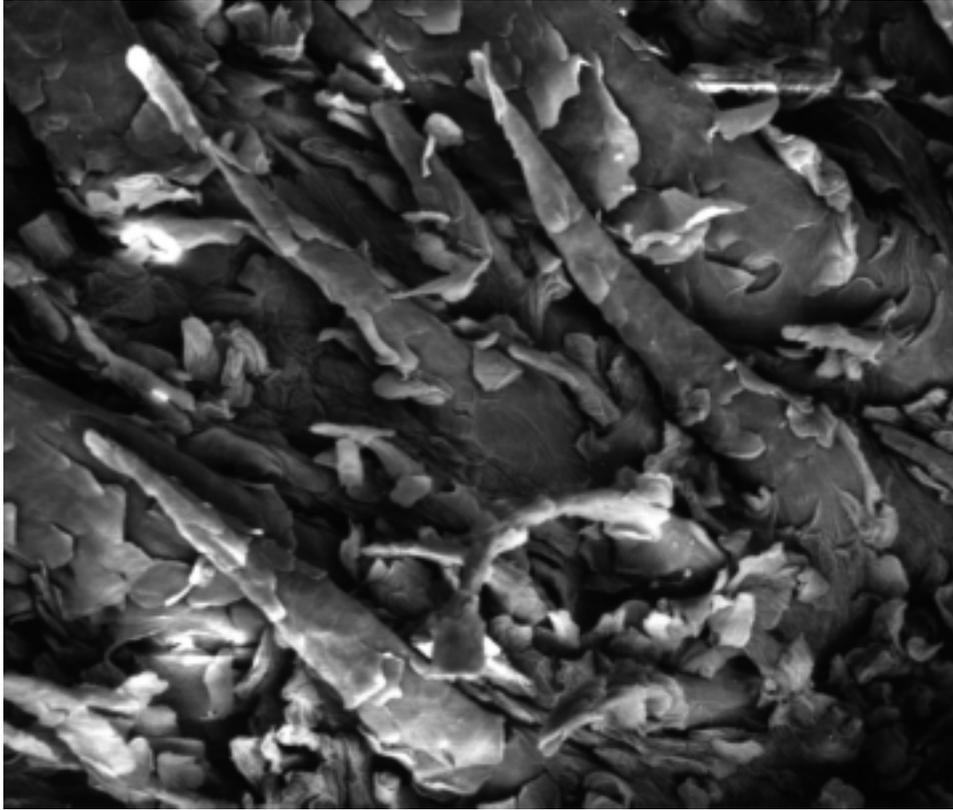
Figure 58. Filiform papillae

Filiform papillae showing conical shape and pointed distal tip. x155 (SEM).
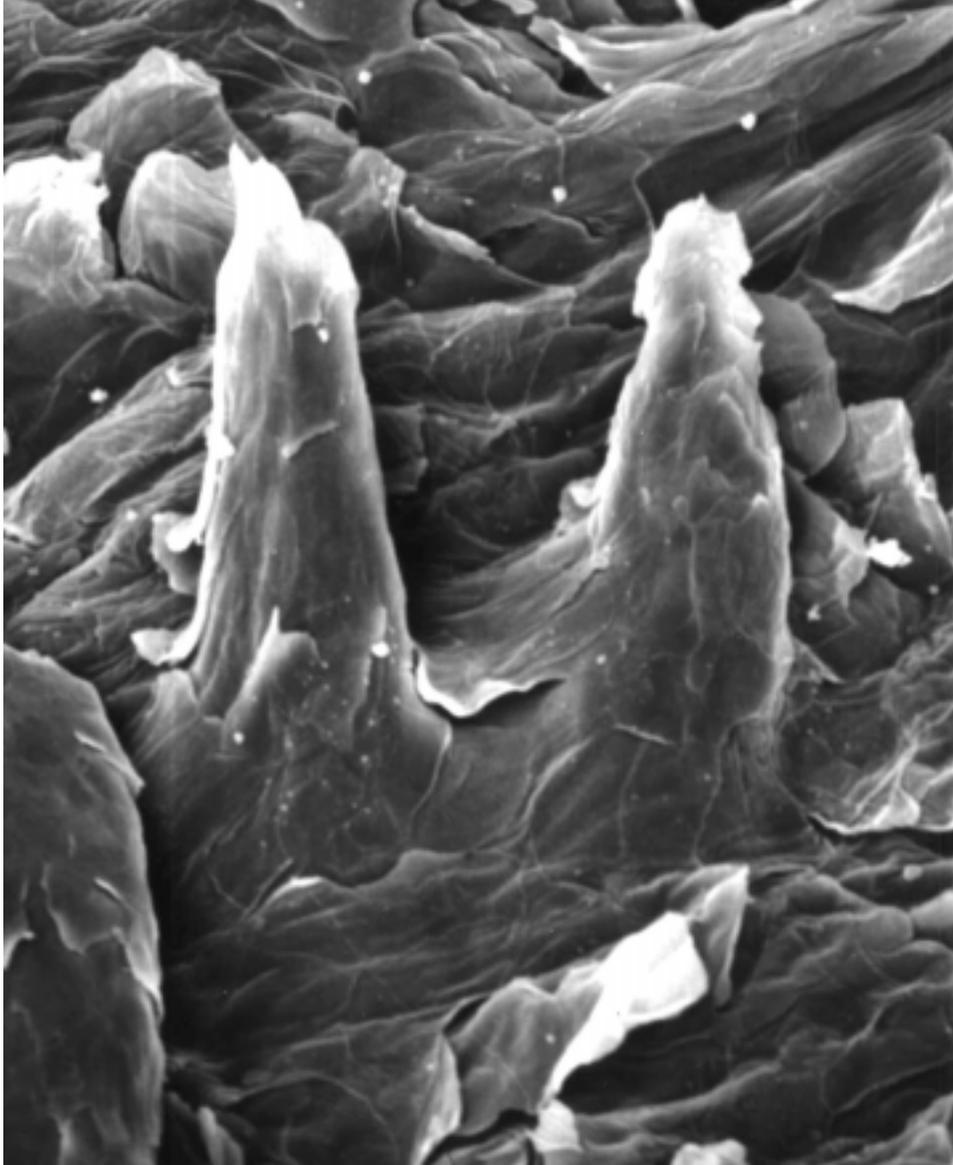
Figure 59. Filiform papillae

Caudal-most filiform papillae showing short filiform papillae. x410 (SEM).
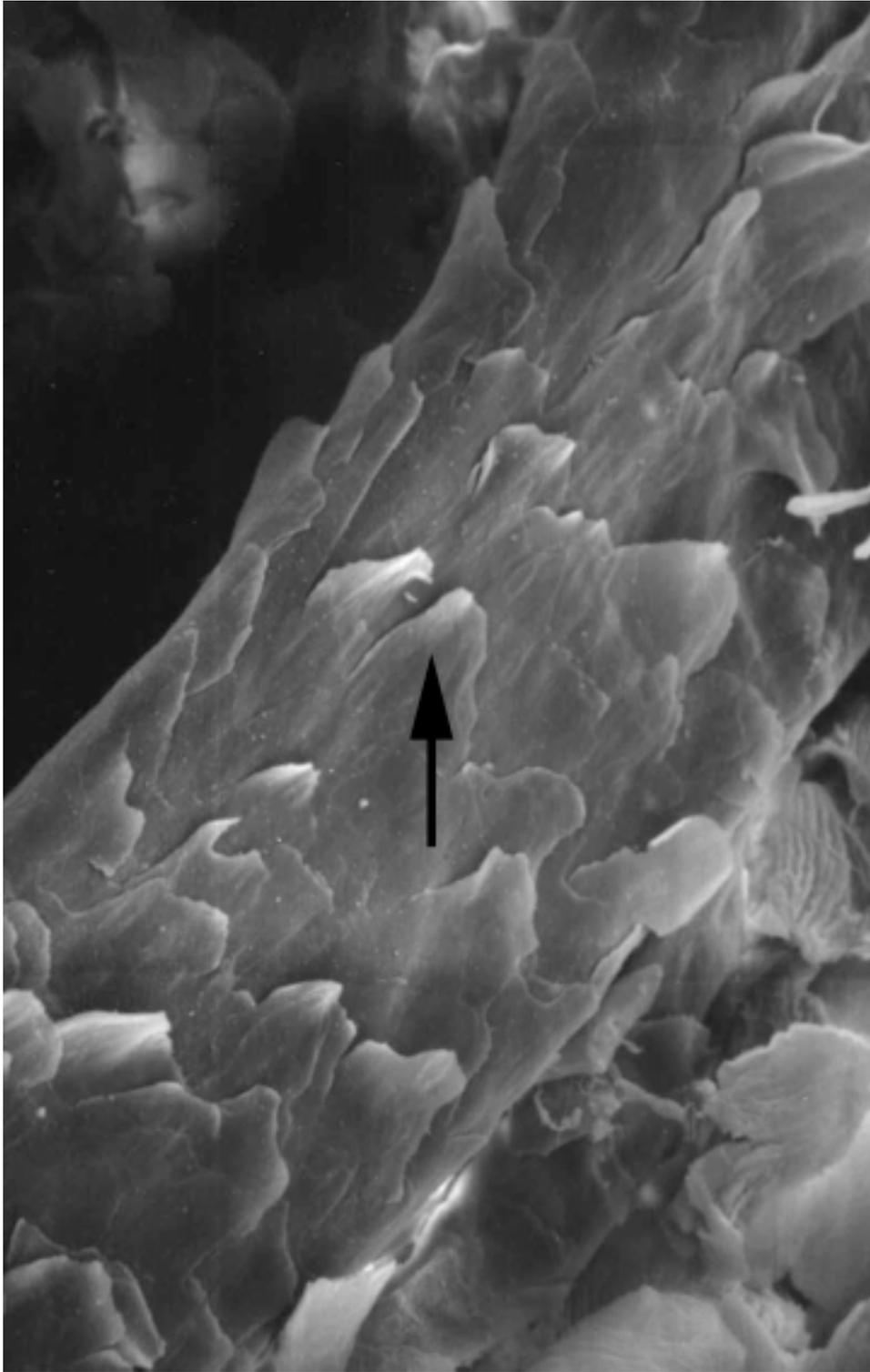
Figure 60. Filiform shaft

Shaft of filiform papilla showing desquamating keratinized layer (arrow). x530 (SEM).
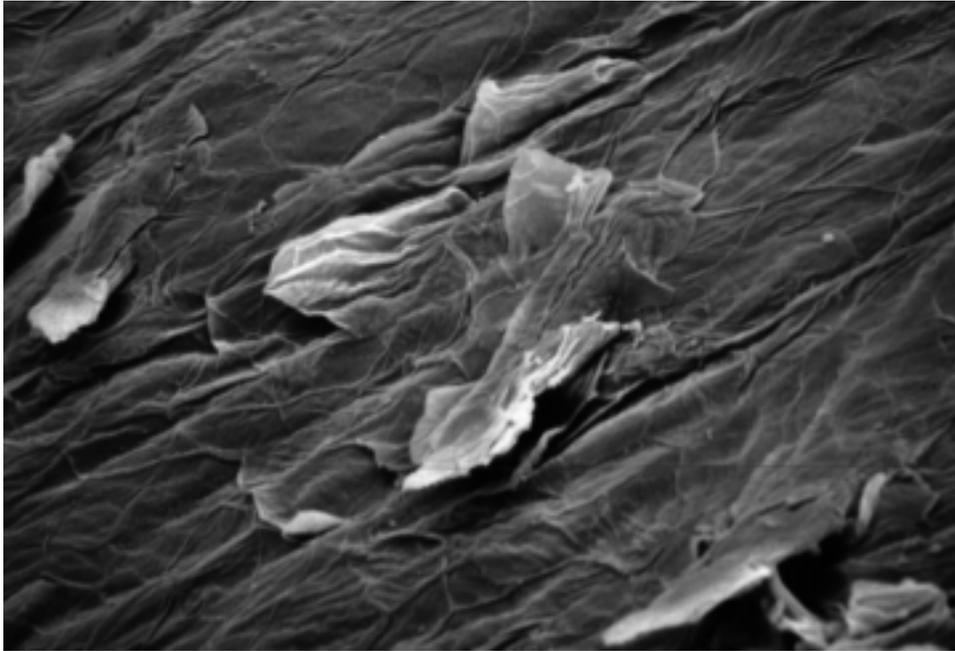
Figure 61. Ventral surface

Papilla free surface on ventral rostral tip. x445 (SEM).
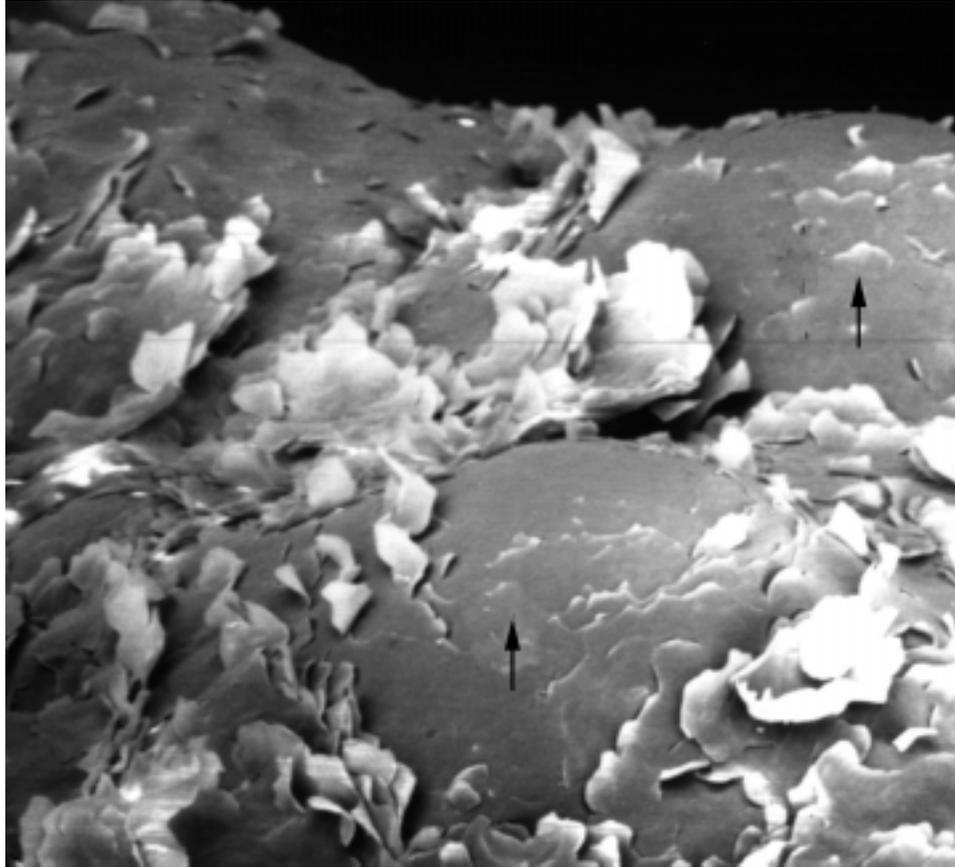
Figure 62. Dorsal papillae

