THE FUNCTIONAL MECHANISMS AND HISTOLOGIC COMPOSITION OF THE LINGUAL APPENDAGE IN THE ALLIGATOR SNAPPING TURTLE, *MACROCLEMYS TEMMINCKI* (TROOST) (TESTUDINES:CHELYDRIDAE)

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Edward Lawrence Spindel

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VITA

Edward Lawrence Spindel, son of Murray and Barbara (Heiz) Spindel, was born May 25, 1956 in Glen Cove, New York. He attended Hicksville Public Schools and graduated from Hicksville High School, Hicksville, New York, in 1974. In September, 1974, he entered the State University of New York at Buffalo and remained there through his sophomore year, 1976. In September, 1976, he entered Cornell University and recieved the degree of Bachelor of Science with Honors (Wildlife Science) in May, 1978. He entered the Graduate School, Auburn University, in September, 1978. On May 5, 1980, he was admitted to the New York State College of Veterinary Medicine for the fall semester of 1980. He married Jacqueline Roman, daughter of Victor and Magdelena (Guzman) Roman, in June, 1978.

THESIS ABSTRACT

THE FUNCTIONAL MECHANISMS AND HISTOLOGIC COMPOSITION OF THE LINGUAL APPENDAGE IN THE ALLIGATOR SNAPPING TURTLE, *MACROCLEMYS TEMMINCKI* (TROOST) (TESTUDINES:CHELYDRIDAE)

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The lingual appendages of 5 juvenile, 2 subadult and 2 adult alligator snapping turtles were examined grossly and microscopically. The appendage is divided into an anterior horn, body, and posterior horn. Adults typically display greater pigmentation of the lingual appendage than do juveniles, although this is not always the case. Melanocyte distribution in the lingual appendage is variable resulting in its mottled appearance. The musculoskeletal components of the hyoid apparatus, presumably responsible for the majority of motion displayed by the appendage, are described with regard to their position and actions. Innervation of the lingual appendage is achieved by the lingual nerve. This nerve divides into three smaller nerves, two coursing rostrally into the anterior horn and one coursing caudally into the posterior horn. These nerves ramify and end in numerous terminals within the lamina epithelialis and lamina propria. Structures, morphologically similar to taste buds described in other species, are present in the lamina epithelialis on the distal two-thirds of the lingual appendage. Blood is transported to the appendage via the lingual artery, a terminal branch of the external carotid artery. Numerous venous sinuses between the predominant bundles of connective tissue of the lingual appendage account for approximately one-fifth of its total volume. Goblet cells, containing acid mucopolysaccarides, are present in the stratified squamous epithelium of the lingual appendage. The morphological data collected support earlier statements regarding the lingual appendage as a luring structure. Engorgment with blood causes swelling and a more pink coloration of the appendage. The coloration, enlargement and wriggling movement combined with the bouyancy of water permits mimicry of small worms and insect larvae. The appearance of melanin through ontogeny may increase the capacity for variation in the pattern displayed by the appendage, presumably resulting in an increase in the number of organisms attracted to it. Goblet cells in the lamina epithelialis, containing acid mucopolysaccaride which presumably is released on the surface, may play an important role in preserving the integrity of the non-keratinized, stratified squamous epithelium. The flexible character of the pseudoerect, active appendage may also aid in the avoidance of injury to the organ. Innervation of the lingual appendage may permit rapid conveyance of chemical and mechanical information to the brain for processing, allowing rapid response to the presence of prey.

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This thesis is dedicated to Barbara Joan (Heiz) Spindel, who, through her genuine concern for the education of her children, inspired me on to completion of this work. To her I am forever indebted.

I gratefully acknowledge the assistance of Alan Smith, HT-ASCP, for his input in the development of a silver stain for reptilian tissue and Mary Ann Louys, for her efforts during production of histologic study materials. I thank Dr. Bruce Gray for his assistance in photomicrography, Dr. Bobby Brown for performing the vinyl acetate injection of a turtle head, Dr. Mary Frances Vaden for assistance in latex injection, and the remainder of the faculty and staff in the Department of Anatomy and Histology, School of Veterinary Medicine, Auburn University for use of their laboratory facilities and continued critique of my work. I thank Franklin Presly and Kenneth Fahey for their assistance in the collection of turtles used in this study. Finally, I extend my heartfelt appreciation to my wife, Jacqueline, who has encouraged, supported and assisted me throughout my M.S. graduate program.

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I. INTRODUCTION

Numerous adaptations for the acquisition of prey exist in the animal kingdom. One trend has been the development of luring structures, anatomical features enabling predators to attract edible species within the reach of some grasping apparatus. Unique in this group of adaptations is the vermiform appendage located inside the oral cavity of the alligator snapping turtle, Macroclemys temmincki, herein called the lingual appendage. The lingual appendage has been described as a bicornuate, pink, filamentous, distensible structure, located in the region of, or extending from, the tongue of the alligator snapper (Gadow, 1901; Ditmars, 1907; Haltom, 1931; Pope, 1939; Allen and Neill, 1950; Carr, 1952; Parmalee, 1954; Cagle, 1957; Hediger, 1963; Breed, 1966; Heusser, 1968; Mount, 1975; Winokur, 1977; Drummond, 1979; Pritchard, 1979). Concerning its function, Ditmars (1907) commented on the turtle's ability to keep the lingual appendage in motion, presumably conveying a more worm or grub-like image to prospective prey. The only reported in vivo observation of appendage activity was made by Haltom (1931). The remainder of the functional information is the result of in vitro observations (Ditmars, 1907; Allen and Neill, 1950; Hediger, 1963; Heusser, 1968; Mount, 1975; Drummond, 1979; Pritchard, 1979). These investigators agreed that the lingual appendage is in fact an adaptation for luring in unsuspecting prey. The most recent study concerning the use of the lingual appendage as a lure was performed by Drummond (1979) who used "ingestively naive" hatchling alligator snappers for a behavioral analysis. He determined four phases to the luring behavior and suggested environmental factors which apparently precipitated each phase.

Although the objective of the luring behavior seems apparent, the mechanics underlying the behavior have yet to be determined. This study was undertaken, therefore, to describe the gross and microscopic structures of the lingual appendage which provide the morphological basis for its function as a luring mechanism in the normal behavioral repertoire of the alligator snapping turtle.

II. MATERIALS AND METHODS

A total of 9 turtles were used in this study: 5 juveniles (each weighing less than 7 kilograms), 2 subadults (each weighing between 7 and 13 kilograms), 2 adults (each weighing more than 13 kilograms). Five specimens were collected from slack water areas along the Alabama River within a 2 mile radius of Boatyard Landing in the town of Tensaw, Baldwin County, Alabama. The other specimens were collected from Hitchee Creek, Crisp County, Georgia; from a slough leading into the Mississippi River, Desha County, Arkansas; from the Coosa River at Childersburg, Talladega County, Alabama; and from a fish market in Lafayette, Louisiana. Five of the nine turtles collected were used for gross anatomical study and the remaining 4 for microscopic analysis of the lingual appendage.

Preparation of Gross Study Materials

The 5 turtles used for gross anatomical study were exsanguinated and subsequently perfused with either formaldehyde or gluteraldehyde fixative. Two of these specimens were then injected with latex. A third individual was injected with vinyl acetate. These vascular injections were performed to facilitate the study of the arterial and venous supply of the lingual appendage. The 2 remaining uninjected specimens were used to examine the basic structure and relationships of the skeletal elements, and the intrinsic and extrinsic musculature of the hyoid apparatus and lingual appendage. Examination of the innervation to the lingual appendage also was performed on these 2 uninjected turtles.

Latex injection of specimens

The tail of the turtle was amputated immediately caudal to the vent. A piece of firm coat hanger wire was inserted into the exposed caudal opening of the vertebral canal and rotated about its long axis as it was passed cranially, destroying the lower portion of the spinal cord. This pithing was continued until the hind legs became flaccid, at which time the femoral vein was isolated and cannulated. Sodium pentobarbital was administered intravenously through the femoral cannula until the turtle reached a surgical plane of anesthesia. A ventrolateral longitudinal incision was made in the neck facilitating isolation of the common carotid artery, which subsequently was cannulated with the cannula directed toward the heart. A 0.65 percent saline solution was injected into the femoral cannula while the turtle bled out through the carotid cannula. Perfusion of the circulatory system was continued until the fluid leaving the carotid cannula was dilute, indicating that most of the blood had been flushed from the vessels. A fixative solution; 10 percent formalin (27%), isopropyl alcohol (54%), glycerine (8%), phenol (7%), and water (4%) then was administered through the carotid cannula. Perfusion of the animal with formalin caused muscle twitching and eventually rigidity of all of the extremities. The turtle subsequently was placed in refrigeration (1° C) for approximately 1 week to permit complete fixation of all tissues of the body. After the week had lapsed, the specimen was removed from the refrigeration unit and processing was continued. The jugular vein, on the side contralateral to the common carotid artery previously cannulated, was isolated and cannulated with the cannula directed rostrally. Red latex, diluted 9 parts latex to 1 part water, was slowly and steadily injected into the carotid artery via the carotid cannula. This was continued until the latex was present in the claws of the hind limb and until the latex filled the carotid artery on the opposite side. A similar procedure was followed for injection of the venous system except that blue latex was injected through the jugular cannula. Approximately 3 weeks post-injection of the latex, solidification was complete and dissection was initiated.

Vinyl acetate injection of specimens

Anesthesia and initial cannulation were performed in the manner described for the latex injected specimens. Gluteraldehyde was used as the fixing agent in this procedure primarily because of its tendency to cause the lumen of non-muscular vessels to remain patent and/or frequently enlarge (Gray, personal comm.). Immediately following the injection of this fixative, red vinyl acetate was injected rostrally into the external and internal carotid arteries. Constant pressure was maintained during the injection of this liquid to ensure complete penetration of the solution throughout the cranial arterial system. Similarly, the veins of the head were perfused with blue vinyl acetate via bilateral injection of the jugular veins. After twenty-four hours the turtle was decapitated and the head was placed in a weak lye solution for maceration. It remained in this solution for approximately

3 weeks with occasional washings in tap water to aid in removal of all soft tissue elements. Upon completion of the maceration process, the skull and vinyl acetate model of the vasculature of the head were removed from the lye solution, washed and air dryed.

Dissection of the four non-macerated specimens was accomplished from the ventral aspect of the head along the midline so that each tissue layer could be photographed and then reflected, revealing the tissue layer beneath it.

Preparation of Histologic Study Materials

The lingual appendage and adjacent tissues extending approximately 0.1 cm laterad, 1 cm rostrad, 1 cm caudad and 0.5 cm ventrad were removed, en bloc, from the four turtles used for histologic analysis. Three of these blocks were fixed in citrate-buffered formalin, adjusted to a pH of 7.2, for a minimum of twenty-four hours. These were subsequently embedded in paraffin. The remaining block was used for nerve and fat study, which, for the procedures selected (Winkelmann, 1960 and Luna, 1968, respectively), required frozen tissue sections. The portion used for nerve study was fixed in 10 percent formalin. The remainder, used for examination of lipid content, was used in a fresh condition. Early in the study it was determined that longitudinal sections of the lingual appendage adequately displayed the relationships between the tissue types within it. For this reason, and because of the limited amount of tissue available, the three paraffin embedded appendages were sectioned longitudinally, 8 um thick. One appendage was cut as serial sections producing two-hundred and fifty consecutive tissue slices. The other 2 were cut at various intervals to confirm findings from the serially sectioned appendage. So that all tissue types would be displayed histologically, 6 staining procedures were employed: (1) a modification of the Sevier-Munger silver stain (1965) for nerve tissue and reticular fibers; (2) Delafield's hematoxylin and eosin stain (Luna, 1968) for general morphology; (3) alcian blue stain (Luna, 1968) for acid mucopolysaccaride; (4) Weigert's resorcin-fuchsin stain (Luna, 1968) for elastic fibers; (5) oil red O stain (Luna, 1968) for fat; (6) azocarmine triple stain (Krajian and Gradwohl, 1952) for collagen and muscle. The azocarmine triple stain used was a modification, developed by J. L. Dusi (personal comm.), of the technique previously cited. Several of these stains overlap in that they display structures appearing with other staining procedures. However, this overlap aided significantly in the differentiation of tissue elements when initial results were not clearly indicative.

Most of these stains are fairly routine. However, when nerve tissue was identified within the lingual appendage using the azocarmine triple stain, it became necessary to select a stain that would indicate the presence of non-myelinated nerve processes and non-encapsulated nerve terminals. Winkelmann's simple silver stain (1957) was attempted on frozen sections but failed to produce adequate results. Eventually, the Sevier-Munger silver stain (1965), developed for mammalian tissue, was modified for use on this reptilian tissue. Two modifications of the Sevier-Munger silver stain (1965) enhanced the impregnation of nerve bundles, fine nerve fibers and nerve terminals with silver so that they could be viewed under the light microscope.