Dorsal surface with two large fungiform papillae (arrows). Note desquamation on free surface. x155 (SEM).
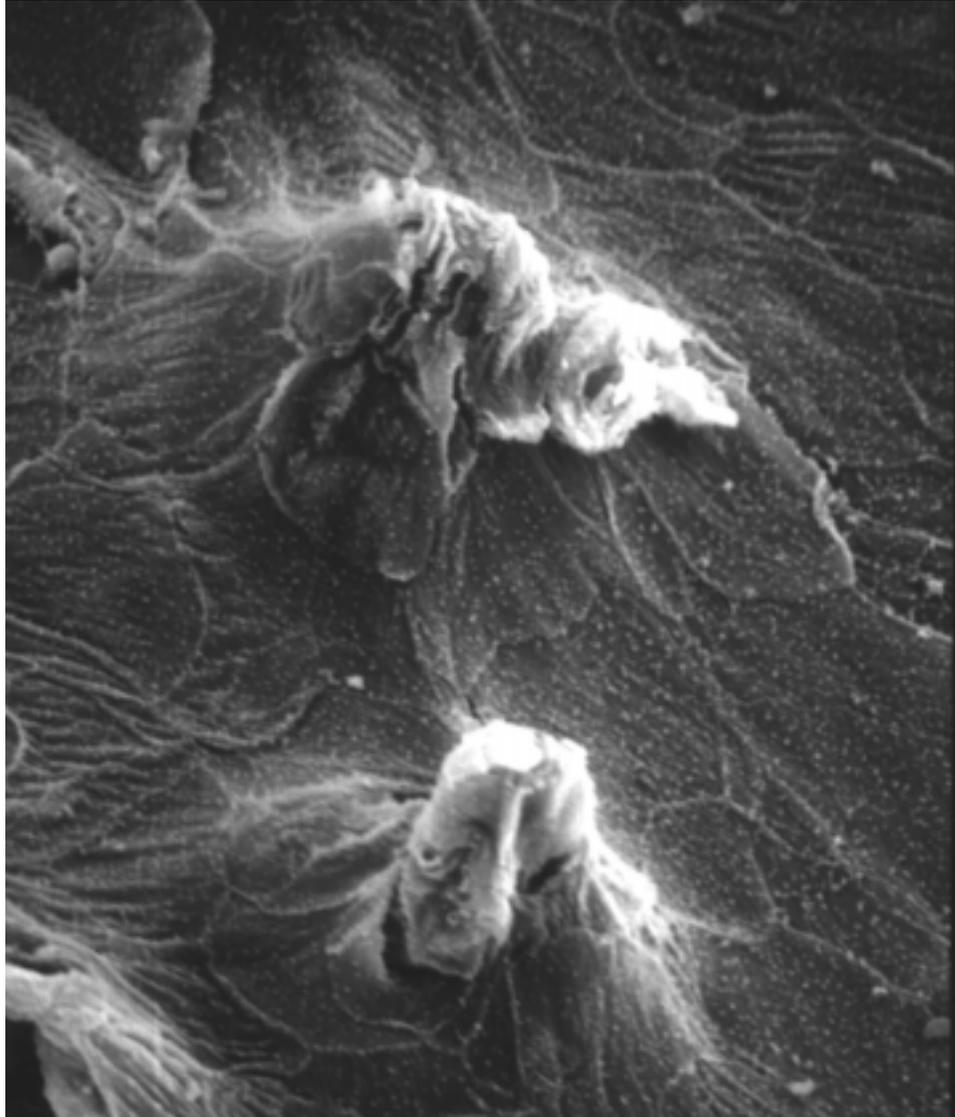
Figure 63. Dorsal papillae

Two small papillae projecting from dorsal lingual surface. x970 (SEM).
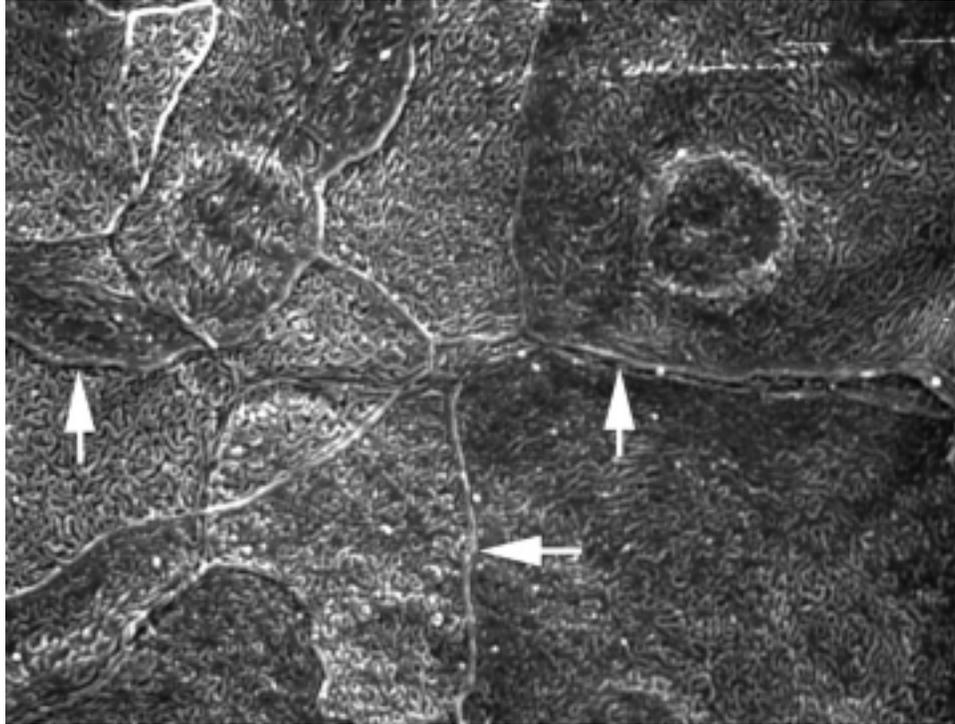
Figure 64. Squamous cells

Surface squamous cells of the dermal lingual surface. Microfolds within cells and cell borders (arrows) can be seen. x2.83K (SEM).
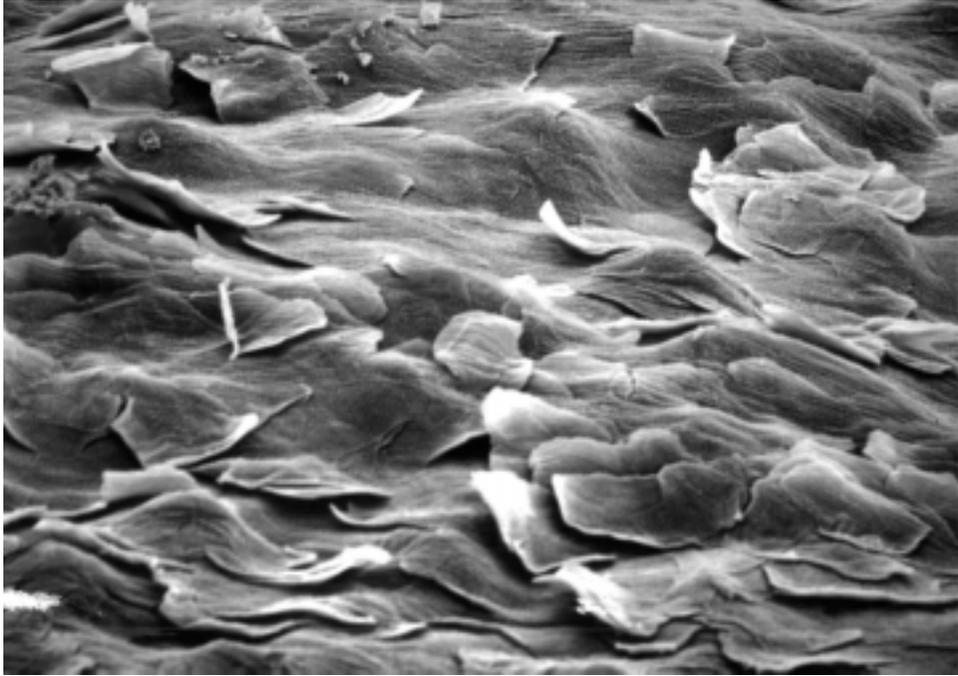
Figure 65. Lateral epithelium

Lateral surface with desquamating epithelium. x270 (SEM).
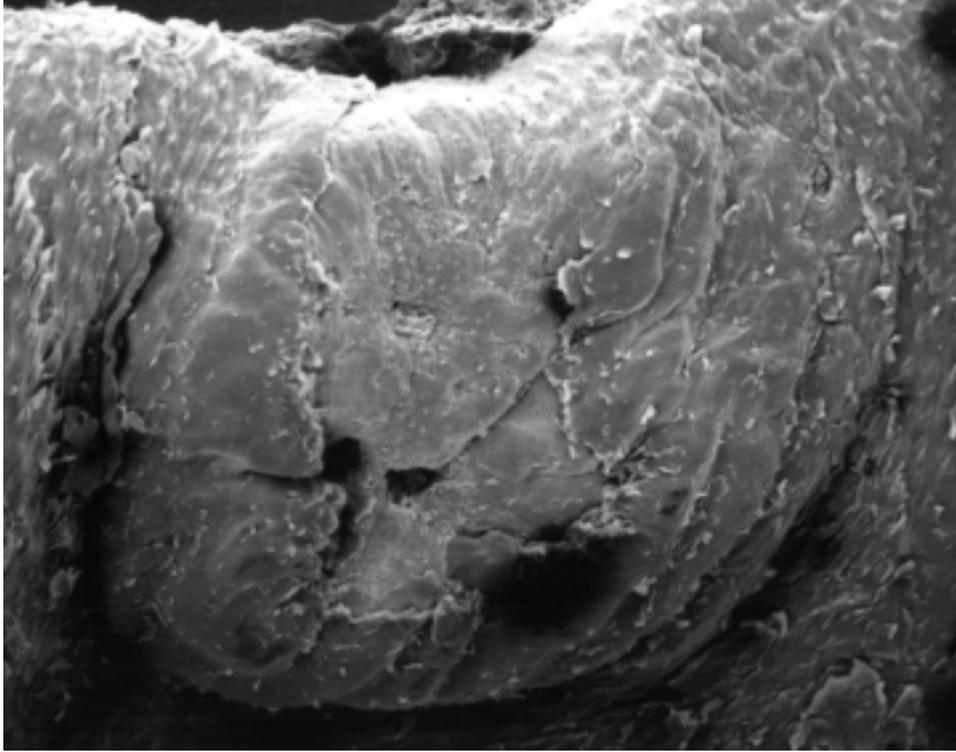
Figure 66. Fungiform papilla

Single fungiform papilla on lateral wall. x35 (SEM).
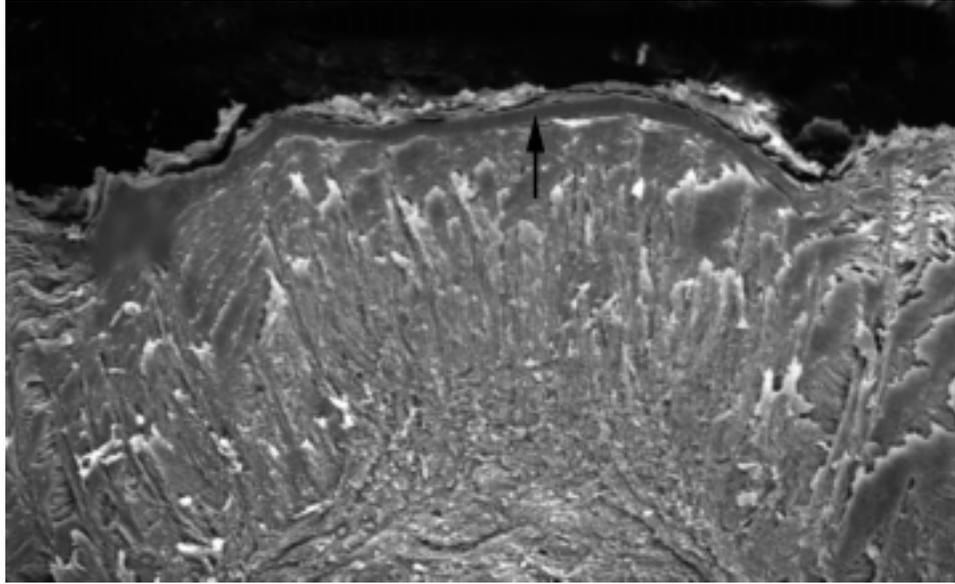
Figure 67. Fungiform papilla