First, rather than adding a standard amount of 10 drops of 2 percent formalin to 50 ml of ammoniacal silver solution and mixing by shaking gently (as reported by Sevier and Munger, 1965), the 2 percent solution was added drop by drop while vigorously swirling the mixture to ensure thorough mixing. An end-point was reached when darkening of the solution first could be detected visually. Further, whereas Sevier and Munger (1965) poured the ammoniacal silver-formalin solution over their slides in a Coplin jar, without prior filtration, in this modified procedure the darkened solution was filtered into a Coplin jar through Whatman #2 paper and then the slides were placed immediately into the solution. The second modification concerned the procedure for determining optimal development time in the ammoniacal silver-formalin solution. Test slide determination of development time (as reported by Sevier and Munger, 1965) was adequate for demonstration of nerve bundles and larger nerve fibers. However, thinner fibers and their terminals stained variably. To ensure adequate impregnation of these smaller nerve processes, it was necessary to microscopically observe each slide so that attention could be focused upon the development of individual, fine nerve fibers and nerve terminals.

Without further treatment, the epithelium and connective tissue of these silver stained tissues appeared light yellow and the basement membrane was brown. At this stage, the non-neural tissue components were not stained sufficiently to interpret relations between neural structures and these surrounding tissues. In an attempt to better visualize the non-neural tissue elements, a routine hematoxylin and eosin stain was applied to the previously silver impregnated tissue sections. These sections were immersed in Delafield's hematoxylin solution (Luna, 1968) for eighteen minutes, washed, destained to the desired intensity in acid-alcohol, rinsed in distilled water, blued in weak ammonia solution and rinsed in deionized water. The sections then were placed in eosin solution for 2 minutes, destained in deionized water to the desired intensity, dehydrated, cleared and mounted. Hematoxylin and eosin counterstaining permitted the accurate interpretation of the morphological relations between peripheral nerve bundles, fibers and their terminals, and the surrounding non-neural tissue elements. As far as is known, this is the first time that the application of a routine hematoxylin and eosin counterstain to tissue sections previously impregnated with silver has been reported.

All histologic preparations were examined under the light microscope. Once this was completed, representative slides were selected for photographing. Approximately forty slides were photographed, using a Zeiss Universal Microscope with an Automated Olympus C-35A camera attachment. Black and white photographs were made using Kodak panatomic-X film. Kodak photomicrography color film 2483 was used for color slides, from which color prints were made.

III. RESULTS

Gross Morphology of the Lingual Appendage

The lingual appendage of Macroclemys temmincki is a bicornuate tissue mass projecting from the anterior third of the floor of the oral cavity, just rostral to the glottis. It may be divided, from anterior to posterior, into an anterior horn, a body, and a posterior horn. Certain individuals may not display a readily visible body segment of the lingual appendage. The appendage is white or pale pink in most juveniles and mottled, smoky gray in subadults and adults. Several juveniles observed displayed a more heavily pigmented appendage, similar to that exhibited by older specimens. In all cases the lingual appendage is readily distinguishable from the gray-black floor of the oral cavity. (Fig. 1)

The Hyoid Skeleton

The hyoid skeleton is the primary supportive structure of the lingual appendage and, by its placement immediately opposing or within the body of the appendage, appears to be intimately involved in the positioning and movement of the appendage. The hyoid skeleton is composed of cartilaginous and bony elements, the latter increasing through ontogeny (Schumacher, 1973). Schumacher (1973), in his review of the hyolaryngeal skeleton of turtles, implied that the hyoid skeleton of M. temmincki is composed of twelve articulating

Figure 1. The lingual appendage of _Macroclemys_ _temmincki_, _in_ _vivo_. Abbreviations: a, anterior horn; b, body; p, posterior horn.

elements: a corpus hyoidis, a hypoglossum and a pair of each of the following - cornu hyale, cornu branchiale I, epibranchiale I, cornu branchiale II, epibranchiale II. In the present study, however, only 10 of these elements could be identified. (Fig. 2) The paired epibranchiale I were not observed. The corpus hyoidis has, extending from its rostral border, a pointed processus lingualis and 3 pairs of lateral extensions; the anterior, intermediate and posterior lateral processes. Articulating with the anterior lateral processes are the paired cornu hyale. To the intermediate lateral processes articulate the paired cornu branchiale I. In most turtles the epibranchiale I articulates with the distal end of the cornu branchiale I, although no such articulating element could be discerned in the specimens of M. temmincki examined. Since no articulation facet was observed at the distal end of the cornu branchiale I, no epibranchiale I was designated. Articulating with the posterior lateral processes are the paired cornu branchiale II to which, at their distal ends, articulate the paired epibranchiale II. Lying ventral in position to the corpus hyoidis is the hypoglossum.

The terminology used in the description of the hyoid skeleton, herein, is that used by Schumacher (1973). A list of synonyms is included in his publication.

Musculature Presumably Responsible for the Movements of the Lingual Appendage

A total of 10 paired muscles, presumably responsible for the movements of the lingual appendage, were identified by dissecting several specimens of M. temmincki. Most of these muscles are

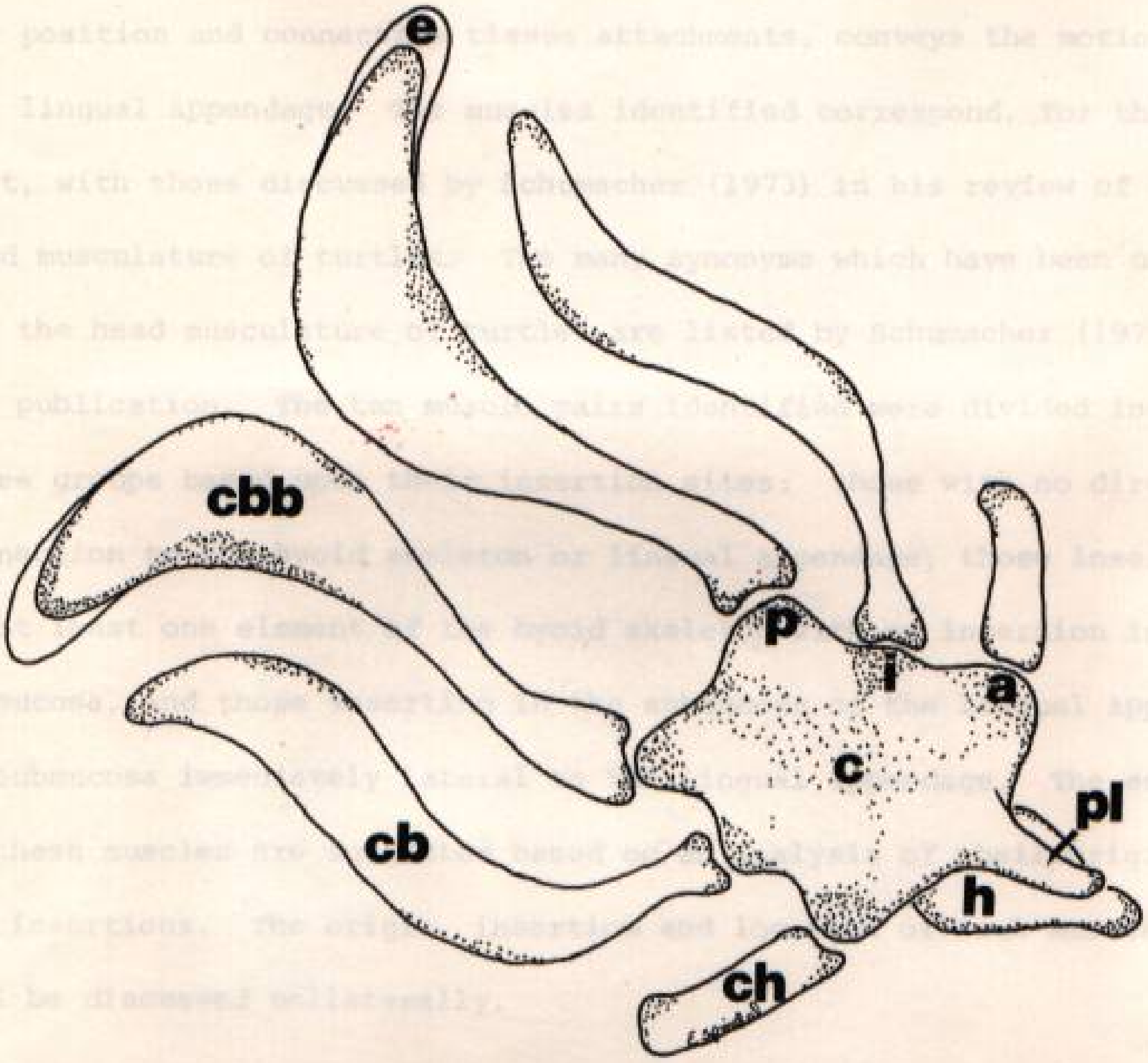

Figure 2. The hyoid skeleton of Macroclemys temmincki. Dorsolateral view. Abbreviations: c, corpus hyoidis; pl, processus lingualis; a, anterior lateral process; i, intermediate lateral process; p, posterior lateral process; ch, cornu hyale; cb, cornu branchiale I; cbb, cornu branchiale II; e, epibranchiale II; h, hypoglossum.

directly associated with the hyoid skeleton. Their contraction presumably results in movement of the hyoid skeleton, which, because of its position and connective tissue attachments, conveys the motion to the lingual appendage. The muscles identified correspond, for the most part, with those discussed by Schumacher (1973) in his review of the head musculature of turtles. The many synonyms which have been used for the head musculature of turtles are listed by Schumacher (1973) in his publication. The ten muscle pairs identified were divided into three groups based upon their insertion sites: those with no direct connection to the hyoid skeleton or lingual appendage, those inserting on at least one element of the hyoid skeleton with no insertion in submucosa, and those inserting in the submucosa of the lingual appendage or submucosa immediately lateral to the lingual appendage. The actions of these muscles are suggested based on an analysis of their origins and insertions. The origin, insertion and location of each muscle pair will be discussed unilaterally.

Musculature with no connection to the hyoid skeleton or lingual appendage

Two muscle pairs are included in this group: the Musculus intermandibularis and the Musculus constrictor colli.

The M. intermandibularis (Fig. 3) is the most superficial muscle on the ventral aspect of the rostral two-thirds of the head. It arises from the ventromedial border of the mandible and joins, by means of a midline connective tissue raphe, its counterpart from the opposite side. The caudal boundary of this muscle serves as an insertion site of the M. constrictor colli. Contraction of the M. intermandibularis

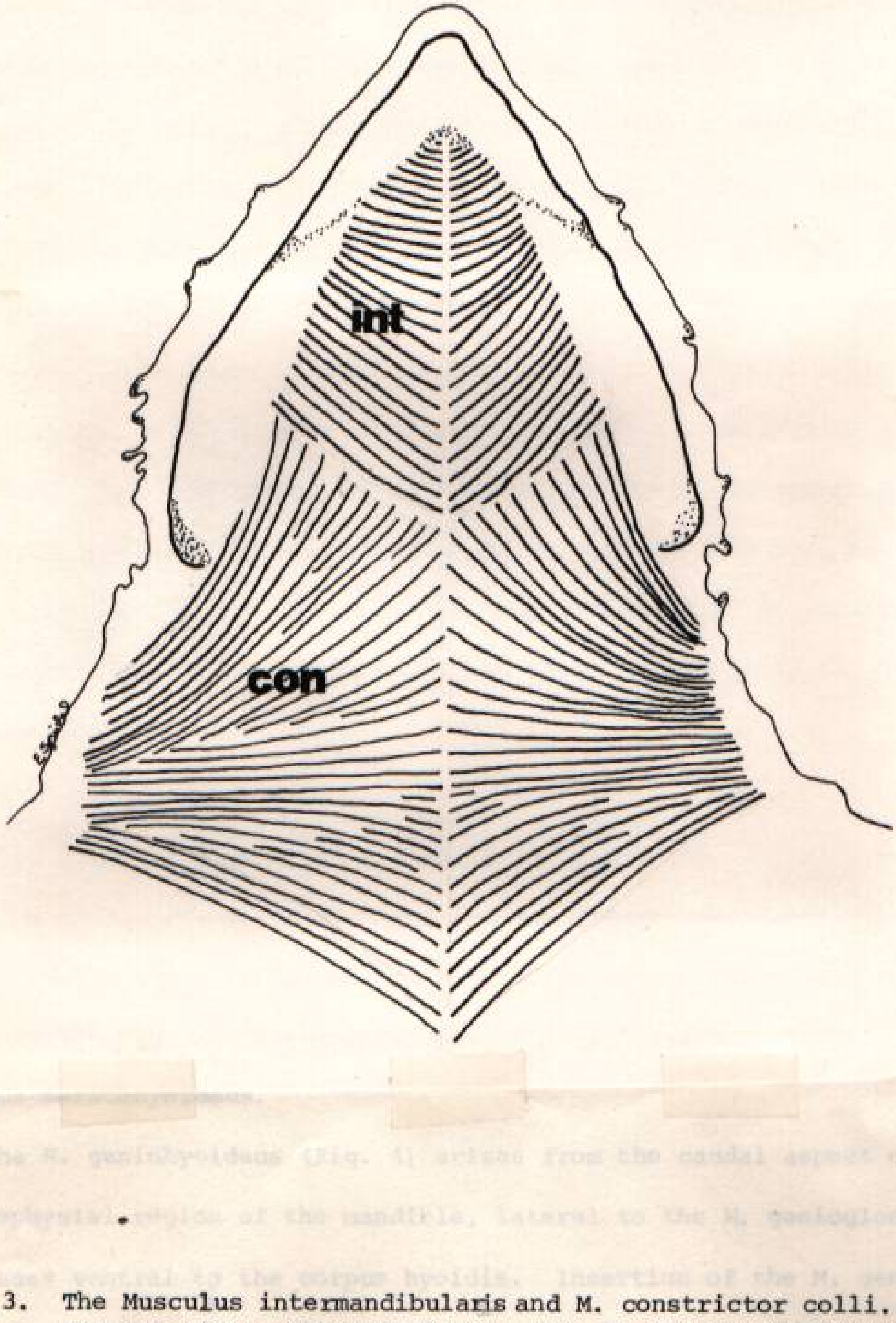

Figure 3. The Musculus intermandibularis and M. constrictor colli. Ventral view. Abbreviations: int, M. intermandibularis; con, M. constrictor colli.

presumably helps support the hyoid skeleton, thus, indirectly supporting the floor of the oral cavity and lingual appendage.