Sectioned lateral fungiform papilla showing thickened keratinized epithelium (arrow).
x50 (SEM).
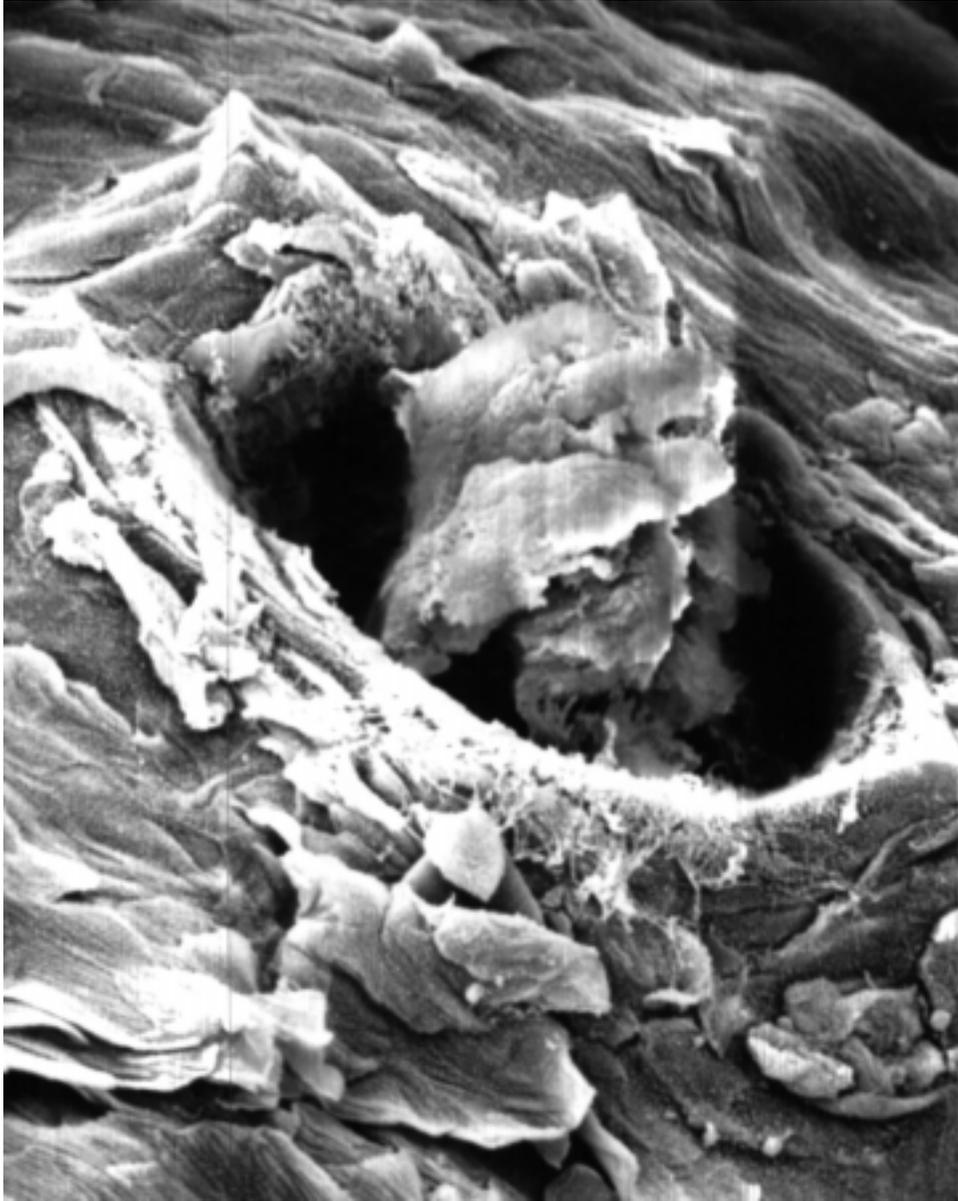
Figure 68. Mucous pit

Lateral pit opening showing mucous secretion within lumen. x433 (SEM).

\
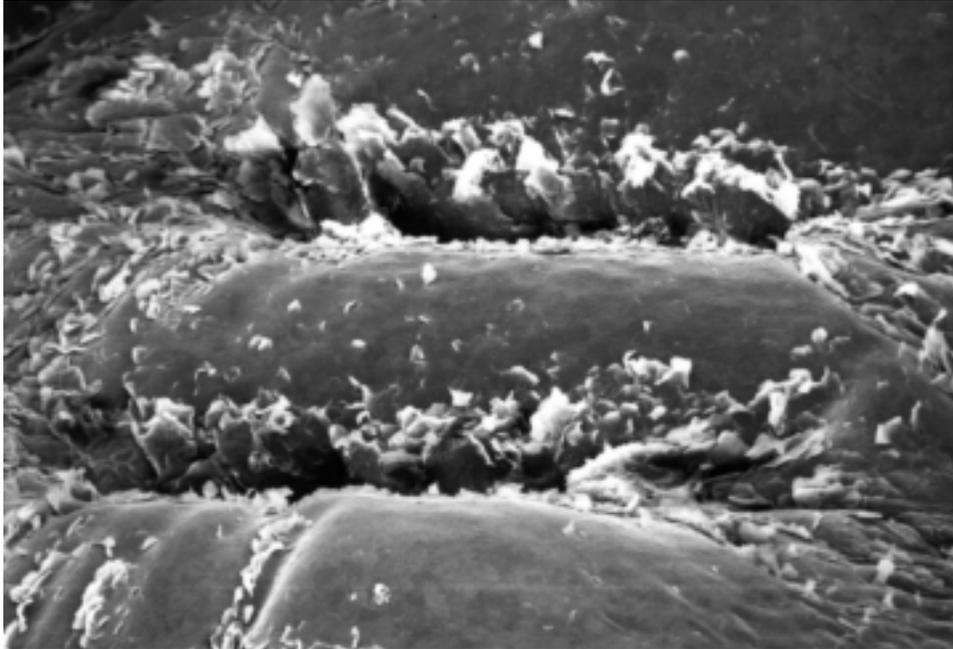
Figure 69. Foliate papilla

Opening into foliate papilla. x48 (SEM).
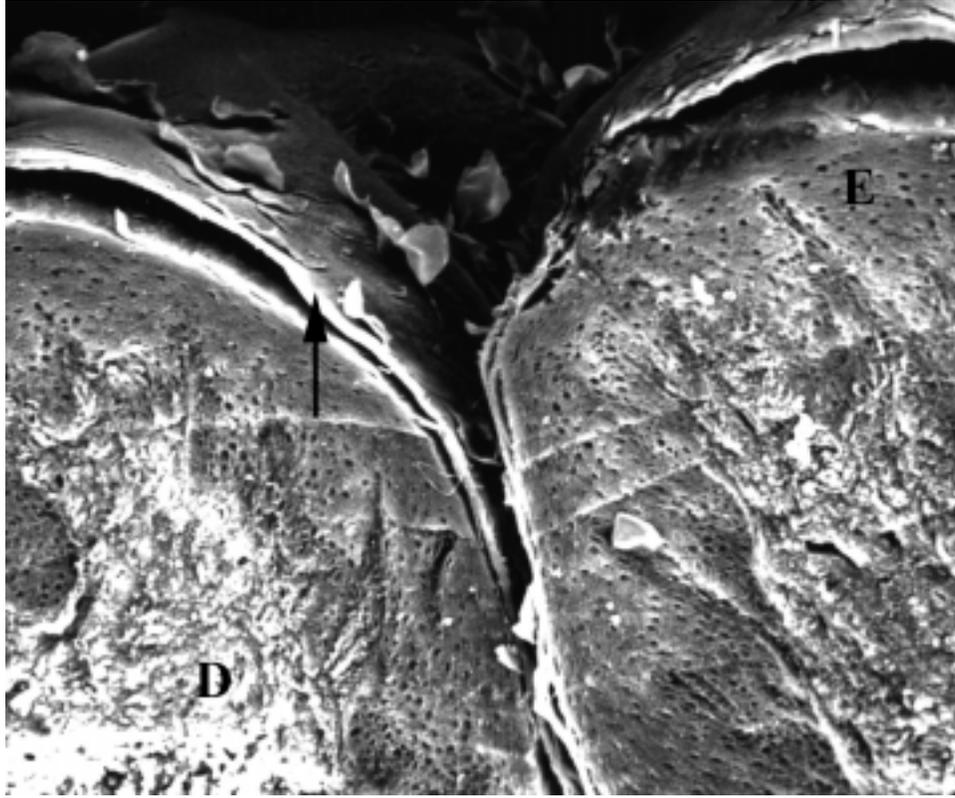
Figure 70. Foliate papilla

Cross-section of foliate papilla with fold. Note epidermis (E) with keratinized layer
(arrow) and dermis (D). x190 (SEM).
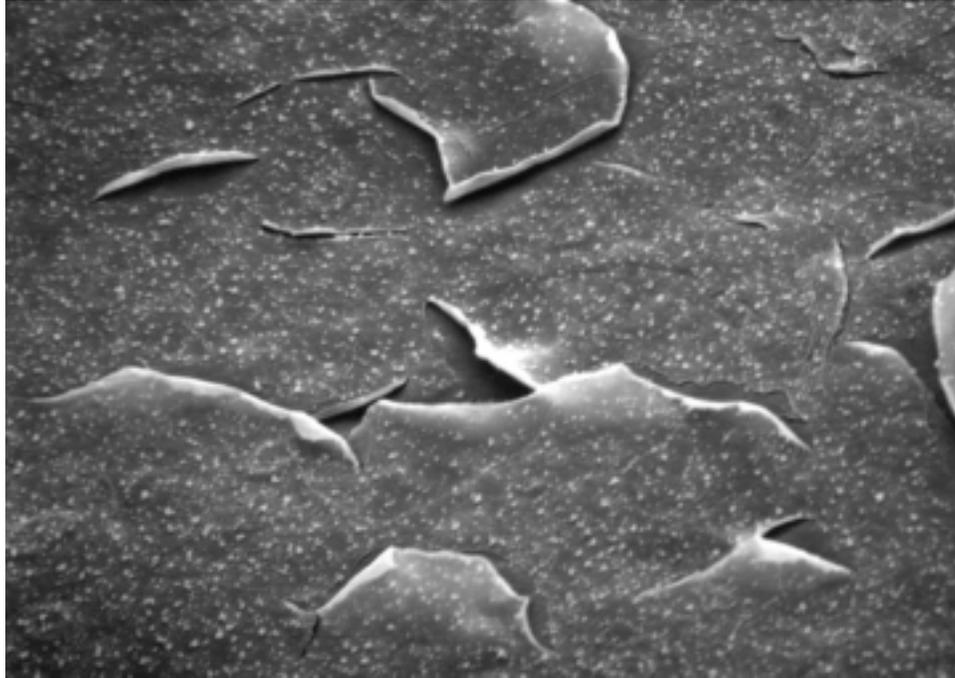
Figure 71. Foliate epithelium

Smooth surface epithelium adjacent to foliate papilla showing small particles (possibly fixative precipitate) on surface. x675 (SEM).

Transmission electron microscopy

Sections from the epidermis and dermis were examined by transmission electron microscopy. Cells of the stratum basale demonstrated round to oval shaped vesicular nuclei occupying the majority of the cytoplasm (Fig. 72). Numerous mitochondria were seen in the cytoplasm surrounding the nuclei of the stratum spinosum layer. Cells of the stratum spinosum were more oval and contained less mitochondria (Fig. 73). Desmosomes connecting adjacent cells were visible. Electron-dense tonofilaments, also known as keratin filaments, were abundant and were attached to desmosomes. In the foliate papillary region, electron-lucent vacuoles lay adjacent to the nuclei in the stratum spinosum (Fig. 74). In some areas, the vacuoles were similar or larger in size (Fig. 75). The stratum corneum was comprised of flattened squamous cells with no recognizable organelles (Fig. 76). Desmosomes could still be seen connecting layers of amorphic keratin material. Keratinized layers could be seen sloughing off the most superficial epidermal layer (Fig. 77).

Collagen fibers (Figs. 78 and 79) were seen in the dermal and hypodermal layers. Striated skeletal muscle (Fig. 80) was seen in the hypodermal layers throughout the lingual substance.
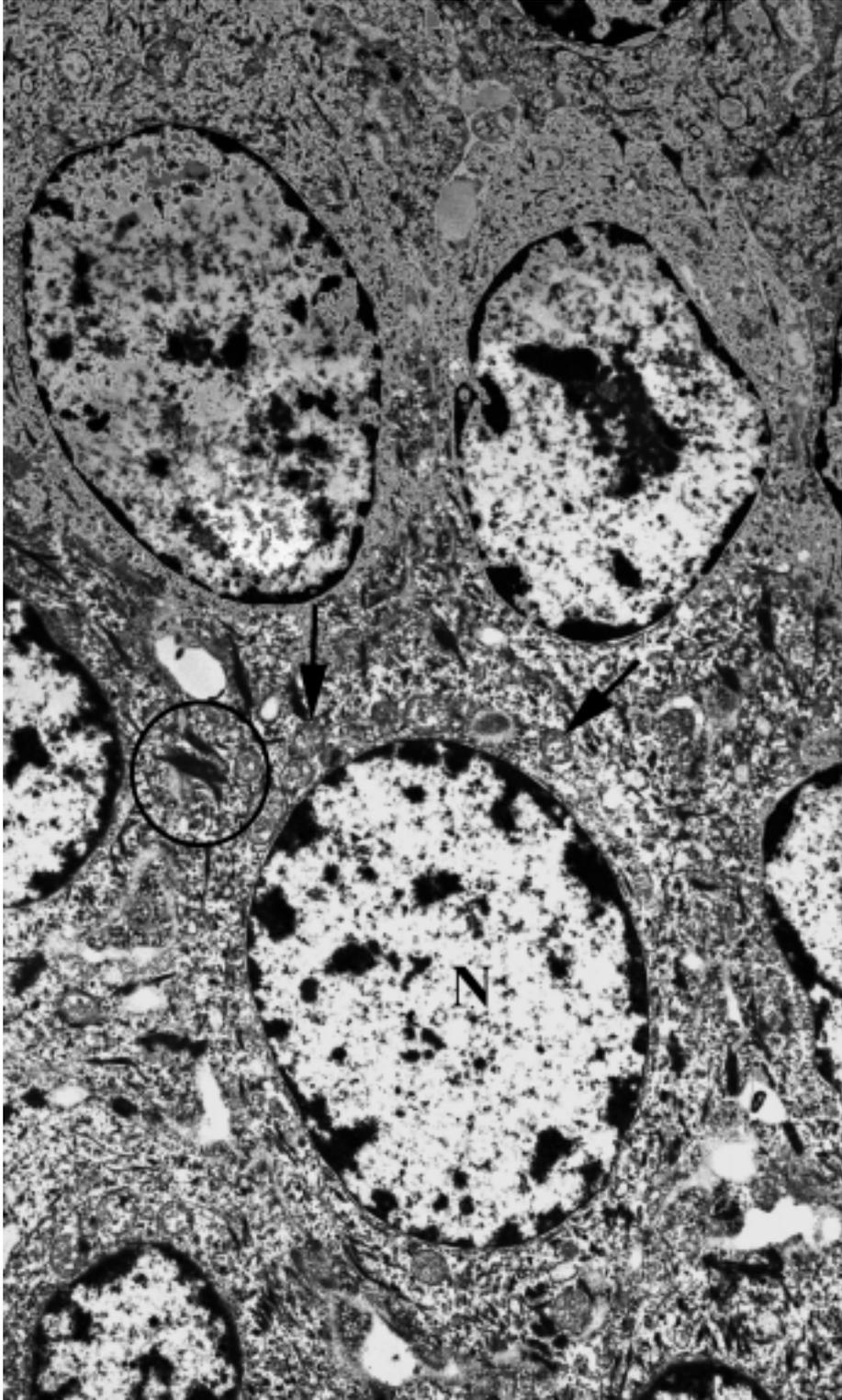
Figure 72. Stratum basale