The M. constrictor colli (Fig. 3) is the most superficial muscle of the caudal one-third of the lower jaw and entire neck of M. temmincki. The origin of this muscle is difficult to determine, based on an anatomical analysis, because it is not affixed to any hard tissue structure. On both the dorsal and ventral aspects of the neck the M. constrictor colli joins its contralateral homologue along a midline connective tissue raphe. Additionally, along its rostral border, the M. constrictor colli forms a tendonous aponeurotic intersection with the caudal border of the M. intermandibularis. Thus, it seems that the caudal region of the oral cavity may be supported by this muscle pair. Upon contraction of the M. constrictor colli, the M. intermandibularis would tend to be drawn caudally, thereby indirectly influencing the lingual appendage.

Musculature with insertion on at least one element of the hyoid skeleton with no insertion in the submucosa

Five muscle pairs are included in this group: the Musculus geniohyoideus, the Musculus branchiohyoideus, the Musculus hypoglossohyoideus, the Musculus branchiomandibularis visceralis, and the Musculus coracohyoideus.

The M. geniohyoideus (Fig. 4) arises from the caudal aspect of the symphysial region of the mandible, lateral to the M. genioglossus, and passes ventral to the corpus hyoidis. Insertion of the M. geniohyoideus occurs on the ventromedial aspect of the ipsilateral cornu branchiale I and the ventrolateral aspect of the ipsilateral cornu

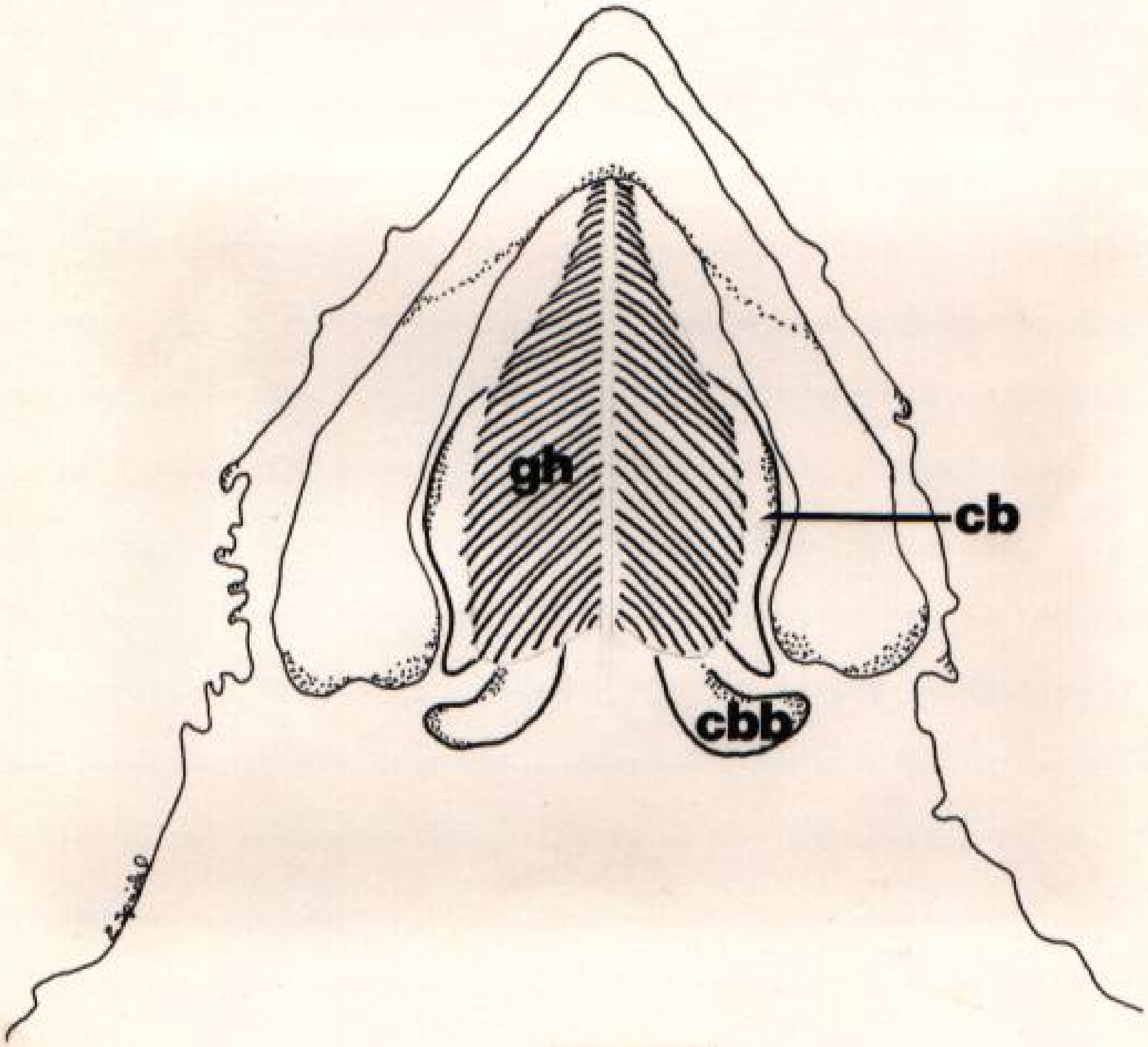

Figure 4. The Musculus geniohyoideus. Ventral view. Abbreviations: gh, M. geniohyoideus; cb, cornu branchiale I; cbb, cornu branchiale II.

branchiale II. This muscle appears to have a major influence on the position of the lingual appendage. Contraction of this muscle pair presumably protracts the hyoid skeleton, causing the lingual appendage lying dorsal to it to protrude within the oral cavity.

The M. branchiohyoideus (Figs. 5 and 6) is one of two intrinsic muscles of the hyoid skeleton, the other being the M. hypoglossohyoideus. The M. branchiohyoideus originates from the proximal end of the rostral border of cornu branchiale I and inserts on the ipsilateral anterior lateral process of the corpus hyoidis; on the proximal, dorsocaudal aspect of the ipsilateral cornu hyale; and on the dorsolateral region of the hypoglossum. Individual contraction of the muscle on either side presumably causes dorsolateral deviation of the anterior components of the hyoid skeleton with a concommitant deviation of the lingual appendage to the same side. Contraction of the muscle pair would appear to result in dorsal flexion of the hypoglossum which, in turn, presumably elevates the processus lingualis lying dorsal to it, bringing the processes lingualis in closer apposition to the body of the lingual appendage.

The M. hypoglossohyoideus (Fig. 7) is a small muscle originating on the hypoglossum. At its origin and throughout its course the muscle fibers of the M. hypoglossohyoideus are mixed with fibers of the M. hypoglossoglossus. The hypoglossohyoideus muscle passes dorsally to insert on the ventrolateral aspect of the processus lingualis. This muscle may produce a lateral deviation and concomitant depression of the processus lingualis or a medial deviation and concomitant elevation of the hypoglossum, depending upon which of the two skeletal structures

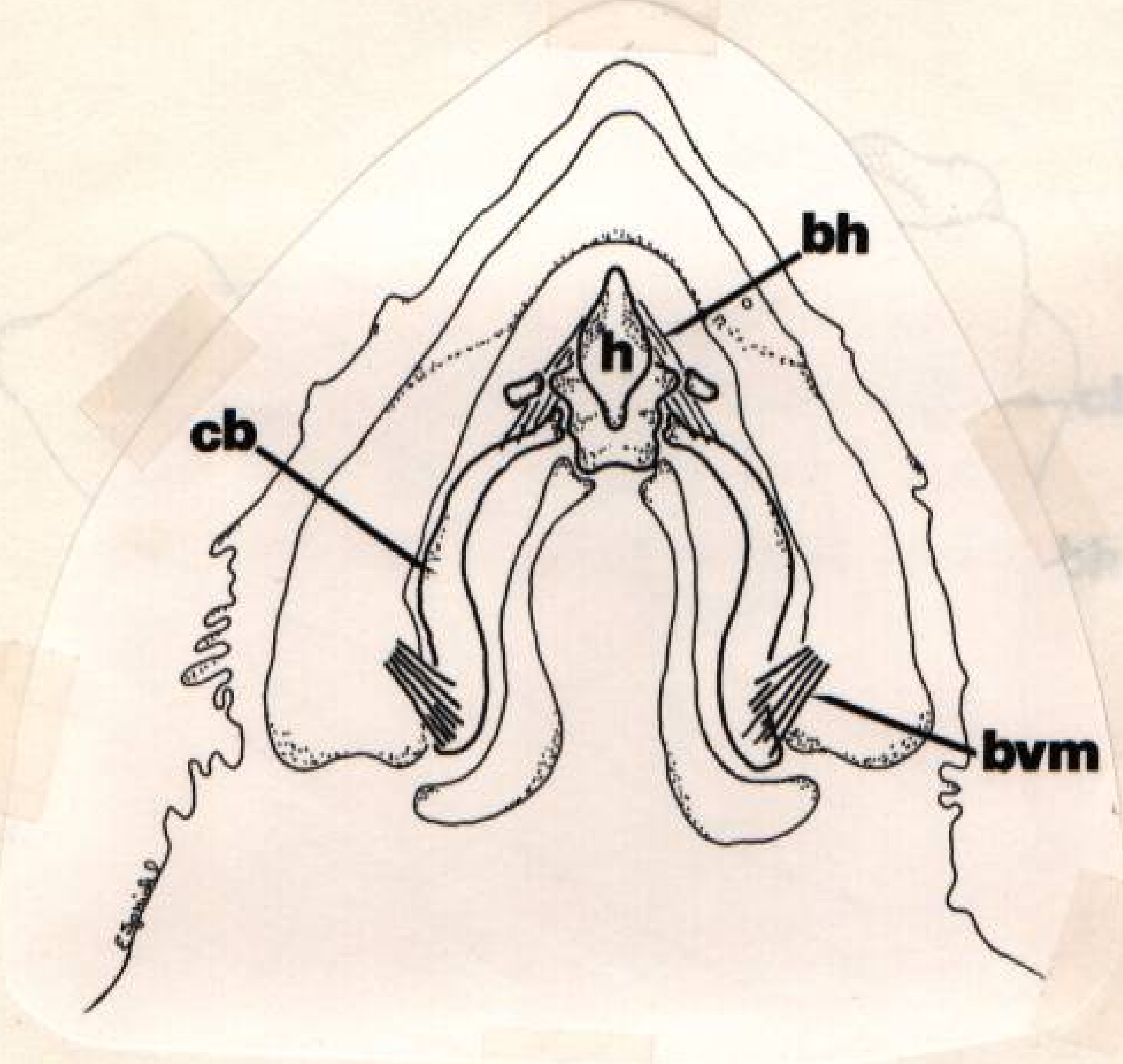

Figure 5. The Musculus branchiohyoideus and M. branchiomandibularis visceralis. Ventral view. Abbreviations: h, hypoglossum; bh, M. branchiohyoideus; bmv, M. branchiomandibularis visceralis; cb, cornu branchiale I.