Cells in the stratum basale layer showing a nucleus (N), mitochondria (arrow), and desmosomes (circle). X9,360 (TEM).
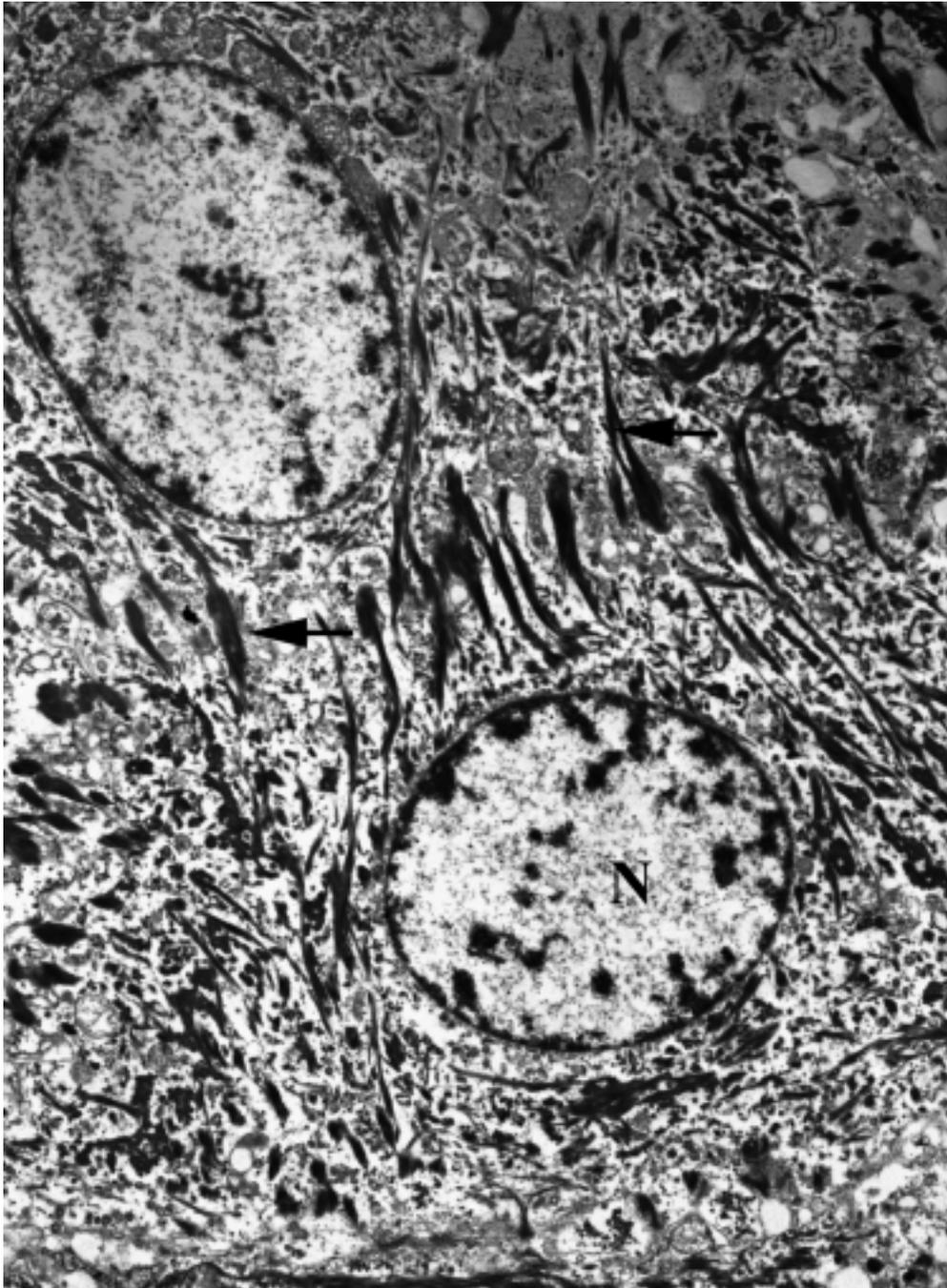
Figure 73. Stratum spinosum

Cells of the stratum spinosum layer showing nucleus (N) and keratin fibers attached to desmosomes (arrows). x9,360 (TEM).
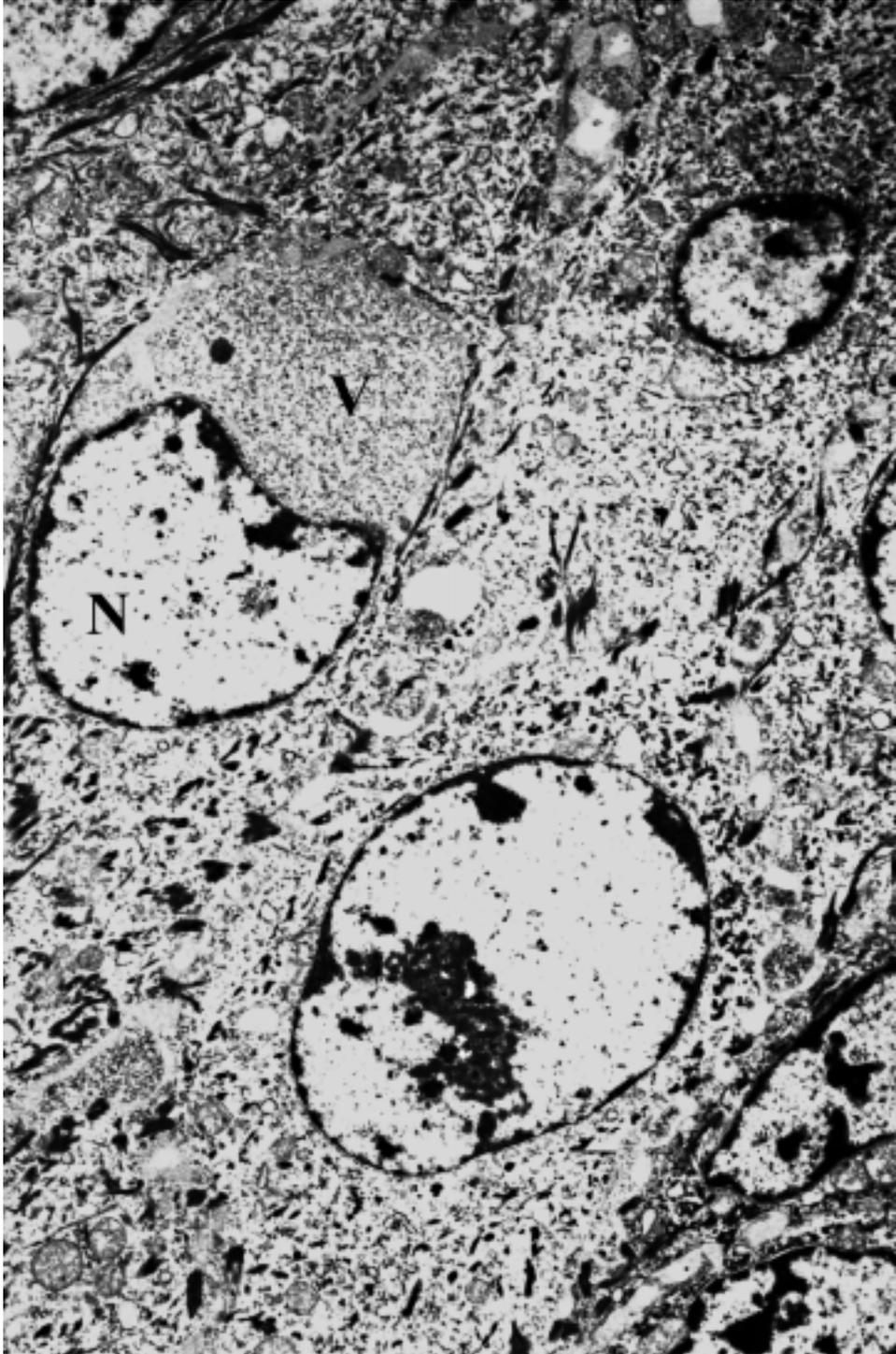
Figure 74. Stratum spinosum

Cells of the foliate papillary stratum spinosum showing nucleus (N) with adjacent
vacuole (V). x9,360 (TEM).
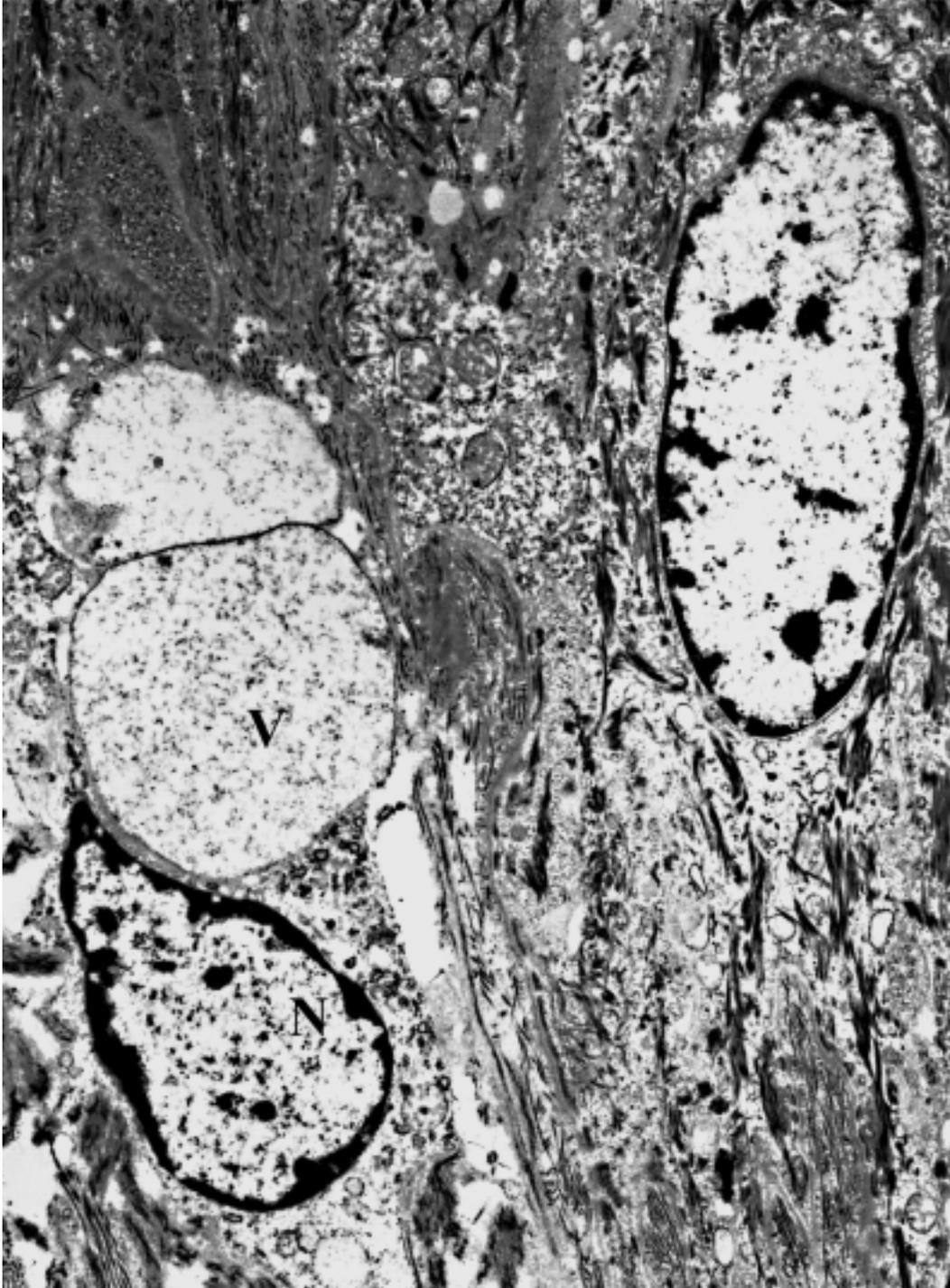
Figure 75. Stratum basale