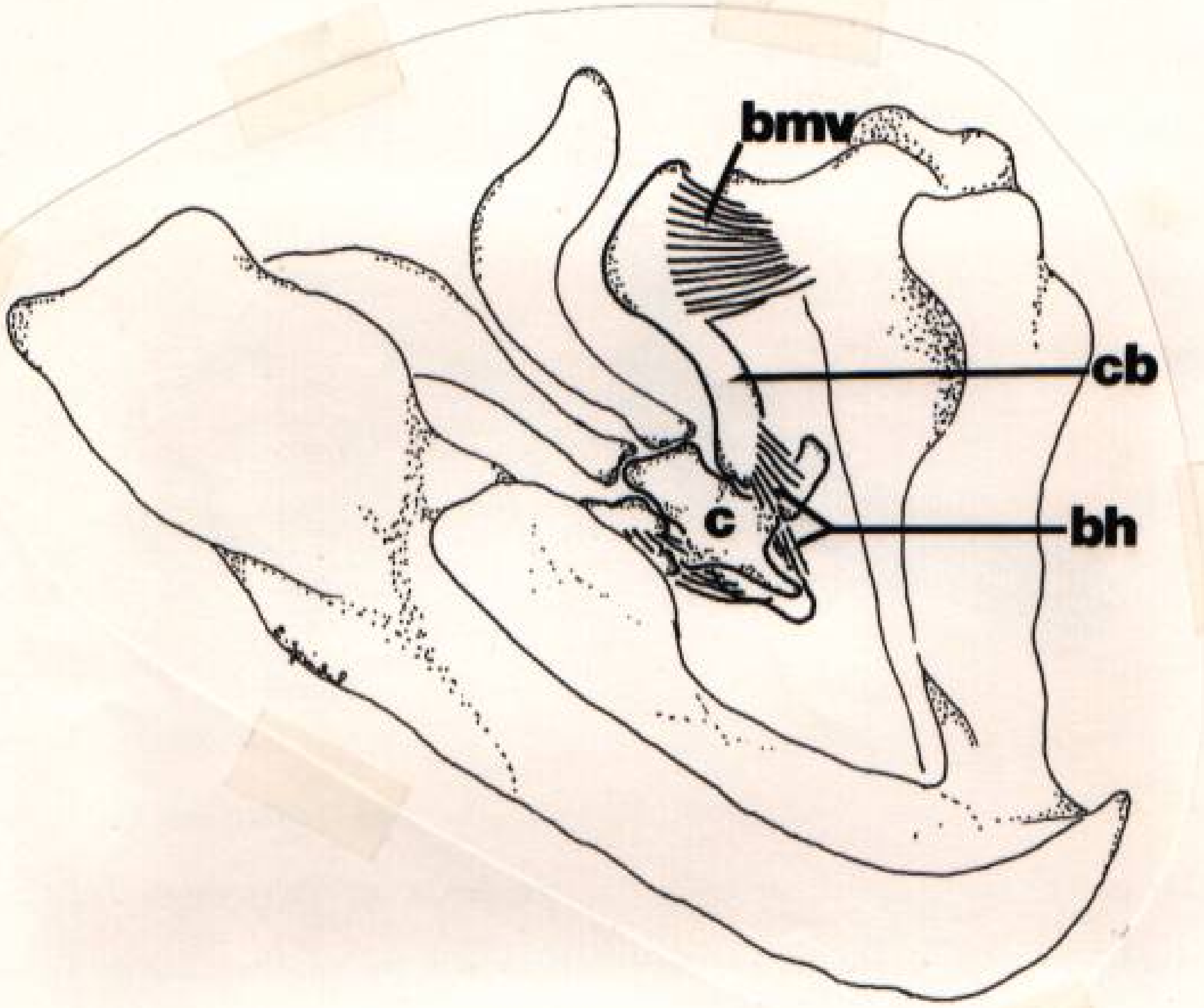

Figure 6. The Musculus branchiohyoideus and M. branchiomandibularis visceralis. Dorsolateral view. Abbreviations: c, corpus hyoidis; bh, M. branchiohyoideus; bmv, M. branchiomandibularis visceralis; cb, cornu branchiale I.

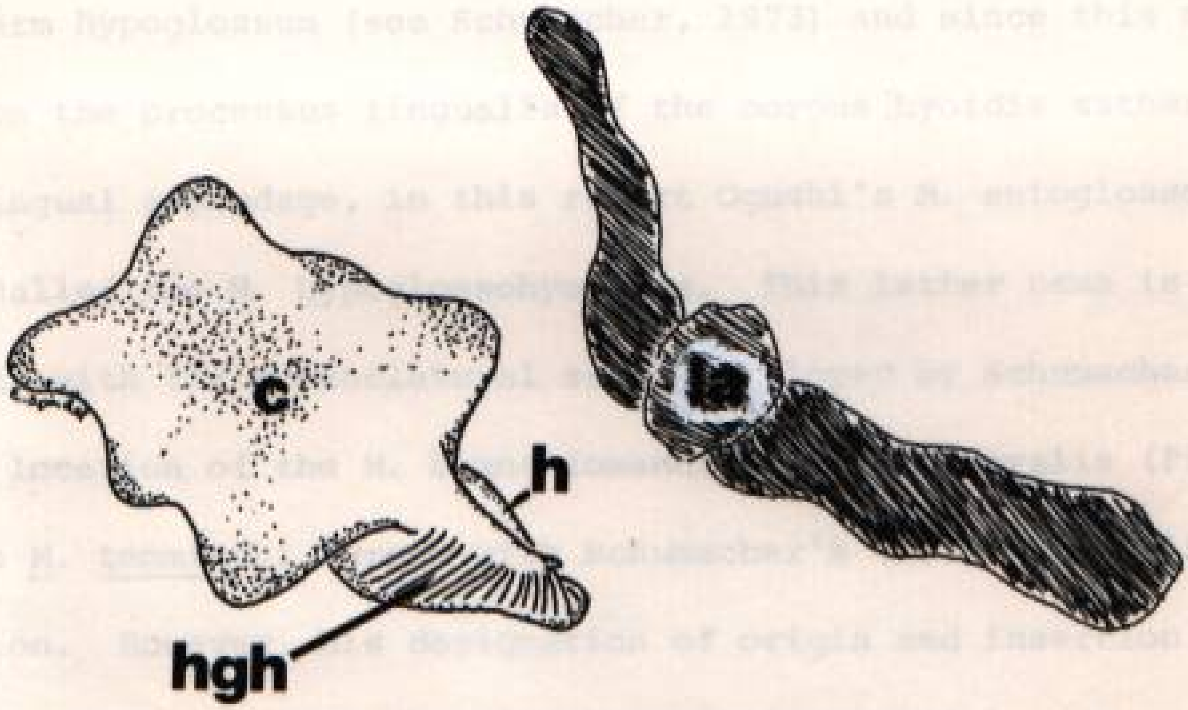

Figure 7. The Musculus hypoglossohyoideus. Dorsolateral view. Abbreviations: c, corpus hyoidis; h, hypoglossum; hgh, M. hypoglossohyoideus; la, lingual appendage.

is more firmly fixed at the time of muscle activation. Contraction of the muscle pair would appear to depress or elevate the aforementioned skeletal elements, again, depending on which was more firmly fixed at the time of muscle activation. This muscle previously has been recognized in Trionyx (see Schumacher, 1973). Ogushi (1913) called it the M. entoglossoglossus. Since the term entoglossum has been replaced by the term hypoglossum (see Schumacher, 1973) and since this muscle inserts on the processus lingualis of the corpus hyoidis rather than in the lingual appendage, in this report Ogushi's M. entoglossoglossus shall be called the M. hypoglossohyoideus. This latter name is more consistent with the nomenclatural scheme employed by Schumacher (1973).

The location of the M. branchiomandibularis visceralis (Figs. 5 and 6) in M. temmincki agrees with Schumacher's (1973) generalized description. However, his designation of origin and insertion does not agree, functionally, with the attachment sites noted in the present study. Schumacher (1973) states, "This muscle originates from the first branchial segment in turtles, runs anteriorly to the capsule of the jaw joint and inserts on the medial side of the angular and subangular". It is difficult to imagine that the contraction of this muscle would result in any substantial movement of the lower jaw when the anchoring skeletal unit is the distal end of the cornu branchiale I. A more appropriate assignment of origin and insertion is just the opposite of that assigned by Schumacher (1973), that is, that the M. branchiomandibularis visceralis originates from the medial aspect of the angular and subangular bones and inserts on the distal end of cornu branchiale I (the first branchial segment). This muscle may be involved in some stabilization function, one which would permit the contraction of other muscles to occur with restricted movement of the cornu branchial I.

The fifth muscle of the group, the M. coracohyoideus (Fig. 8), arises from the rostromedial one-half of the coracoid bone of the pectoral girdle. It courses rostrally along the ipsilateral, ventromedial aspect of the neck and head, and inserts on the caudal border and posterior lateral process of the corpus hyoidis and the proximal end of the cornu branchiale II. Contraction of the paired muscle appears to act as the antagonist of the M. geniohyoideus, presumably by retracting and drawing caudally the hyoid apparatus and connected lingual appendage.

Musculature inserting in the submucosa associated with the lingual appendage

Three muscle pairs are included in this group: the Musculus genioglossus, the Musculus hypoglossoglossus and the Musculus hypoglossus.

The M. genioglossus (Fig. 9) has its primary origin on the caudal aspect of mandible at the symphysis, medial to the origin of the M. geniohyoideus. Additionally, an aponeurotic sheet of connective tissue extends from the rostral one-third of the caudomedial aspect of the mandible to the lateral border of the M. genioglossus. Most fibers of the M. genioglossus course ventrocaudally to insert on the ipsilateral rostral border of the cornu hyale and corpus hyoidis, inclusive of the dorsolateral region of the processus lingualis. The remainder of the fibers run dorsocaudally to insert ipsilaterally in

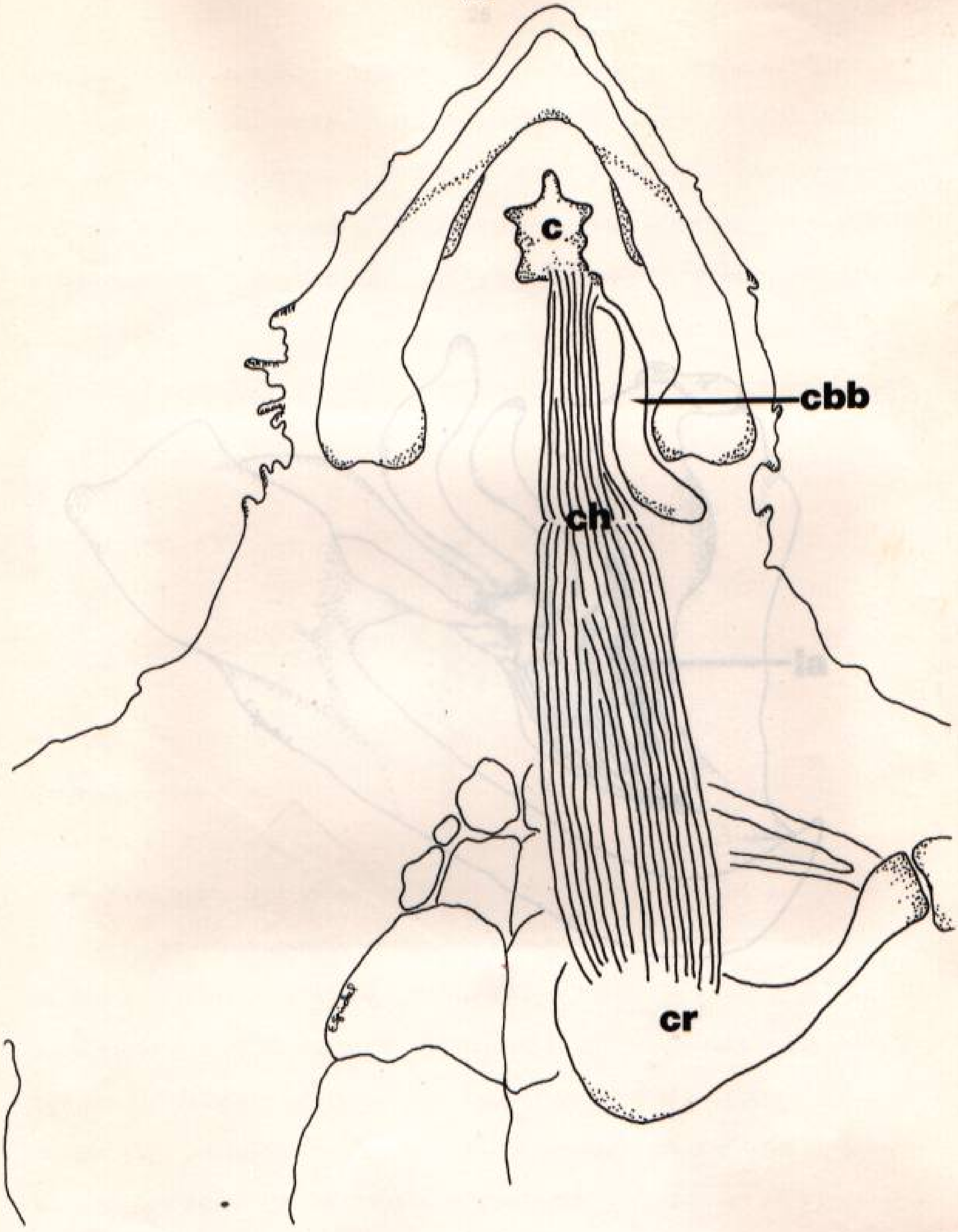

Figure 8. The Musculus coracohyoideus. Ventral view. Abbreviations: c, corpus hyoidis; cbb, cornu branchiale II; ch, coracohyoideus; cr, coracoid.

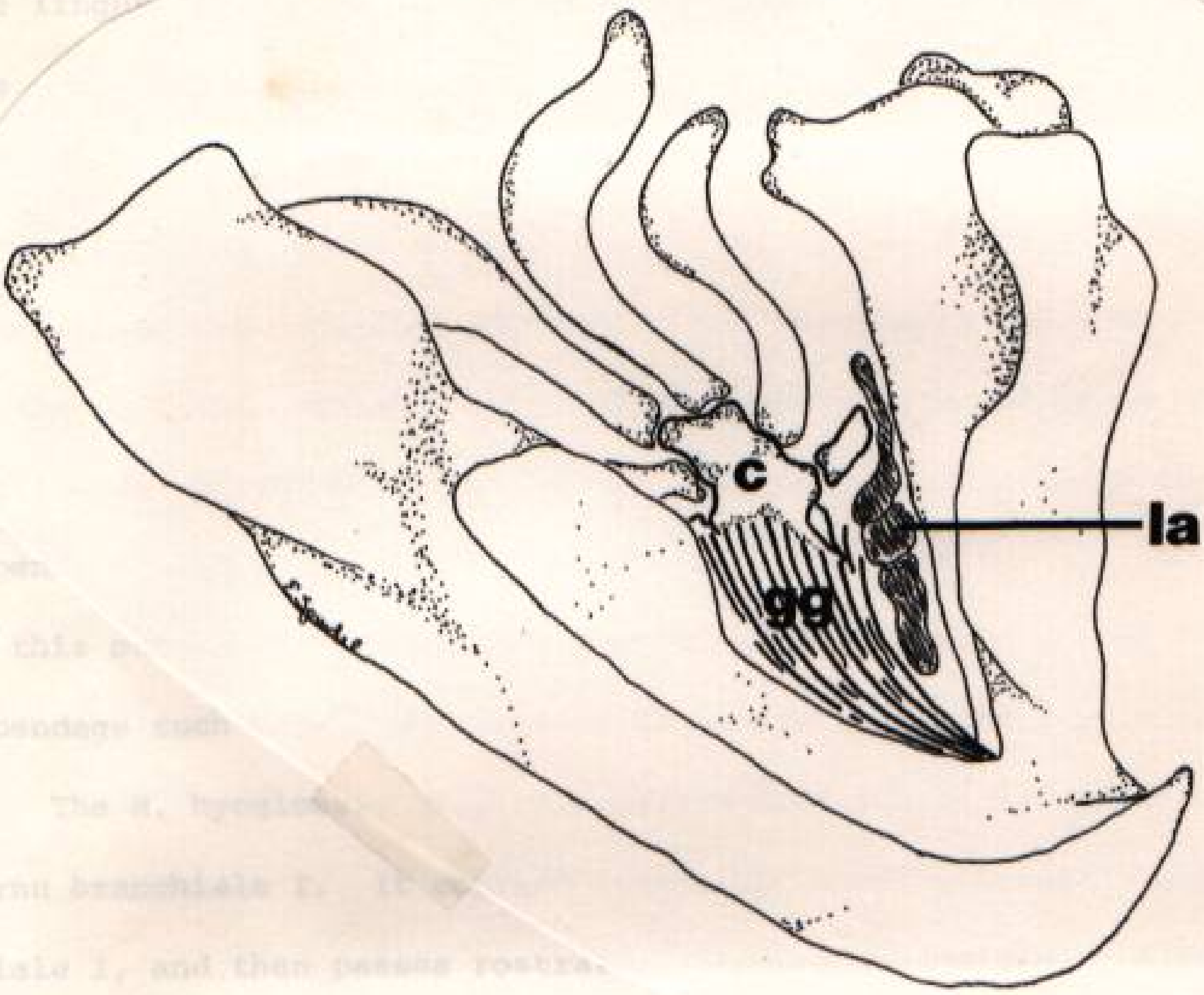

Figure 9. The Musculus geniohyoideus. Dorsolateral view. Abbreviations: gg, M. genioglossus; la, lingual appendage; c, corpus hyoidis.

the submucosa lateral to the body of the lingual appendage. Contraction of the M. genioglossus presumably results in an ipsilateral protraction and lateral deviation of both the anterior hyoid skeleton and the lingual appendage. Contraction of both genioglossus muscles simultaneously would appear to protract the hyoid skeleton and draw the lingual appendage to a point immediately apposing the apex of the processus lingualis.

The M. hypoglossoglossus (Fig. 10) originates on the rostral one-half of the dorsal side of the hypoglossum. It runs dorsally, ensheathing the processus lingualis, and inserts in the submucosa of the body and, variably, the caudal region of the anterior horn of the lingual appendage. This is the only skeletal muscle in the lingual appendage, and is quite limited in that regard. (Fig. 11) Contraction of this muscle pair presumably results in positioning of the lingual appendage such that the processus lingualis is inserted into its body.

The M. hyoglossus (Fig. 12) arises from the medial aspect of the cornu branchiale I. It courses ventrally, running beneath cornu branchiale I, and then passes rostrally, picking up additional fibers originating from the cornu hyale. These fibers continue rostrodorsally, passing dorsal to the processus lingualis, and insert ipsilaterally in the submucosa immediately lateral to the body of the lingual appendage. Contracting individually, each M. hyoglossus may produce ipsilateral lateral deviation of the lingual appendage. Contraction of the muscle pair presumably brings the lingual appendage in closer apposition to the apex of the processus lingualis.

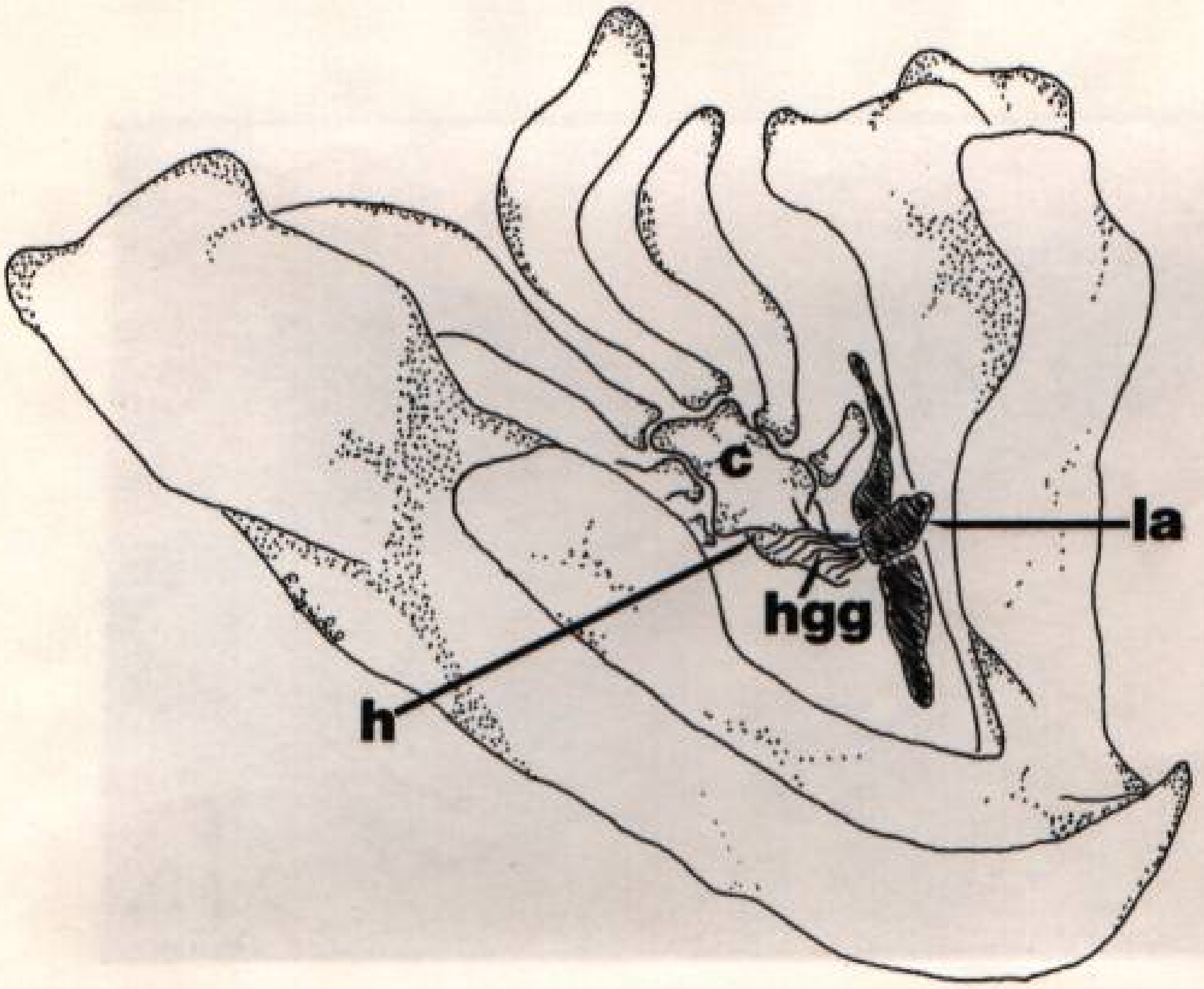

Figure 10. The Musculus hypoglossoglossus. Dorsolateral view. Abbreviations: c, corpus hyoidis; hgg, M. hypoglossoglossus; la, lingual appendage; h, hypoglossum.

Figure 11. Insertion of the Musculus hypoglossoglossus in the submucosa of the lingual appendage. Azocarmine triple stain. 4.5x Abbreviations: hgg, M. hypoglossoglossus; a, anterior horn; b, body.

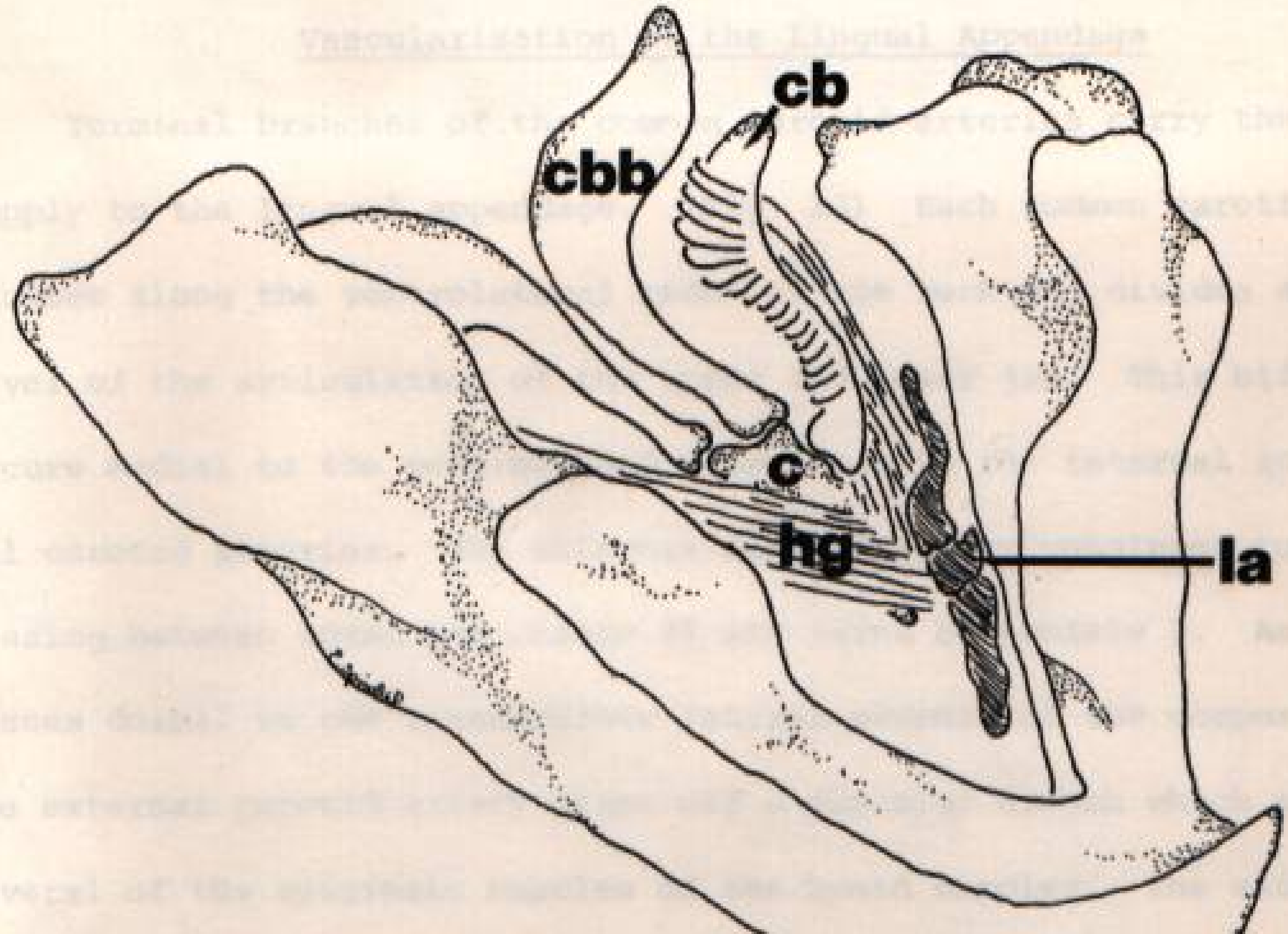

Figure 12. The Musculus hyoglossus. Dorsolateral view. Abbreviations: cbb, cornu branchiale II; cb, cornu branchiale I; c, corpus hyoidis; hg, M. hyoglossus; la, lingual appendage.

The alternate contraction and relaxation of each muscle of these three paired muscles, 180° out of phase with its homologue, may be responsible for the waving motions of the appendage from side to side. This activity also may be the product of muscular activity directly associated with movement of the processus lingualis.

Vascularization of the Lingual Appendage

Terminal branches of the common carotid arteries carry the blood supply to the lingual appendage. (Fig. 13) Each common carotid artery courses along the ventrolateral side of the neck and divides at the level of the articulation of the upper and lower jaw. This bifurcation occurs medial to the mandible and gives rise to the internal and external carotid arteries. The external carotid artery continues rostrally, passing between cornu branchiale II and cornu branchiale I. As it passes dorsal to the intermediate lateral process of the corpus hyoidis, the external carotid artery gives off a muscular branch which enters several of the extrinsic muscles of the hyoid complex. The external carotid artery then continues in a rostromedial path dorsal to the corpus hyoidis. It terminates at the level of the first tracheal ring as a bifurcation into 2 arteries. One artery descends along the trachea and the other courses rostrally, immediately ventral to the mucosa of the oral cavity. The rostrally coursing artery is, herein, called the lingual artery. The lingual artery proceeds rostrally, lateral and ventral to the glottis, giving off muscular branches along its course. Rostral to the glottis each lingual artery curves toward the midline and anastomoses with its homologue of the opposite side.