Cells of the foliate papillary region showing nucleus (N) with large adjacent vacuole (V).
x12,480 (TEM).
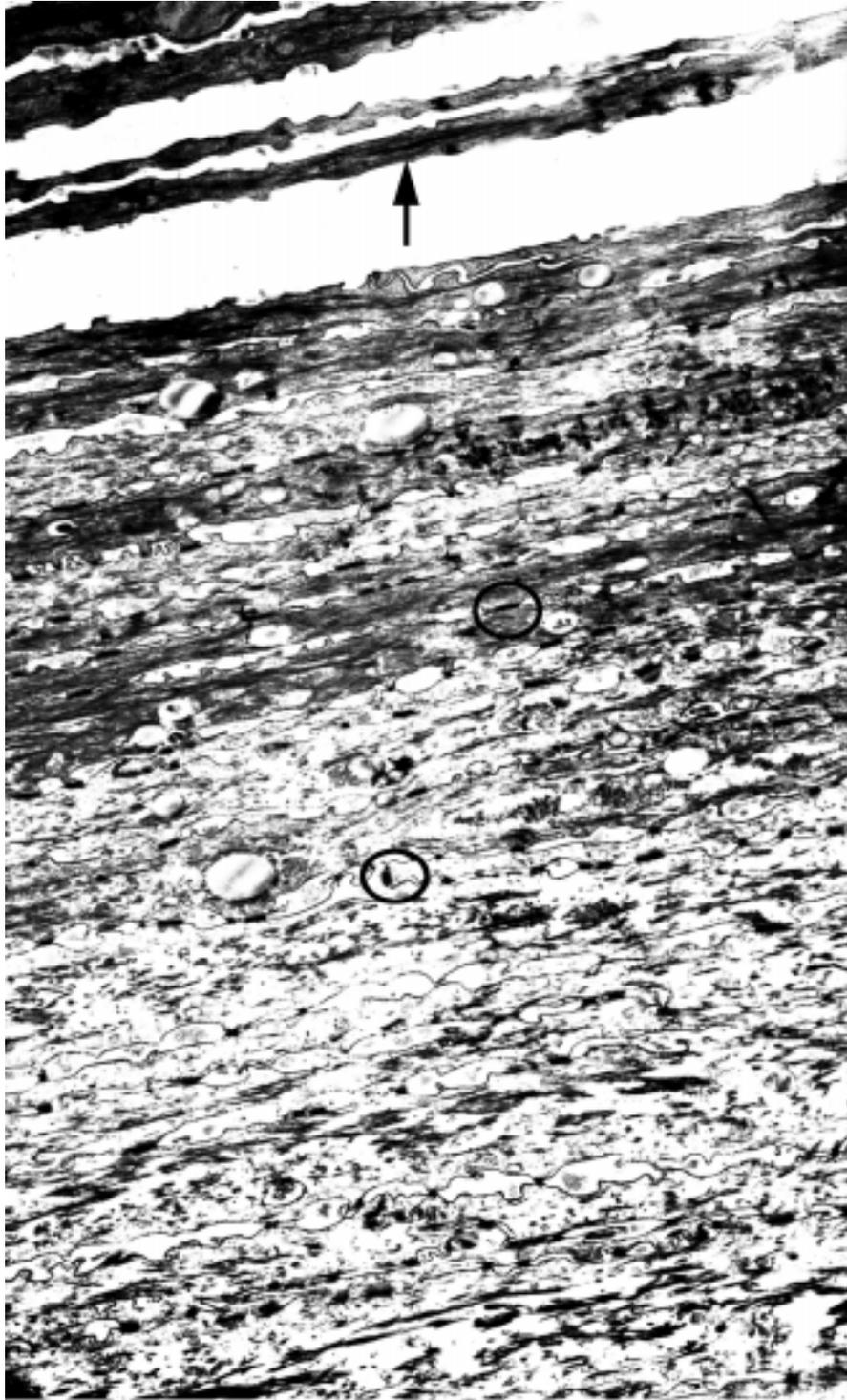
Figure 76. Stratum corneum

Flattened squamous cells of the stratum corneum showing desquamating keratin layers
(arrow) and desmosomes (circles). x9,360 (TEM).
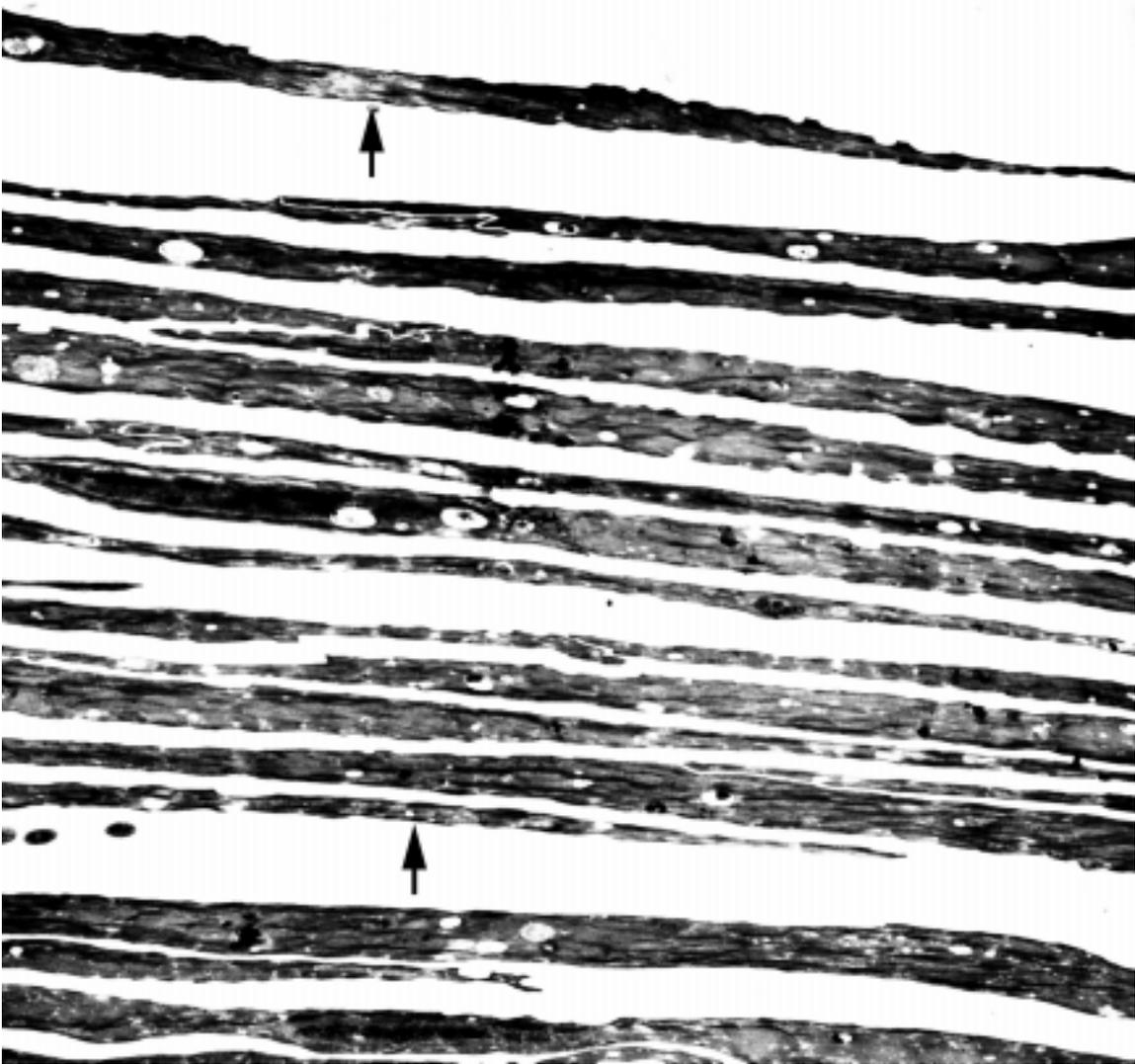
Figure 77. Keratinized layer

Desquamating keratin layers (arrows). x9,360 (TEM).
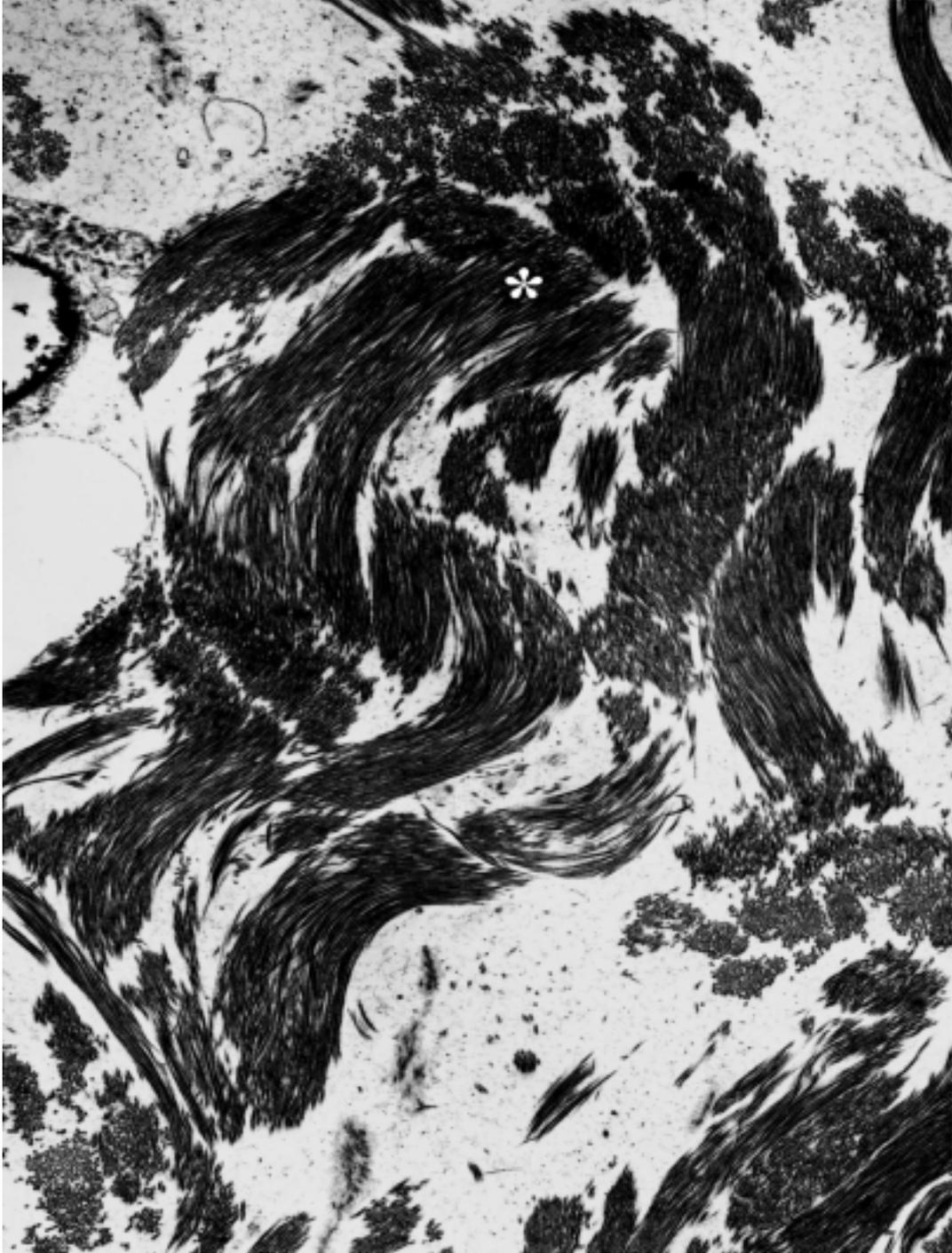
Figure 78. Collagen fibers

Electron-dense collagen fibers (asterisk) in the dermis. x9,360 (TEM).
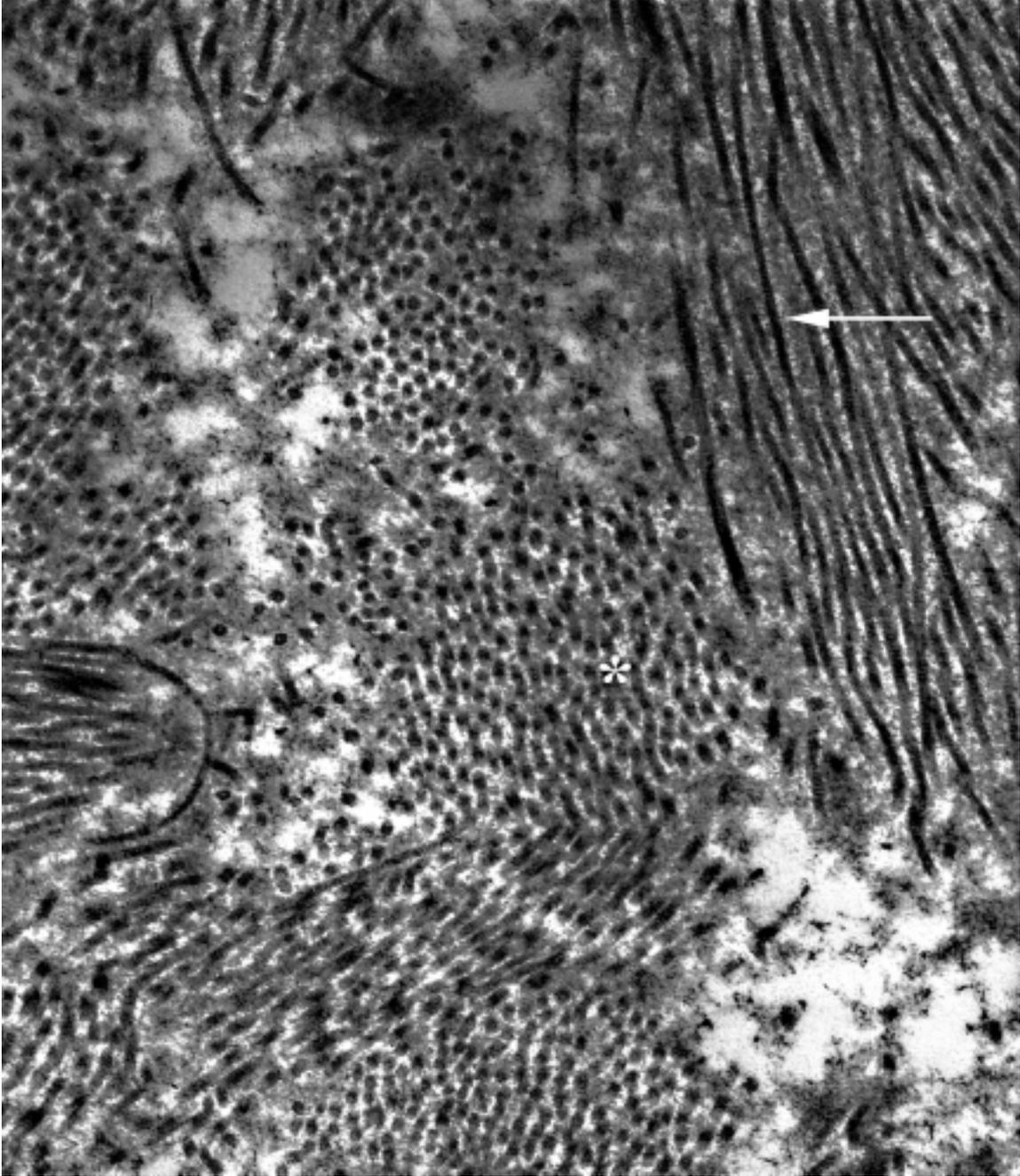
Figure 79. Collagen fibers