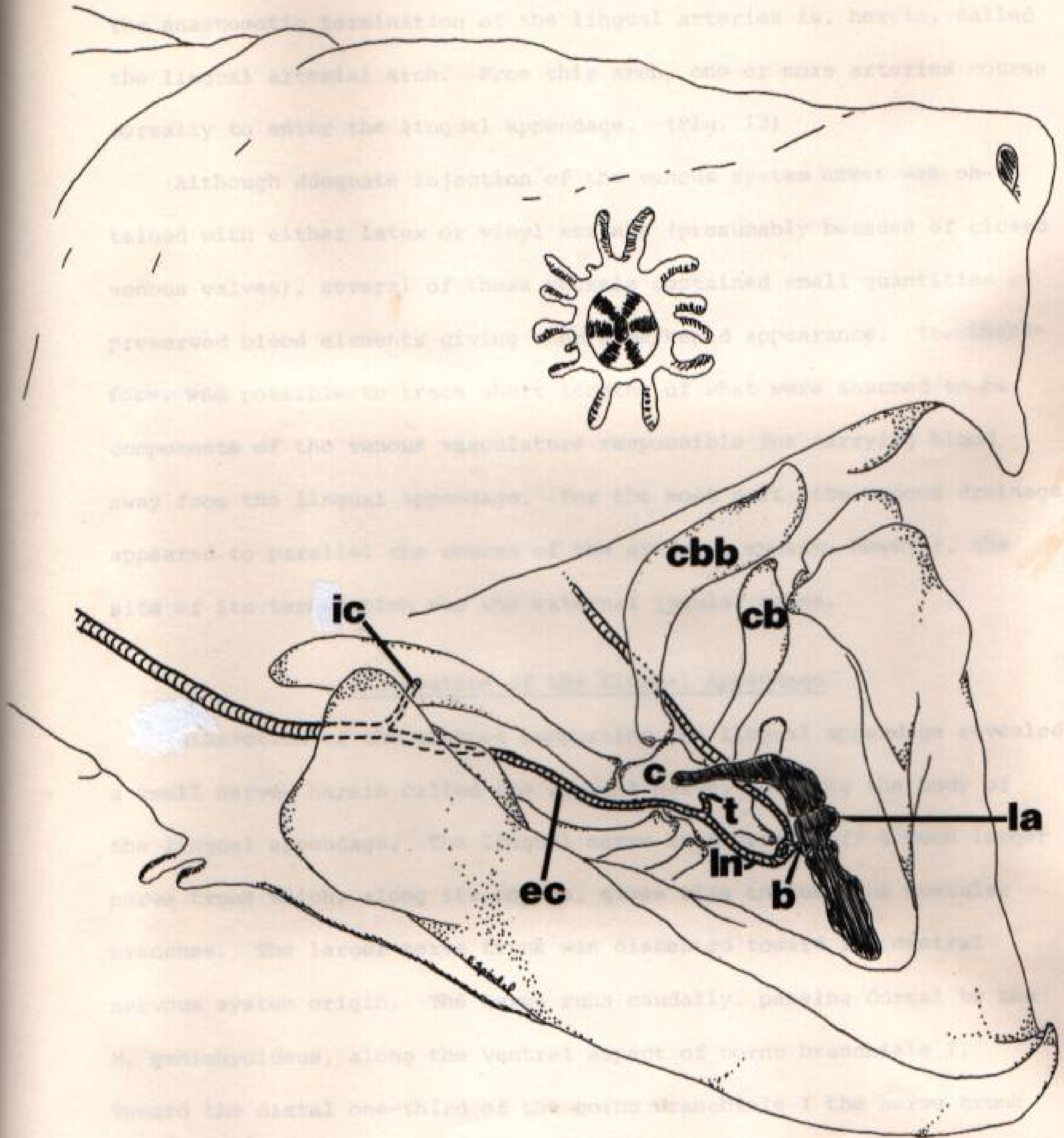

Figure 13. The gross blood supply to the lingual appendage. Dorsolateral view. Abbreviations: ec, external carotid artery; ic, internal carotid artery; t, tracheal artery; ln, lingual artery; b, branch to lingual appendage; cbb, cornu branchiale II; cb, cornu branchiale I; c, corpus hyoidis; la, lingual appendage.

The anastomotic termination of the lingual arteries is, herein, called the lingual arterial arch. From this arch, one or more arteries course dorsally to enter the lingual appendage. (Fig. 13)

Although adequate injection of the venous system never was obtained with either latex or vinyl acetate (presumably because of closed venous valves), several of these vessels contained small quantities of preserved blood elements giving them a darkened appearance. It, therefore, was possible to trace short lengths of what were assumed to be components of the venous vasculature responsible for carrying blood away from the lingual appendage. For the most part, the venous drainage appeared to parallel the course of the arterial system, however, the site of its termination was the external jugular veins.

Innervation of the Lingual Appendage

Dissection of the tissues supporting the lingual appendage revealed a small nerve, herein called the lingual nerve, entering the body of the lingual appendage. The lingual nerve is a branch off a much larger nerve trunk which, along its course, gives rise to numerous muscular branches. The larger nerve trunk was dissected toward its central nervous system origin. The nerve runs caudally, passing dorsal to the M. geniohyoideus, along the ventral aspect of cornu branchiale I. Toward the distal one-third of the cornu branchiale I the nerve trunk turns dorsally, passing for a short distance medial to the external carotid artery. The nerve eventually approaches the external, caudal region of the cranial vault, where it is joined by cranial nerve X and several other unidentified nerves. These all appear to enter the skull through a group of foramina within approximately 1 mm of each other. Removal of a section of the cranial vault, exposing the brain, revealed a complex origin of this nerve trunk from the middle and caudal regions of the medulla. Based on drawings and descriptions of cranial nerve origins (Thompson, 1932; Noble and Noble, 1940; Cruce and Nieuwenhuys, 1974; Northcutt, 1979) it appears that this nerve trunk is composed of cranial nerves IX, X, XI and XII. The actual components extending through the length of this larger nerve trunk and continuing into the lingual nerve were not determined. Further, whether the lingual nerve contained sensory fibers, motor fibers or both could not be determined by the techniques used in the present study.

Histologic Composition of the Lingual Appendage

The lingual appendage of M. temmincki is composed of 2 main layers: the tunica mucosa and the tunica submucosa. The tunica mucosa is further divided into the lamina epithelialis, basement membrane and lamina propria. There is no lamina muscularis in the tunica mucosa lining the oral cavity of the alligator snapper; thus, the only skeletal muscle present within the lingual appendage is that of the M. hypoglossoglossus, as stated previously. (Fig. 11)

The lamina epithelialis is composed of a non-keratinized, stratified squamous epithelium (Fig. 14) with possible variations. Several sections displayed epithelial types resembling pseudostratified and possibly stratified columnar epithelium at various locations on the lingual appendage. Within the epithelium are found numerous

Figure 14. The epithelium of the lingual appendage. Silver, hematoxylin and eosin stain. 440x Abbreviations: ps, pseudostratified squamous epithelium; g, goblet cell; bm, basement membrane; lp, lamina propria.

goblet cells. The presence of goblet cells in the turtle tongue was first noted by Andrews (1959). Carmignani and Zaccone (1975) and Nalavalde and Varute (1976) performed histochemical analyses on the contents of goblet cells in the turtles *Geomyda trijuga* and *Pseudemys scripta*, respectively. They found that the goblet cells in these 2 aquatic turtles contained 3 different mucopolysaccarides. Application of an alcian blue stain to tissue sections cut from the lingual appendage revealed the presence of acid mucopolysaccaride within the goblet cells. (Fig. 15) The specific mucopolysaccaride(s) was not determined by this technique. Additionally, the alcian blue stain revealed a layer of mucopolysaccaride overlying the outermost cell layer of the lamina epithelialis. Melanocytes also are present in the lamina epithelialis (Fig. 16) and are partially responsible for the pigmented appearance of the lingual appendage. The distribution of melanocytes is such that certain areas display high concentrations while other areas remain completely devoid of their presence. Few melanocytes were seen in the lingual appendage of juvenile specimens examined. In contrast, a very high density of melanocytes was noted in adult specimens. However, several juvenile specimens displayed substantial pigmentation of the lingual appendage upon gross examination, deviating from the typical amelanistic lingual appendage commonly observed in this age group. Taste buds, another feature of the lamina epithelialis, will be discussed in context with the nerve supply to the appendage.

Melanocytes also were found in the lamina propria and tunica submucosa. (Fig. 16) The greatest density of melanin, when present, was observed in the lamina propria, immediately ventral to the basement

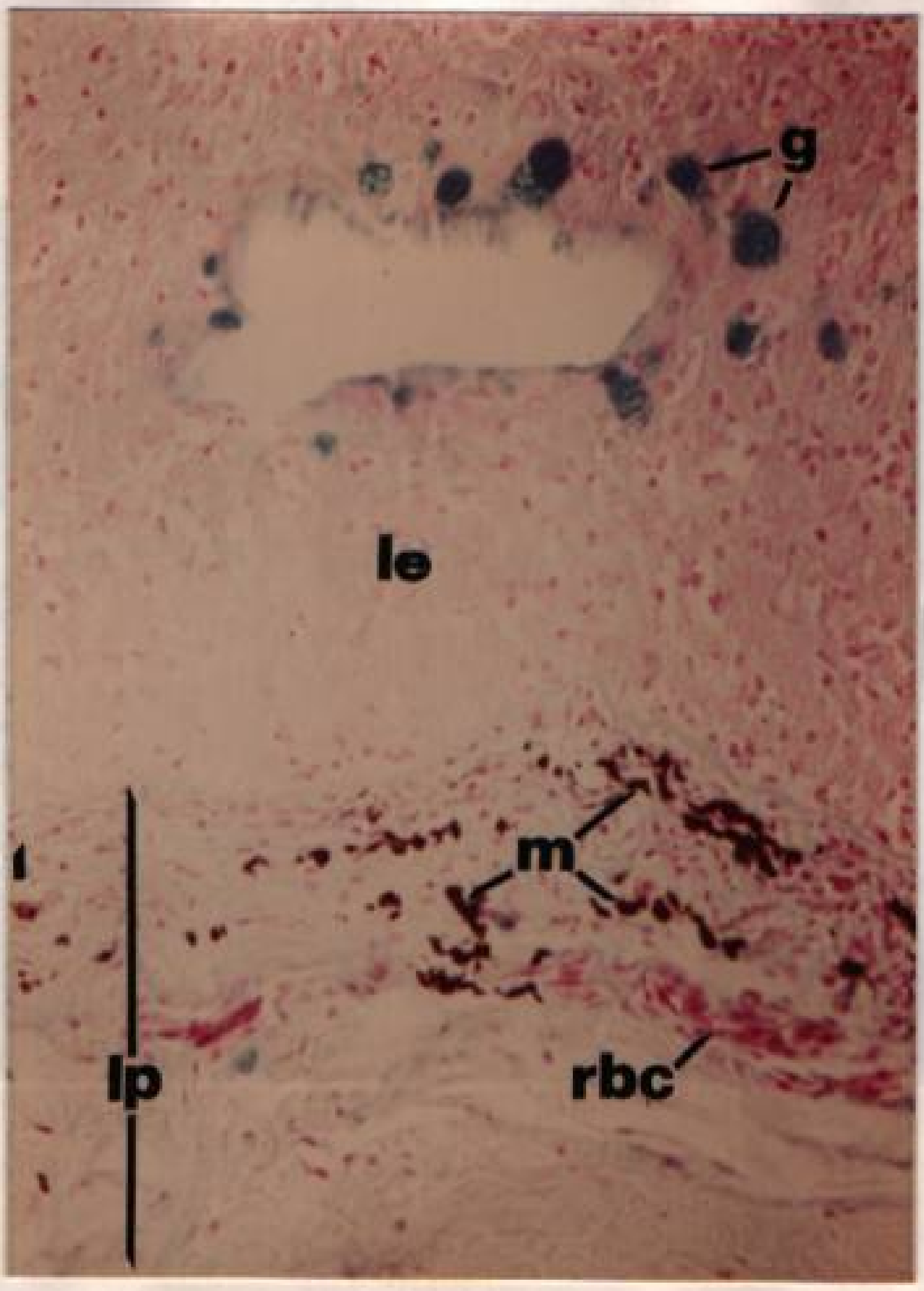

Figure 15. Goblet cells containing acid mucopolysaccaride. Alcian blue stain. 310x Abbreviations: g, goblet cell; le, lamina epithelialis; lp, lamina propria; rbc, red blood cell; m, melanin.

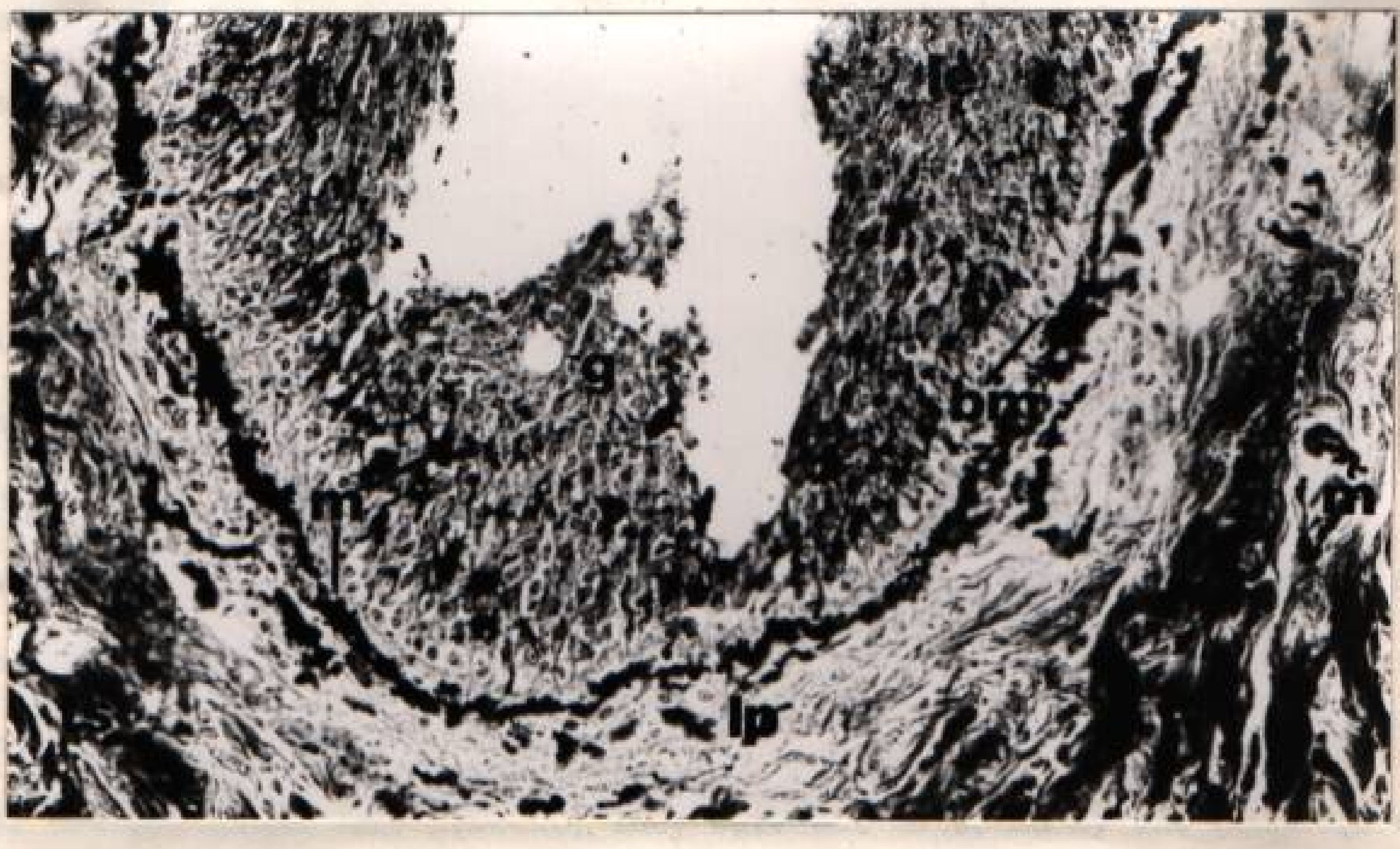

Figure 16. Melanin distribution in the layers of the lingual appendage. Silver, hematoxylin and eosin stain. 340x Abbreviations: le, lamina epithelialis; m, melanin; g, goblet cell; bm, basement membrane; lp, lamina propria; ts, tunica submucosa.

membrane. Located throughout the lamina propria and submucosa, between the predominant connective tissue bundles, are large venous sinuses composing greater than one-fifth of the total volume of the lingual appendage. Several arterioles, presumably branches of the lingual artery, also were observed in the lamina propria and submucosa. The connective tissue of the submucosa contains a limited number of elastic fibers. Relatively few submucosal elastic fibers are present in the lingual appendage when compared to the number present in the submucosa of adjacent areas. No fat cells were observed in the appendage of any specimen.

The lingual nerve, traced by gross dissection into the body of the lingual appendage, was observed histologically dividing into 3 smaller nerves. Two of these nerves enter the anterior horn and 1 enters the posterior horn. The largest nerve observed in the lingual appendage was approximately one hundred um in diameter. (Fig. 17) The diameters of individual axons comprising the larger nerves ranged from approximately 5 um down to the limit of light microscopic resolution, 0.2 um. If smaller axons were present, they were not detected by the techniques used in this study. As these larger nerves coursed through the horns of the lingual appendage (Fig. 18), they ramified.

Nerve terminals were observed in both the lamina epithelialis and the lamina propria. Those in the former layer display bulbous endings ranging in magnitude from 2.8 um by 4.2 um to 3.0 um by 3.2 um. (Figs. 19, and 20) Terminals within the lamina propria display a great deal more variation in shape, although their sizes fall into the same range as those of the lamina epithelialis. Glomerular bulbs were

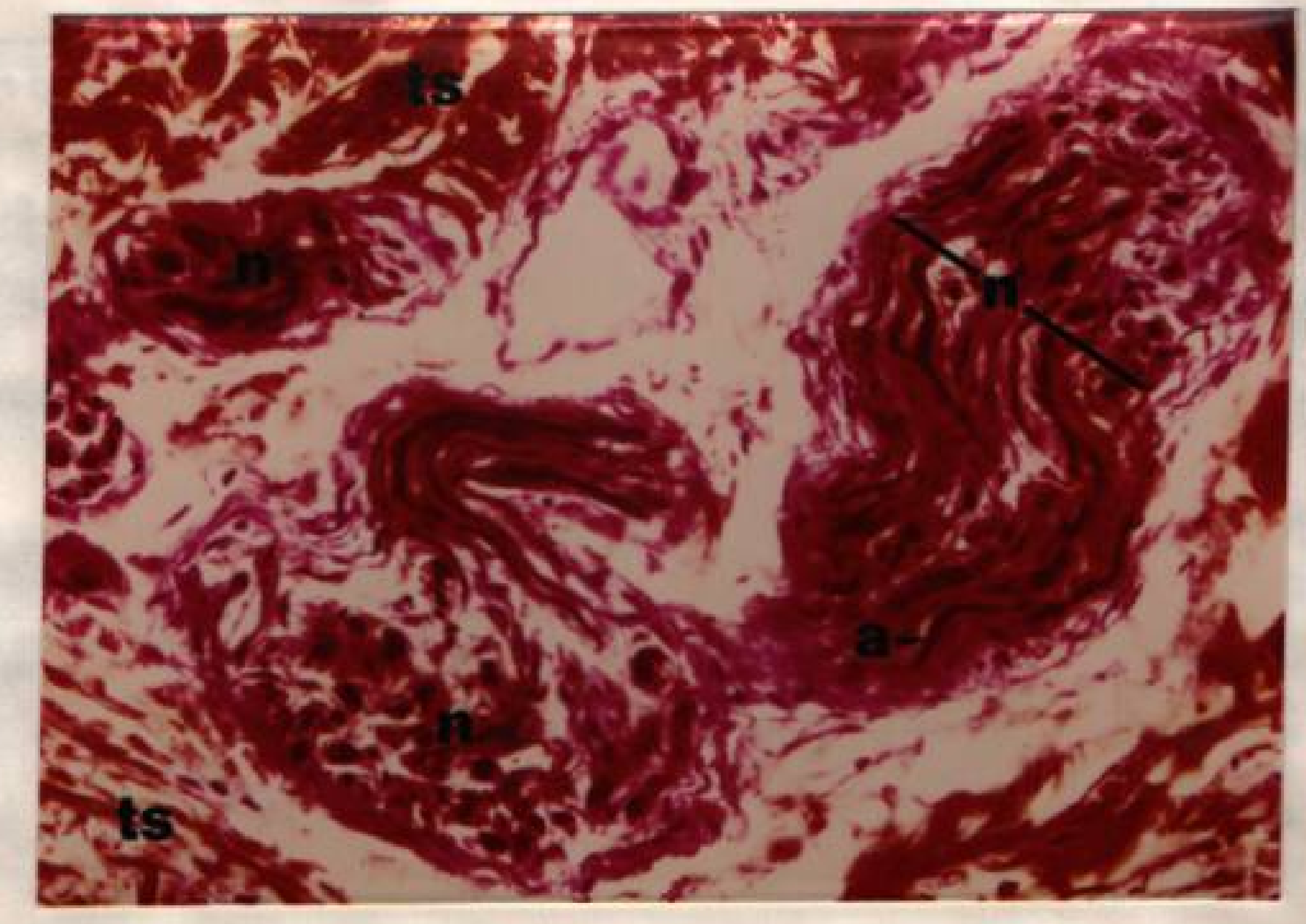

Figure 17. Large nerve of the lingual appendage. Silver, hematoxylin and eosin stain. 400x Abbreviations: ts, tunica submucosa; a, axon; n, nerve.

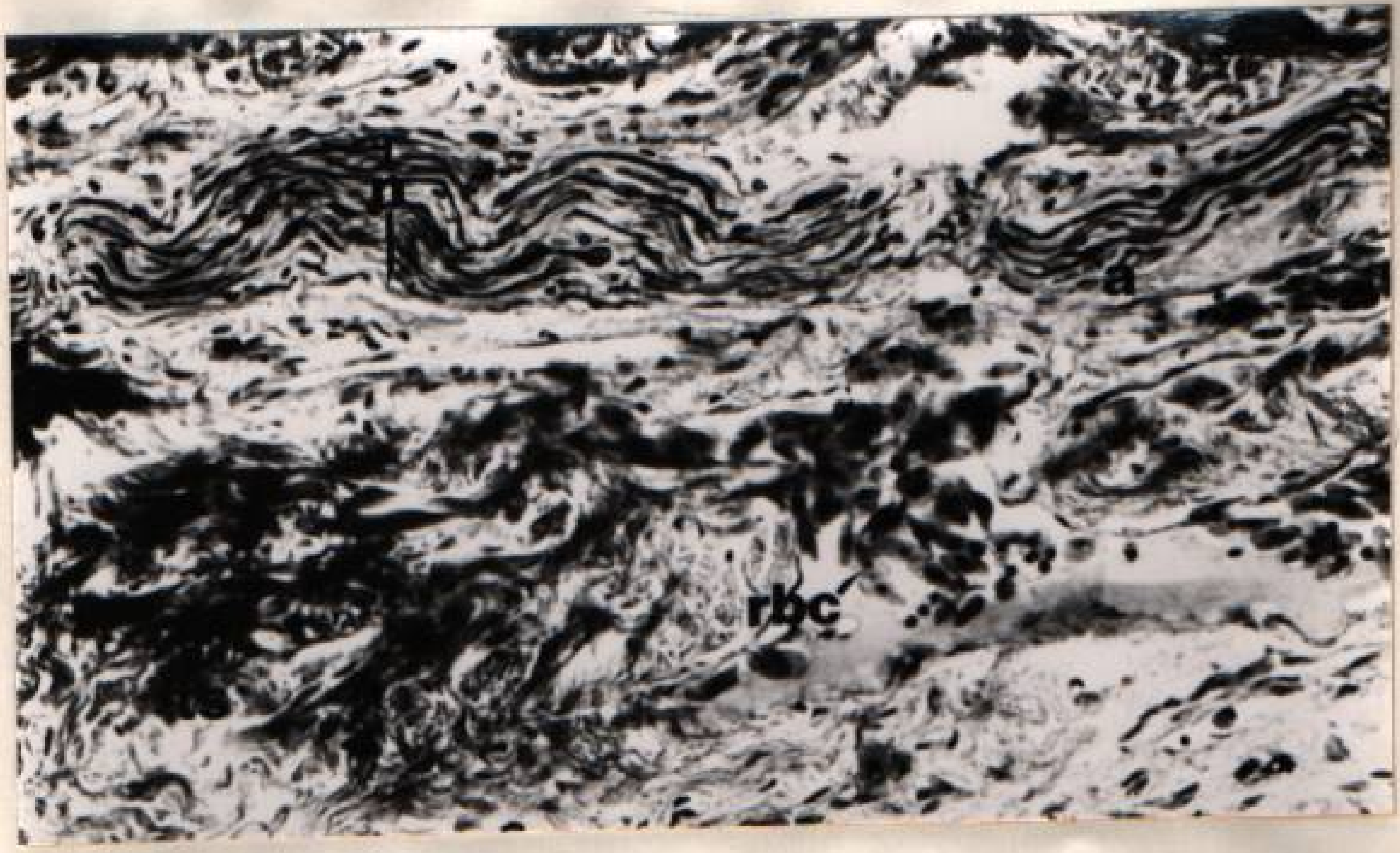

Figure 18. Nerve bundle coursing through the tunica submucosa. Silver, hematoxylin and eosin stain. 340x Abbreviations: n, nerve; a, axon; ts, tunica submucosa; rbc, red blood cell.