Collagen fibers cut in longitudinal (arrow) and cross sections (asterisk). x49,400 (TEM).
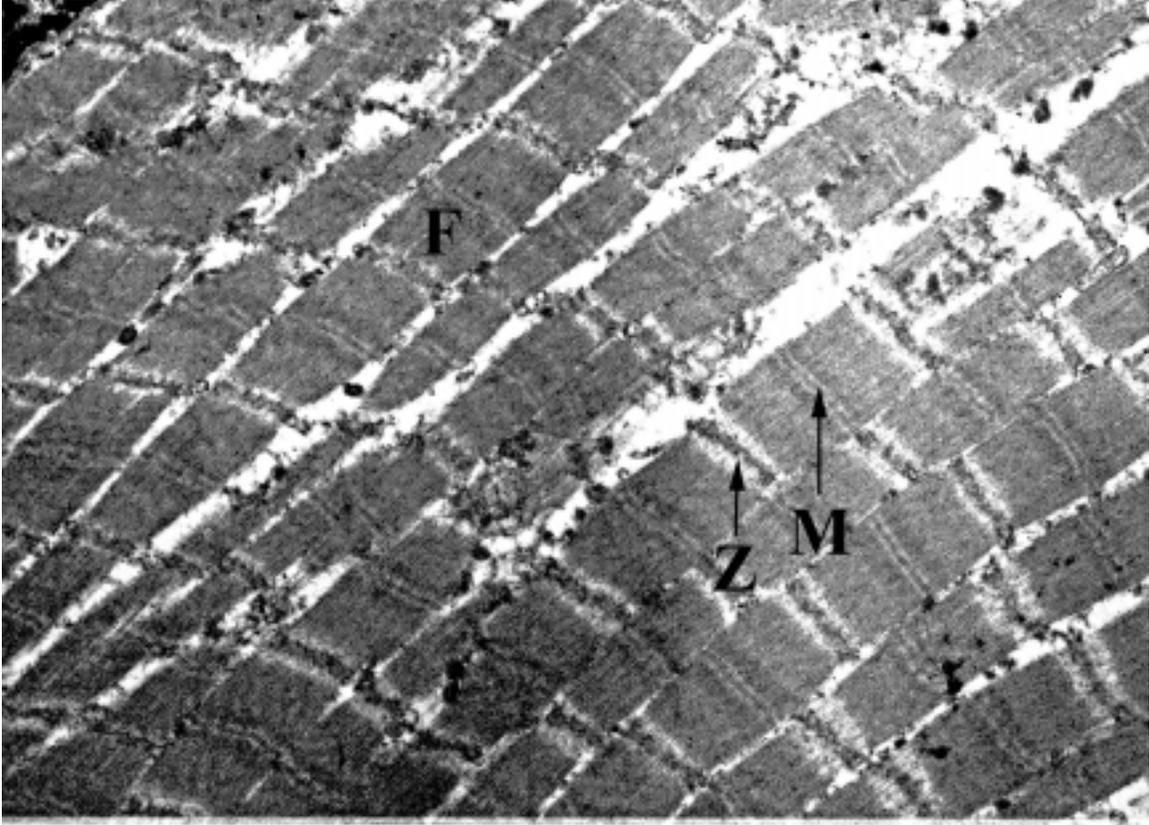
Figure 80. Intrinsic lingual musculature

Striated skeletal muscle (longitudinal sections) showing individual fibril (F) and Z and M
lines. x18,720 (TEM).

Discussion

The following discussion will focus on the functional roles, both mechanical and chemoreceptive, of the manatee tongue. These roles will then be compared and contrasted with other mammals, including marine and terrestrial species. Investigative techniques, such as electron microscopy and immunohistochemistry, will also be discussed.

General lingual functions

The shape and function of an animal's tongue are adapted for prehension and/or manipulation of its foods. Beyond this perhaps most obvious function, the highly muscular tongue also participates in multiple other tasks such as drinking, grooming, and vocalization. The shape and function of tongues thus varies tremendously from species to species. For example, some terrestrial herbivores, such as cows and giraffes, can protrude their tongues, and use it in prehension to gather vegetation. Cats use their tongues as a tool for grooming. Panting by dogs allows for the dissipation of body heat. Baleen whales manipulate their tongues to force water through baleen in order to filter out small invertebrates, such as krill. Heat exchangers embedded throughout the tongue of Grey whales (Heyning and Mead, 1997) have been suggested as participating in thermoregulation, reducing heat loss in cold waters.

Mechanical role of the manatee tongue

The size, shape, and mobility of the manatee tongue relate to its mode of feeding. The primary dietary function of the tongue is mastication of its vegetative diet and subsequent swallowing. The manatee's molars are also important in the cropping of seagrasses. The tongue most likely positions food in the oral cavity in the area of the molars for grinding. In addition, salivary glands within the substance of the tongue moisten the often-gritty food prior to swallowing.

The most distinguishing characteristics of the manatee's tongue are its position so far caudally in the mouth and being entirely non-protrusible. These features clearly reflect

103

the tongue's minimal role in prehension of food. The manatee instead uses its very flexible lips and pectoral flippers for this purpose. Molars, the manatee's only teeth replaced continuously over the its life, are used to grind food into smaller pieces.

The main ingestive function of the tongue thus relates mainly to manipulation of food during mastication, formation of a food bolus, and swallowing. In addition, mucous glands within the substance of the tongue assist in moistening food during mastication and preparation for swallowing.

Certain characteristics of the intrinsic lingual musculature demonstrated its importance in mastication. Muscle fibers coursed through all planes of orientation, transversely, longitudinally, and obliquely. Though the tongue cannot be protruded from the oral cavity, the combination of extrinsic and intrinsic muscles allow it to move between the mandibular rami. Together with its movement, the lingual salivary glands and lingual papillae aid in the mastication of foodstuffs.

Most papillae on the manatee's tongue appear to perform mechanical rather than gustatory functions. Filiform papillae on the free rostral tip of the tongue likely serve to direct food caudally in the oral cavity. The fungiform papillae of the manatee differ from those of many terrestrial mammals in lacking taste buds. Their function is therefore likely mechanical, assisting to positioning food and retaining it in the mouth in order for the molars to grind the diet into smaller particles.

The two types of salivary glands present in the tongue likely have different roles. The purely mucous glands figure most significantly in lubricating food for mastication and swallowing. The seromucous glands, restricted to the foliate papillae, most likely play a role in rinsing the taste pores of food particles and/or chemically modifying food particles in preparation for transduction by the taste bud.

Chemoreceptive role of manatee tongue

Very little research, anatomical or physiological, has been directed towards the chemoreceptive abilities of the manatee. Other marine mammals such as cetaceans and pinnipeds have been found to possess the structural components for chemoreception, such as taste buds and olfactory tissues. Nerve tracts supporting those tissues were found to be reduced in size or lost in cetaceans (Lowell and Flanigan, 1980). The West Indian manatee was found to lack a vomeronasal organ (Mackay-Sim et al., 1985), possibly giving the tongue a greater chemoreceptive role. Welker (Welker et al., 1996) has investigated the brain of the manatee and found taste circuits were prominent. Therefore, the manatee tongue may a play a role in chemoreception, such as tasting, detecting hormones, or other chemicals released from other manatees, and detecting salinity changes. The numbers of taste buds were not as great as in terrestrial mammals, but were more numerous than other marine mammals, such as dolphins (Yamasaki and Komatsu, 1980); manatees are nonetheless believed to be able to taste. In the wild, manatees have been known to avoid eating plants containing biotoxins.

Beyond tasting, the use of chemoreceptors, including those found on taste buds, may help the manatee to recognize individual manatees and also to identify female manatees in estrus through hormones or other chemicals released in the water. Chemoreception may also aid manatees during seasonal migrations: differences in water salinity may act as chemical cues to direct manatees to warm water springs or power plant discharges during the winter months. Chemosignaling was thought to occur in toothed whales such as belugas, which empty their perianal glands to provide a chemical cue to other conspecifics (Lowell and Flanigan, 1980). Future work with manatees should include work on their chemoreceptive abilities.

Comparative Sirenia lingual anatomy

The tongues of the three manatee species are similar in size, shape, and mobility. Dugong lingual structure is slightly different, which may reflect its environment (mostly marine) and feeding strategy (mostly bottom feeding). Several anatomical differences between the manatee and dugong tongue exist. First, the lingual apex is round in the

manatee and truncated in the dugong. Second, filiform papillae are restricted to the rostral free tip in the manatee and extended more caudally in the dugong. Third, gustatory organs housing taste buds were found in the multifossulate swellings of the foliate papillae in the manatee and in pits on the dorsal sidewalls (no swellings) in the dugong. Lastly, lingual glands were mostly mucous in the manatee and mostly serous in the dugong. The difference in lingual glands between the manatee and the dugong may reflect feeding preference. While both eat a variety of seagrasses, the dugong diet is more marine while the manatee diet is both fresh and marine water vegetation. More investigations are needed to elucidate this difference.

Comparative terrestrial lingual anatomy

As mentioned earlier, the manatee's closest living relatives include the elephant (Order Proboscidae), rock hyrax (Order Hyracoidea) (Kleinschmidt et al., 1986), and aardvark (Order Tubulidentata). Sonntag (1992) suggested that the manatee tongue resembled that of the rock hyrax, but included no evidence. As mentioned earlier, the manatee tongue was similar in shape and function to the elephant.

Aardvarks have a long tongue with a pointed apex with filiform papillae. Fungiform papillae are located along the lateral walls along its length. Three vallate papillae were arranged in an inverted triangle near the base; there were no foliate papillae.

Due to similar diets, Sonntag (1922) had suggested that sirenian tongues resembled those of ungulata, such as the cow and horse. [In a study on the digestive strategy and efficiency of the West Indian manatee (Burn and Odell, 1987), manatees had a higher digestibility of cellulose than any terrestrial herbivore, most likely due to the low lignin content found in seagrasses as compare to terrestrial grasses.]. Though all three are herbivores, the tongues differ in shape, papillae distribution, and especially mobility. The cow uses its tongues for prehension, and the tongues of both cow and horse are protrusible. The manatee neither uses its tongue in prehension nor is the tongue protrusible. Vallate papillae (2-3) are found in both the cow and horse, and not in the

manatee. Taste buds are found in the vallate and foliate papillae of both the cow and horse.

Comparative marine mammal lingual anatomy

Though manatees are marine mammals, their tongues differ greatly from other marine mammals such as dolphins, whales, and walruses. Again, differences in biology, food intake, and food preference are major factors for these differences.

Cetacea

According to Sonntag (Sonntag, 1922), cetacean tongue anatomy varies between the toothed (Odontoceti) and baleen (Mysticeti) whales in several ways. First, the tongue is firm and hard in the toothed whales and soft in the baleen whales. Second, lingual glands are numerous in the toothed whales and less numerous in the baleen whales. Third, lingual muscles are well developed in toothed and slight in the baleen whales. Finally, oil is absent in the toothed whale but abundant in the baleen whale.

In general, toothed whales eat mainly fish and invertebrates by swallowing their food whole, without chewing. The numerous lingual mucous glands in toothed whales may help to lubricate its food prior to swallowing (Yamasaki et al., 1976). Baleen whales filter water through their baleen and then manipulate its tongue to collected and swallow the remaining food.

The tongues in both suborders of whales are simpler than the manatee's. Few to no lingual papillae are present in most species of cetaceans (Sonntag, 1922). Some papillae in dolphins, such as the striped dolphin (*Stenella coeruleoalba*), attain maximum development in the early postnatal period and disappear after the weaning period (Yamasaki et al., 1978). These papillae on the craniolateral margin of the tongue may play a mechanical function by preventing the loss of milk while suckling, and do not contain any taste buds. Taste buds are thought to be few or absent in most cetacea (Lowell and Flanigan, 1980; Sonntag, 1922; Yamasaki et al., 1976). However, taste sensation in the harbour porpoise (Berhrmann, 1988) and the bottlenose dolphin

(Nachtigall and Hall, 1984) was thought to be well developed. Taste buds in tongues of bottlenose and common dolphins were found in the floor of small cavities in the root of the tongues (Suchowskaja, 1972). Generally, the cetacean tongue is the simplest of all the mammals.

Seals and Sea Lions

The lingual shape varies greatly among the pinnipeds (Bradely, 1971). Tongues are usually short and tapering, while the apex is highly variable between seals and sea lions. The numbers of filiform and fungiform papillae also vary among pinnipeds, with the grey seals having no fungiform papillae and the hooded seal having numerous fungiform papillae. In seals, the vallate papillae are the chief gustatory organs, while absent in sea lions. Foliate papillae were only found in seals (Tuckerman, 1890).

Walrus

The tongues of walruses are broad, thick, short, and smooth. Musculature within the lingual substance is well developed. The tongues are well adapted for its feeding strategy of consuming bivalve molluscs. Unlike manatees and cetaceans, the walrus can protrude its tongue out of the oral cavity, and uses it as a tactile organ to search for food in the sediment (Kastelein et al., 1997). The tip of the tongue is well innervated and can determine the position of objects within its oral cavity. Lamellated corpuscles are found subjacent to the epidermis. When the tongue is depressed in the buccal cavity, the walrus creates negative pressure and can extract the edible parts of the bivalve mollusc from its shell. The function of suction may also aid in suckling, as in cetaceans. Taste buds, few but large, were found in the seven circumvallate papillae in V-shaped row in the caudal portion of the tongue. No foliate papillae were observed. Mechanoreception, for its feeding strategy, was thought to be more important that gustation (Kastelein et al., 1997).

Comparative microscopic observations

Epithelium

The epidermis of the manatee was a stratified, keratinized squamous epithelium. This type of epithelium most likely protects the tongue against the abrasive forces due to

masticating its vegetative diet. Other marine mammals, such as dolphins (Yamasaki et al., 1976) northern fur seals (Kubota, 1968), and walruses (Kastelein et al., 1997), also demonstrated this type of epithelium. The filiform papillae in the manatee were essentially a core of keratin and most likely received the greater amount of abrasive forces. Epithelium covering the fungiform papillae was more keratinized than the adjacent non-papillary epithelium. Similar findings were reported for the northern fur seal (Kubota, 1968) and the horse (Pfeiffer et al., 1999). Cell layers in the epidermis, including the stratum basale, stratum spinosum, and stratum corneum, were similar to other mammalian epidermal architectures. One distinguishing feature was noted in the stratum spinosum of the foliate papillary epidermis, clear to translucent vacuoles were seen adjacent to the nuclei in this layer. These cells were termed lipokeratinocytes by Pfeiffer (Pfeiffer and Rowntree, 1996). Similar findings were reported in several cetaceans (Pfeiffer and Jones, 1993; Pfeiffer and Rowntree, 1996) including the bottlenose dolphin, long-finned pilot whale, humpback whale, fin whale, and the southern right whale. In southern right whales, vacuoles were found be to lipid in nature, in cetacean skin, and were thought to be involved in nuclear or cytoplasmic metabolism, rather than insulation or secretion. The lipokeratinocytes were thought to be an adaptation in marine mammal skin related to its aquatic environment as a mechanism for dealing with the stresses of migration, food shortages, and diving. The appearance of the vacuoles were similar in manatees and cetaceans. The vacuole composition in the manatee was not determined in this study. The vacuoles were believed to be lipid in nature.

Muscle

The tongue of the manatee was composed mostly of striated skeletal muscle, as in the tongues of all vertebrates. Intrinsic muscle fibers were seen in all planes of orientation, thus giving the tongue its mobility (as well as extrinsic muscles).

Taste buds

In mammals, taste buds can be found in the foliate, fungiform, and/or vallate papillae. Of the three papillae, the vallate usually contains the greatest number of taste buds. Taste buds, other than in the tongue, can also be found in the rodent palate (Bradely, 1971), rat

pharynx and larynx (Travers and Nicklas, 1990), and canine larynx (Yamamoto et al., 1997). Taste buds are also found in a variety of non-mammalian species, including amphibians, reptiles, birds, and fish. The mammalian taste bud is usually constructed of 3 types of cells, Type I (dark cells-gustatory), Type II (light cells-supporting), Type III (special gustatory) (Murray, 1986). The apical end of a taste bud projects microvilli from the taste pore, which binds to molecules in the oral cavity such as food particles and hormones. Taste bud shapes range from spherical to spindle. Shape of taste buds vary widely among mammals, being oval in manatees, ovoid in the bovine (Davies et al., 1979), ovoid to pear-shaped in the mouse (foliate papillae) (Royer and Kinnamon, 1988), barrel-shaped in humans (Burkitt et al., 1993), and disk-shaped in the frog (Bradely, 1971). The number of taste buds varies greatly amount the mammals, from nearly none in cetaceans, to over 15,000 in rabbits (Bradely, 1971) and 20,000 in bovines (Davies et al., 1979). Further studies are needed to accurately determine the number of taste buds in manatees and dugong.

Comparative immunohistochemistry

To investigate the innervation within the lingual substance including its taste buds, the antibody neuron specific enolase (NSE) was selected. The antibody was previously used in several studies including the birds (Toyoshima, 1989), fish (Toyoshima, 1989), amphibians (Toyoshima, 1989), rats (Montavon and Lindstrand, 1991; Yoshie et al., 1989), pigs (Montavon and Lindstrand, 1991), guinea pigs (Yoshie et al., 1988), and humans (Takami et al., 1994). In the mammalian species, NSE reacted in both taste buds and subepithelial connective tissue, and basal cells of teleost fish and amphibians. NSE reactivity was noted among muscle bundles, glands, and blood vessels in the rat (Montavon and Lindstrand, 1991). Similar findings were noted in the manatee. Although NSE has been used to track nerve fibers penetrating the epithelium and into the taste bud, no tracts were demonstrated in the present study, most likely due to the sub-optimal fixation and processing of the tissue. To fully describe neural elements surrounding and penetrating taste buds, fresh tissue will be needed.

Comparative scanning electron microscopy

A variety of scanning electron microscopic studies has been performed on the lingual papillae of several species, including horses and cows (Chamorro et al., 1986), goats (Kumar et al., 1998), rodents (Liu and Lee, 1982), rabbits, (Watanabe et al., 1988), opossums (Krause and Cutts, 1982), and humans (Azzali et al., 1996). General papillary shape was similar in most species; however, distinguishing differences in distribution and papillary types were noted. For instance, the goat had fungiform papillae on the ventral, as well as the dorsal surface. Also, filiform papillae had 3-6 projections at the base and were surrounded by a shallow groove, not seen in the manatee. Opossums, as well as other marsupials, displayed three vallate papillae, small and scattered fungiform papillae, and no foliate papillae. Numerous compound filiform papillae, arranged in a horseshoe shape cavity, covered the majority of the dorsum. Epithelium covering the fungiform papillae appeared thinner and less keratinized than other papillae, opposite that of the manatee. The horse and cow both showed fungiform papillae similar to manatees, though the manatee papillae lacked taste pores. Filiform papillae surrounded the fungiform papillae in the cow. Foliate papillae in the horse were similar in shape and location to the manatee; however, the cow foliate papillae were much smaller and also lacked taste pores.

Comparative transmission electron microscopy

Limited transmission electron microscopy was performed in this study due to the limited number of very freshly fixed tissues received. Also, tissue quality was sub-optimal to adequately describe ultrastructural features in detail. Thus, limited information on epidermal and dermal structures were included in this report.

Dermal features including collagen fibers and skeletal muscle showed ultrastructural features previously described in other mammals. Epithelial features were also similar to those of other mammals, except for nuclei-associated vacuoles seen in the foliate papillary stratum spinosum region. Again, these vacuoles were reported as being lipid in nature when examined ultrastructurally. Due to the quality of tissue, composition of the

vacuoles could be not determined in this report, although the vacuoles of the manatee appeared similar to those of the other species by both light and electron microscopy. In particular, vacuoles were nestled within a nuclear concavity as reported in the southern right whale (Pfeiffer and Rowntree, 1996). Fresh tissue will be necessary to elucidate these features in the manatee in future works.

Summary

A variety of techniques was used to describe the lingual structure of the manatee tongue in more detail than previously published works. Gross findings described in this report closely match those of Yamasaki (Yamasaki and Komatsu, 1980). The most noteworthy features of the manatee tongue included its caudal position in the oral cavity, inability to be protruded, and the short free rostral tip. Functionally, the manatee uses its tongue for moving food in the oral cavity, cropping its vegetative diet, and swallowing the food bolus. The tongue is not used for prehension or vocalization. Filiform, fungiform, and foliate papillae were found, but no circumvallate papillae were located. Open pits on the lateral side-walls and dorsal caudal region led to mucous salivary glands. With the use of histochemistry, the nature and distribution lingual structures, such as collagen, keratin, and mucous salivary glands were clearly described. Immunohistochemistry with neuron specific enolase revealed subepidermal nerve fibers and the innervation of salivary glands, taste buds, and blood vessels more clearly. For the first time, electron microscopic (scanning and transmission) techniques were applied to detail surface and cytoplasmic features at high resolution. Vacuole-associated nuclei in the foliate papillary stratum spinosum were described for the first time in manatees using transmission electron microscopy. Though this and other reports clearly show manatees possess taste buds, the ability for manatees to taste is still unclear.

Though more detailed lingual structure was reported here than in previously published works, certain basic cell structures such as taste buds could not be elucidated due to the lack of freshly fixed tissues. This was especially evident when processing tissues for electron microscopy. The majority of tissues were fixed after a period of several hours,

and in some cases, after a period of 24 hours. Several factors accounted for poor tissue quality including poor body condition, delays in carcass retrieval, and delays in necropsy after carcass retrieval. Due to limited personnel and the time required to locate and retrieve a carcass and perform a necropsy, and typical high ambient temperatures at carcass sites rendered quick removal and fixation of tissues for microscopic examination extremely difficult. Due to the endangered status of the manatee, euthanization of an animal for sample collection is impossible. Performing limited on-site necropsies to remove small sections of lingual tissue was suggested, but never occurred during the study period. Therefore, the majority of tissues considered to be in good condition upon arrival in our laboratory were processed. One approach to obtain fresh tissues would involve any of the several parks and aquaria in Florida that maintain manatees in captivity. If the imminent death of a manatee was anticipated, tissues could be collected and fixed immediately after the death.

More studies are needed in the future to completely describe the lingual structure of the West Indian manatee. This can occur only when fresh tissues are collected and fixed. In particular, the structure of taste buds needs more investigation. Features of particular interest include individual cell types and numbers within each bud, innervation within and around taste buds, the total number of taste buds. The stratum spinosum in the foliate papillary region also warrants future investigation into the composition of the vacuoles associated with the nuclei. Histochemistry with special stains such as oil red O may reveal the possible lipid composition of the vacuoles. As mentioned earlier, freshly fixed tissue will be the key to further study the manatee tongue in detail.

Table 1. Study animals

Manatees examined by the Marine Mammal Pathobiology Lab whose tongues were
included in the study.

ID Number	Sex	Length (cm)	Weight (kg)	Carcass Condition
MEC9815	Female	223	253	Fresh
MEC9819	Male	87	11	Fresh
MEC9854	Female	n/a	not available	not available
MNE9818	Male	303	476	Moderately decomposed
MSE9820	Male	199	not available	not available
MSW9821	Male	206	not taken	Moderately decomposed
MSW9825	Male	127	44	Fresh
MSW9826	Male	97	17	Fresh
SWFTM9727	Female	326	not taken	Fresh

Table 2. Tongue Dimensions

Dimensions of tongue and number of lateral fungiform papillae.

	Length of Tongue (cm)	Breadth (cm)	Thickness (cm)	Lateral Papillae
MEC9815	11.5	3	3.5	L-37; R-33
MEC9819	6	2.3	2.5	L-36; R-35
MEC9854	n/a	n/a	n/a	n/a
MNE9818	14	3.5	5.5	L-51; R-?
MSE9820	n/a	n/a	n/a	n/a
MSW9821	15	3.6	5.3	L-61; R-61
MSW9825	10	2.4	3	L-39; R-43
MSW9826	8.5	2.4	3.4	L-38; R-42
SWFTM9727	15.6	4	6	L-42; R-32

L-left

R-right

n/a-not available

References

Azzali, G., Gennari, P. U., Maffei, G., and Ferri, T. (1996). Vallate, foliate and fungiform human papillae gustatory cells. An immunocytochemical and ultrastructural study. Minerva Stomatol *45*, 363-79.

Beddard, F. E. (1897). Notes upon the anatomy of a manatee (Manatus inunguis) lately living in the society's garden. Proc. Zool. Soc., London, 47-53.

Berhrmann, G. (1988). The Peripheral Nerve Ends in the tongue of the Harbour Porpoise Phocoena phocoena (Linne, 1758). Aquatic Mammals *14*, 107-112.

Bradely, R. M. (1971). Tongue Topography. In Handbook of Sensory Physiology, L. M. Beidler, ed. (Berlin, Heidelberg, New York: Springer-Verlag), pp. 1-30.

Burkitt, H. G., Young, B., and Heath, J. W. (1993). Wheater's Functional Histology A text and colour atlas, third Edition: Churchill Livingstone).

Burn, D. M., and Odell, D. K. (1987). Volatile fatty acid concentrations in the digestive tract of the West Indian manatee, Trichechus manatus. Comp Biochem Physiol [B] *88*, 47-9.

Caceci, T. (1984). A Gravimetric Formula for Erlandson's Maraglas, D.E.R. 732 Embedding Medium. TSEMJ *15*, 26-27.

Chamorro, C. A., de Paz, P., Sandoval, J., and Fernandez, J. G. (1986). Comparative scanning electron-microscopic study of the lingual papillae in two species of domestic mammals (Equus caballus and Bos taurus). 1. Gustatory Papillae. Acta Anat *125*, 83-7.

Cohen, J. L., Tucker, G. S., and Odell, D. K. (1982). The photoreceptors of the West Indian manatee. J. Morphol *173*, 197-202.

Davies, R. O., Kare, M. R., and Cagan, R. H. (1979). Distribution of taste buds on fungiform and circumvallate papillae of bovine tongue. Anat Rec *195*, 443-446.

Forbes, W. A. (1879). On the anatomy of the african elephant (Elephas africanus, Blum). Proc Zool Soc, London, 420-435.

Gohar, H. A. F. (1957). The Red Sea dugong. Publ. Marine Biol. Station Al-Ghardaqa Red Sea *9*, 3-49.

Griebel, U., and Schmid, A. (1997). Brightness discrimination ability in the West Indian manatee (Trichechus manatus). J. Exp Biol *200*, 1587-92.

Griebel, U., and Schmid, A. (1996). Color vision in the manatee (Trichechus manatus). Vision Res *36*, 2747-57.

Heyning, J. E., and Mead, J. G. (1997). Thermoregulation in the mouths of feeding gray whales [see comments]. Science *278*, 1138-9.

Hill, D. A., and Reynolds, J. E. d. (1989). Gross and microscopic anatomy of the kidney of the West Indian manatee, Trichechus manatus (Mammalia: Sirenia). Acta Anat *135*, 53-6.

Home, E. (1820). Particulars respecting the anatomy of the dugong, intended as a supplement to Sir T.S. Raffles' account of that animal. Philos. Trans. Roy. Soc. London *110*, 315-323.

Humphrey, C. D., and Pittman, F. E. (1974). A simple methylene blue-azure II-basic fuchsin stain for epoxy-embedded tissue sections. Stain Technol *49*, 9-14.

Kastelein, R. A., Dubbeldam, J. L., and de Bakker, M. A. G. (1997). The anatomy of the walrus head (Odobenus rosmarus). Part 5: The tongue and its function in walrus ecology. Aquatic Mammals *23*, 29-47.

Kleinschmidt, T., Czelusniak, J., Goodman, M., and Braunitzer, G. (1986). Paenungulata: a comparison of the hemoglobin sequences from elephant, hyrax, and manatee. Mol Biol Evol *3*, 427-35.

Krause, W. J., and Cutts, J. H. (1982). Morphological observations on the papillae of the opossum tongue. Acta Anat *113*, 159-68.

Kubota, K. (1968). Comparative anatomical and neurohistological observations on the tongue of the northern fur seal (Callorhinus ursinus). Anat Rec *161*, 257-65.

Kumar, P., Kumar, S., and Singh, Y. (1998). Tongue papillae in goat: a scanning electron-microscopic study. Anat Histol Embryol *27*, 355-7.

Liu, H. C., and Lee, J. C. (1982). Scanning electron microscopic and histochemical studies of foliate papillae in the rabbit, rat and mouse. Acta Anat *112*, 310-20.

Lowell, W. R., and Flanigan, W. F. (1980). Marine mammal chemoreception. Mammal Rev. *10*, 53-59.

Mackay-Sim, A., Duvall, D., and Graves, B. M. (1985). The West Indian manatee (Trichechus manatus) lacks a vomeronasal organ. Brain Behav Evol *27*, 186-94.

Maluf, N. S. (1995). Kidney of elephants. Anat Rec *242*, 491-514.

Maluf, N. S. (1989). Renal anatomy of the manatee, Trichechus manatus, Linnaeus. Am J Anat *184*, 269-86.

Montavon, P., and Lindstrand, K. (1991). Immunohistochemical localization of neuron-specific enolase and calcitonin gene-related peptide in pig taste papillae. Regul Pept *36*, 235-248.

Murie, J. (1885). Futher observations of the Manatee. Trans. Zool. Soc. Lond. *11*, 19-48.

Murie, J. (1872). On the form and structure of the manatee (Manatus americans). Trans. Zool. Soc. Lond. *8*, 127-202.

Murray, R. G. (1986). The mammalian taste bud type III cell: a critical anaylsis. J. Ultra Mol Struct Res *95*, 175-188.

Nachtigall, P. E., and Hall, R. W. (1984). Taste Reception in the Bottlenosed Dolphin. Acta Zool. Fennica *172*, 147-148.

Pfeiffer, C. J., and Jones, F. M. (1993). Epidermal lipid in several cetacean species: ultrastructural observations. Anat Embryol (Berl) *188*, 209-18.

Pfeiffer, C. J., Levin, M. L., and Lopes, M. (1999). Ultrastructure of the Horse Tongue: Further Observations on the Lingual Integumentary Architecture. (submitted).

Pfeiffer, C. J., and Rowntree, V. J. (1996). Epidermal ultrastructure of the southern right whale calf (Eubalaena australis). J Submicrosc Cytol Pathol *28*, 277-86.

Reep, R. L., Johnson, J. I., Switzer, R. C., and Welker, W. I. (1989). Manatee cerebral cortex: cytoarchitecture of the frontal region in Trichechus manatus latirostris. Brain Behav Evol *34*, 365-86.

Reep, R. L., Marshall, C. D., Stoll, M. L., and Whitaker, D. M. (1998). Distribution of innervation of facial bristles and hairs in the Florida manatee (Trichechus manatus latirostris). Marine Mammal Science *14*, 257-273.

Reep, R. L., and O'Shea, T. J. (1990). Regional brain morphometry and lissencephaly in the Sirenia. Brain Behav Evol *35*, 185-94.

Reynolds, J. E., 3rd, and Rommel, S. A. (1996). Structure and function of the gastrointestinal tract of the Florida manatee, Trichechus manatus latirostris. Anat Rec *245*, 539-58.

Reynolds, J. E. d., and Krause, W. J. (1982). A note on the duodenum of the West Indian manatee (Trichechus manatus), with emphasis on the duodenal glands. Acta Anat *114*, 33-40.

Royer, S. M., and Kinnamon, J. C. (1988). Ultrastructure of mouse foliate taste buds: synaptic and nonsynaptic interactions between taste cells and nerve fibers. J Comp Neurol *270*, 11-24, 58-9.

Sonntag, C. F. (1922). The comparative anatomy of the tongue of mammlia. VII Cetacea, Sirenia and Ungulata. Proc. Zool. Soc. Lond., 639-646.

Suchowskaja, L. I. (1972). The Morphology of the Taste Organs in Dolphins. In Investigations on Cetacia, G. Pilleri, ed., pp. 201-206.

Takami, S., Getchell, T. V., McLaughlin, S. K., Margolskee, R. F., and Getchell, M. L. (1994). Human taste cells express the G protein alpha-gustducin and neuron- specific enolase. Brain Res Mol Brain Res *22*, 193-203.

Toyoshima, K. (1989). Chemoreceptive and mechanoreceptive paraneurons in the tongue. Arch Histol Cytol *52*, 383-8.

Travers, S. P., and Nicklas, K. (1990). Taste bud distribution in the rat pharynx and larynx. Anat Rec *227*, 373-9.

Tuckerman, F. (1892). Further Observations on the Gustatory Organs of the Mammalia. J. Morph *7*, 69-94.

Tuckerman, F. (1890). On the Gustatory Organs of some of the Mammalia. J. Morphol *4*, 152-193.

Watanabe, I., Ogawa, K., and Yamada, E. (1988). Taste buds of the rabbit foliate papillae. A scanning electron microscopy study. Ciencia E Cultura *40*, 787-790.

Welker, W., Reep, R., and Johnson, J. I. (1996). The Brain of the Florida Manatee.

Yamamoto, Y., Atoji, Y., and Suzuki, Y. (1997). Innervation of taste buds in the canine larynx as revealed by immunohistochemistry for the various neurochemical markers. Tissue Cell *29*, 339-46.

Yamasaki, F., and Komatsu, S. (1980). A comparative morphological study on the tongues of manatee and dugong (sirenia). Sci Rep Whales Res Inst *23*, 127-144.

Yamasaki, F., Komatsu, S., and Kamiya, T. (1981). An anatomical note on the tongue of the Dugong, Dugong dugong, H. Marsh, ed. (James Cook University of North Queensland, pp. 182-191.

Yamasaki, F., Komatsu, S., and Kamiya, T. (1978). Papillary projections at the lingual margin in the striped dolphin, Stenella coeruleoalba, with special reference to their development and regression. J. Morphol *157*, 33-47.

Yamasaki, F., Satomi, H., and Kamiya, T. (1976). The tongue of Franciscana (La Plata dolphin), Pontoporia blainvillei. Okajimas Folia Anat Jpn *53*, 77-92.

Yoshie, S., Teraki, Y., Iwanaga, T., and Fujita, T. (1989). Immunocytochemistry of neuron-specific proteins and neuropeptides in taste buds and associated nerves. Arch Histol Cytol *52*, 389-96.

Yoshie, S., Wakasugi, C., Teraki, Y., Iwanaga, T., and Fujita, T. (1988). Immunocytochemical localizations of neuron-specific proteins in the taste bud of the guinea pig. Arch Histol Cytol *51*, 379-84.

MILTON JAY LEVIN
1800 Mt Tabor Road
Blacksburg, Virginia 24060
540-951-4917
milevin@vt.edu

OBJECTIVE   Position in Immunotoxicology

EDUCATION   Masters of Science, Veterinary Medical Sciences
Virginia-Maryland Regional College of Veterinary Medicine
Department of Biomedical Sciences and Pathobiology
College of Veterinary Medicine
Blacksburg, VA
Graduate Summer 1999
Current GPA: 3.80

Bachelor of Science, Marine Biology
Florida Institute of Technology (F.I.T.)
Melbourne, FL
Graduated June 1990
GPA: 3.23; Dean's List (11 of 13 quarters)
Graduated with Honors

RESEARCH EXPERIENCE

2/92-9/97   **COVANCE LABS, Contract Toxicology**
Research Assistant, Vienna, Virginia
- Necropsied laboratory animals including mice, rats, dogs, rabbits, and primates
- Described and recorded macroscopic pathological findings
- Weighed organs, prepared blood and bone marrow smears, and reviewed and quality checked data
- Served as member of rodent surgical catheterization team
- Worked in other areas including neurotoxicology, teratology, and histology

3/95-3/97   **Marion duPont Scott Equine Medical Center**
Foal Watch Volunteer**,** Leesburg, Virginia
- Performed duties including sitting with premature and full term foals
- Milked the mares, hand fed the foals, monitored I.V. lines and basic vital signs
- Handled patients during examination/procedures

6/95-6/97   **Leesburg Animal Hospital**
Volunteer**,** Leesburg, Virginia
- Observed veterinarians in the examination rooms

- Reviewed past and present animal health records
- Assisted with handling and restraint of patients for examinations and injections
- Trained to collect blood samples, performed and interpreted clinical tests (fecal, heartworm, FIV, urine, hematology, chemistry, etc.), and performed physical examinations.

3/91-11/91	**Applied Biology, Inc.** Environmental Consultant, Jensen Beach, Florida • Supported field and laboratory duties in environmental program at the Fort Myers, Florida field office • Performed water quality monitoring, benthic invertebrate collection and sampling, fish collection and identification, and surveys of recreational fishermen
10/89-6/90	**Florida Institute of Technology** Senior Undergraduate Research, Melbourne, Florida • Collected manatee population numbers at various sites in Florida • Snorkeled with manatees to collected data including location, sex, body measurements, and activity • Made hand drawings, still photos, and videos of scar markings • Analyzed data to determine if manatees repeatedly used these sites during consecutive winter months
10/89-3/91	**Marine Mammal Stranding Network** Volunteer, Melbourne, Florida • Collected dead dolphins and whales in Brevard County, Florida • Necropsied animals and collected tissue samples • Examined species included bottlenose dolphins, spinner dolphins, and pygmy sperm whales
TEACHING	Teaching Assistant, Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA Spring, 1999 • Assist professor with various duties for veterinary neurobiology Teaching Assistant, Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA Fall, 1998 • Assisted in weekly laboratory sections for veterinary histology

THESIS
"Gross and Light Microscopic Description of the Lingual Structure of the West Indian Manatee (*Trichechus manatus latirostris*)" (in progress)

PRESENTATIONS
Marine Mammal Biomedicine, College of Veterinary Medicine, Virginia Tech, February, 1999
"Manatee Biology"

Environmental Health Toxicology, College of Veterinary Medicine, Virginia Tech, December, 1998
"Aquatic Toxicology and the St. Lawrence River Beluga Whales"

PUBLICATIONS
Levin, M.J., Pfeiffer, C.J. (1998). Analysis of Cyamid Frontal "Eye" Photoreceptors. In *Proceedings of the Sixth Annual Atlantic Coastal Dolphin Conference*, St. Petersburg, FL.

Levin, M.J., Pfeiffer, C.J., Smith, B.J., Caceci, T. (1998). Gross and Microscopic Description of the West Indian Manatee (*Trichechus manatus latirostris*) Tongue. In *Proceedings of the American Association of Veterinary Anatomist*. Blacksburg, VA.

Levin, M.J., Pfeiffer, C.J. (1999). Photoreceptor Ultrastructure of the Amphipod *Cyamus ceti* (Linne, 1758), an Ectoparasite of Bowhead, Right, and Gray Whales. *Journal of Submicrpscopic Cytology and Pathology*, 31(**3**), 1-9.

Lewis, M.C., Levin, M.J., Klein, B.G. (1999). Alterations of Cortical Dopamine Concentrations by a Sub-clinical Dose of the Anticholinesterase Insecticide Chlorpyrifos in Rat. *Society of Neuroscience Absracts*, 25 (in print).

Pfeiffer, C.J., Levin, M.J., Lopes, M. (1999). Ultrastructure of the Horse Tongue: Further Observations on the Lingual Integumentary Architecture. (submitted).

Levin, M.J., Pfeiffer, C.J., Freeman, L.F. (1999). Observations on the Lingual Structure of the West Indian Manatee (Trichechus manatus latirostris). In *Proceedings of the American Association of Veterinary Anatomist*, Baton Rouge,

LA. (in print).

**PROFFESIONAL
AFFILIATIONS**   Member, Society for Marine Mammalogy

Member, American Association for Laboratory Animal Science (AALAS); Certified Assistant Laboratory Animal Technician, December, 1992

Member, Beta Beta Beta National Biological Honor Society; Vice-President, 1989-1990

**UNIVERISTY
SERVICES**   Member, Graduate Student Cabinet, College of Veterinary Medicine, Virginia Tech

REFERENCES   Furnished upon request