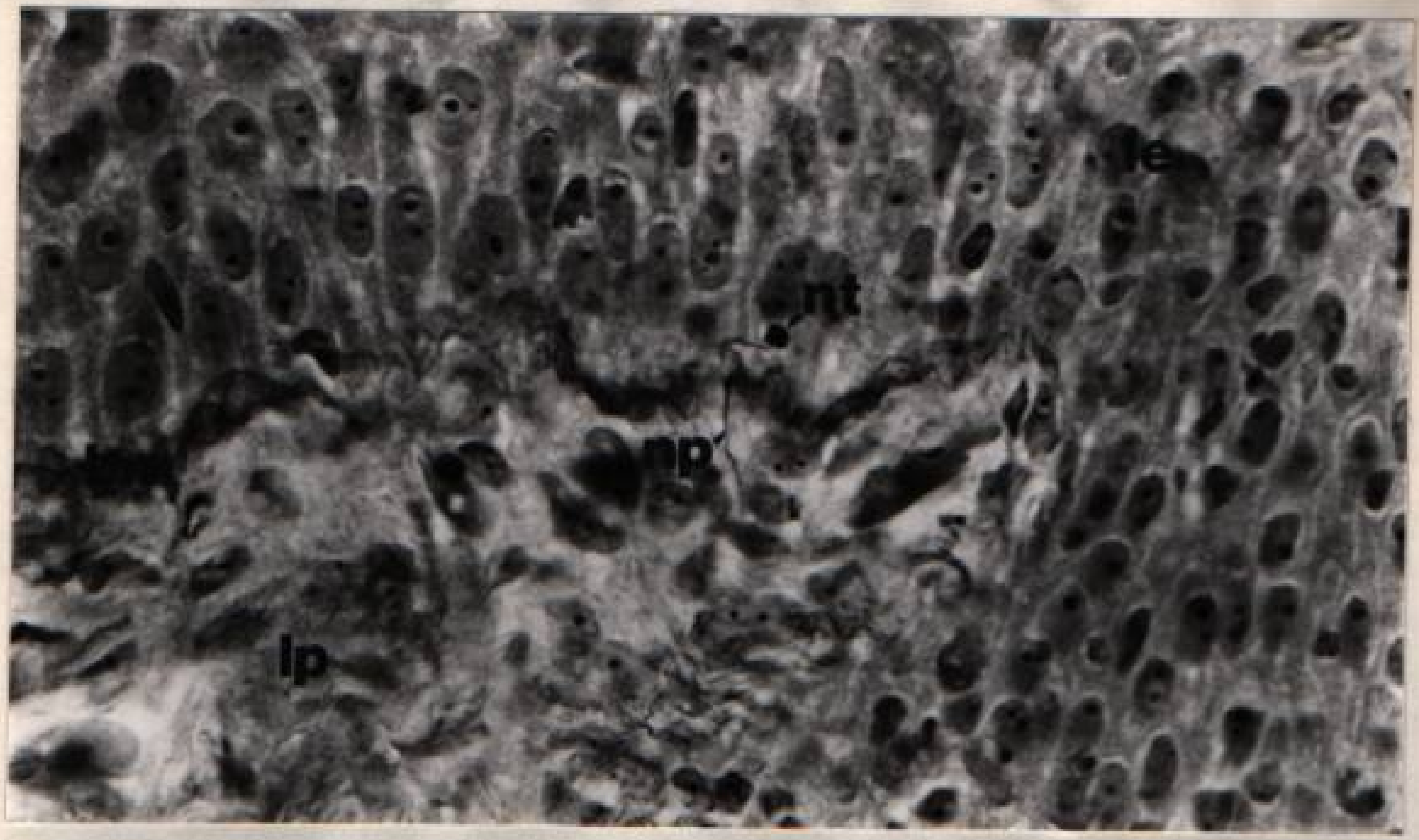

Figure 19. Epithelial nerve terminal. Silver, hematoxylin and eosin stain. 740x Abbreviations: le, lamina epithelialis; np, nerve process; nt, nerve terminal; bm, basement membrane; lp, lamina propria.

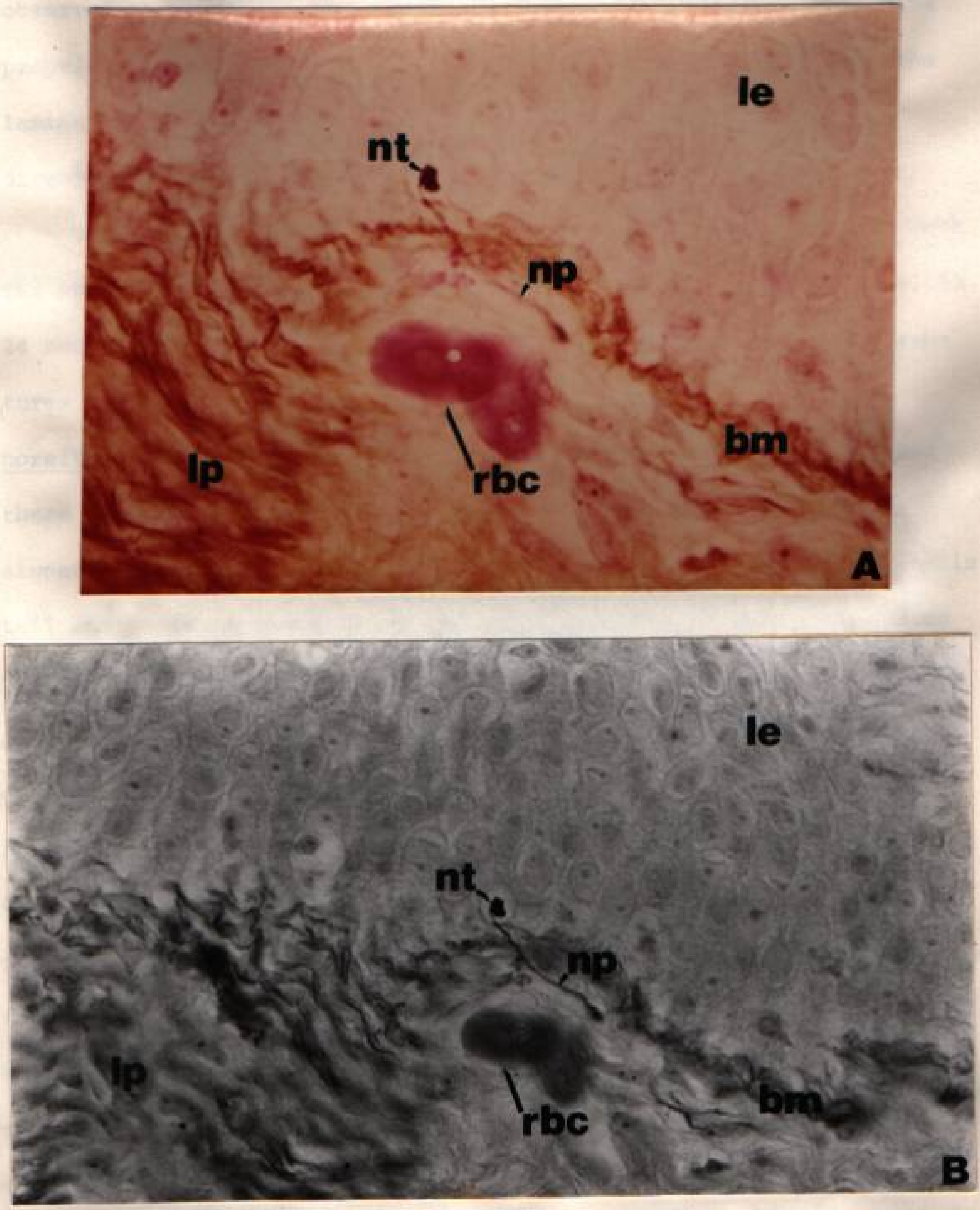

Figure 20. Epithelial nerve terminal. Silver, eosin stain. A-820x; B-780x Abbreviations: le, lamina epithelialis; np, nerve process; nt, nerve terminal; rbc, red blood cell; bm, basement membrane; lp, lamina propria.

observed on several nerve fibers as they coursed through the lamina propria. (Figs. 21 and 22) Other neuronal enlargements within the lamina propria appeared mace-like, with spikes radiating in all directions. (Fig. 22)

Taste buds are distributed over the distal three-fourths of both the anterior and posterior horns of the lingual appendage. (Figs. 23, 24 and 25) Pevzner and Tikhonova (1978) reported on the fine structures of these morphological features in two turtles, Testudo horsfieldi and Emys orbicularis. Based on their histologic studies these presumed sensory receptors observed in M. temmincki were designated as taste buds. These epithelial structures are several cells tall and ovoid in shape. A slight depression is present surrounding the cluster of outermost cells, resulting in the formation of a small papilla on the surface of the lingual appendage. Argyrophilic granules were observed throughout the taste bud. (Figs. 24 and 25) Similar granules were described by Pevzner and Tikhonova (1978) within the supporting cells of the taste bud. However, distribution of the granules seen in M. temmincki taste buds did not appear to be restricted to any particular group of cells, and, further, appeared to be located both intra- and intercellularly. Nerve fibers, presumed to convey information from the taste bud to the central nervous system, were observed in the lamina propria immediately ventral to the epidermal receptor. (Figs. 24 and 25) However, these fibers never were observed penetrating the basement membrane of the taste bud. The apparent absence of direct innervation of the epidermal "receptor", and the particular distribution of granules within the "receptor" suggests that the designation of these structures as "taste buds" might be questionable.

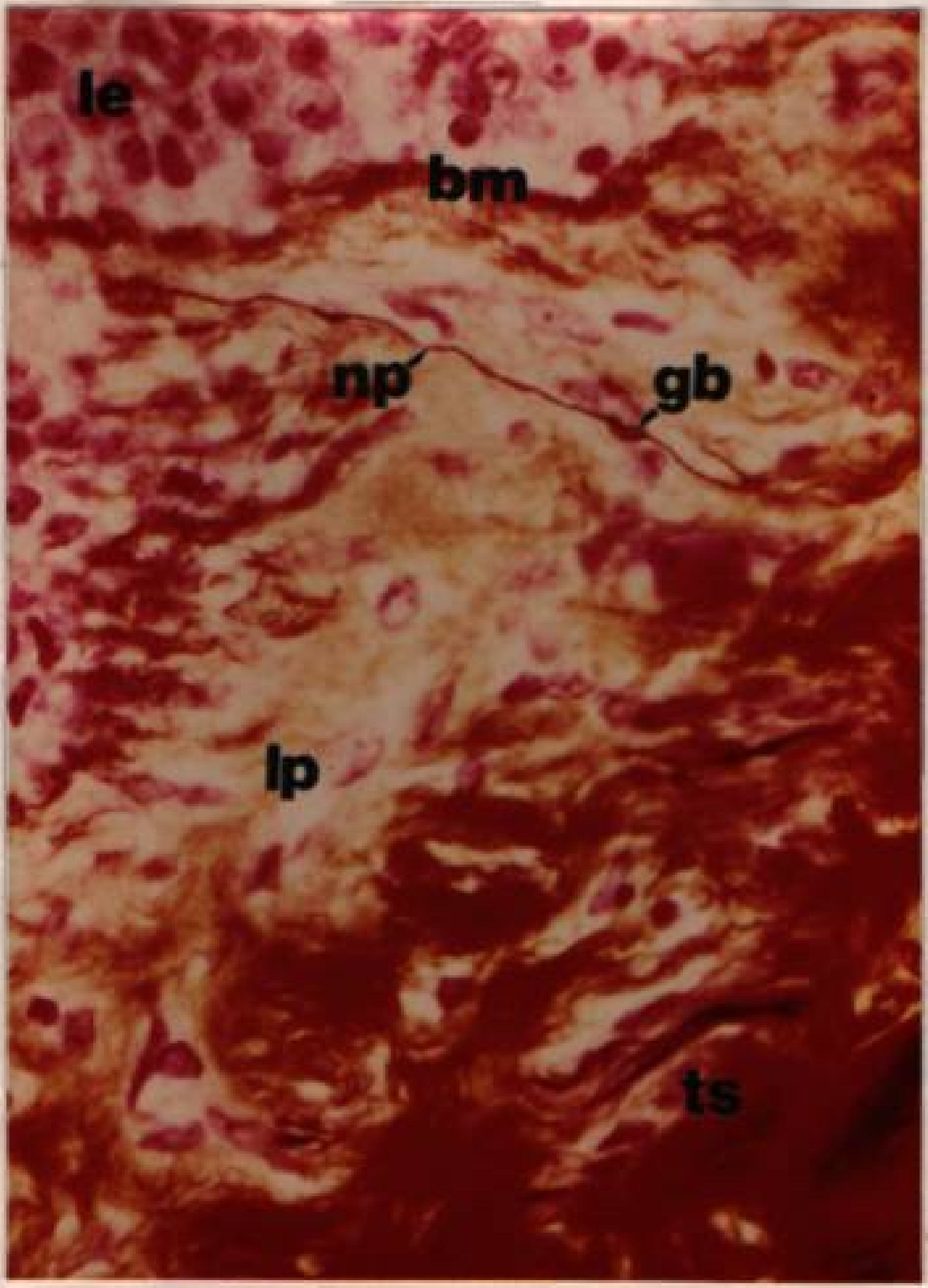

Figure 21. Glomerular bulb along a nerve process in the lamina propria. Silver, hematoxylin and eosin stain. 720x Abbreviations: gb, glomerular bulb; np, nerve process; le, lamina epithelialis; bm, basement membrane; lp, lamina propria; ts, tunica submucosa.

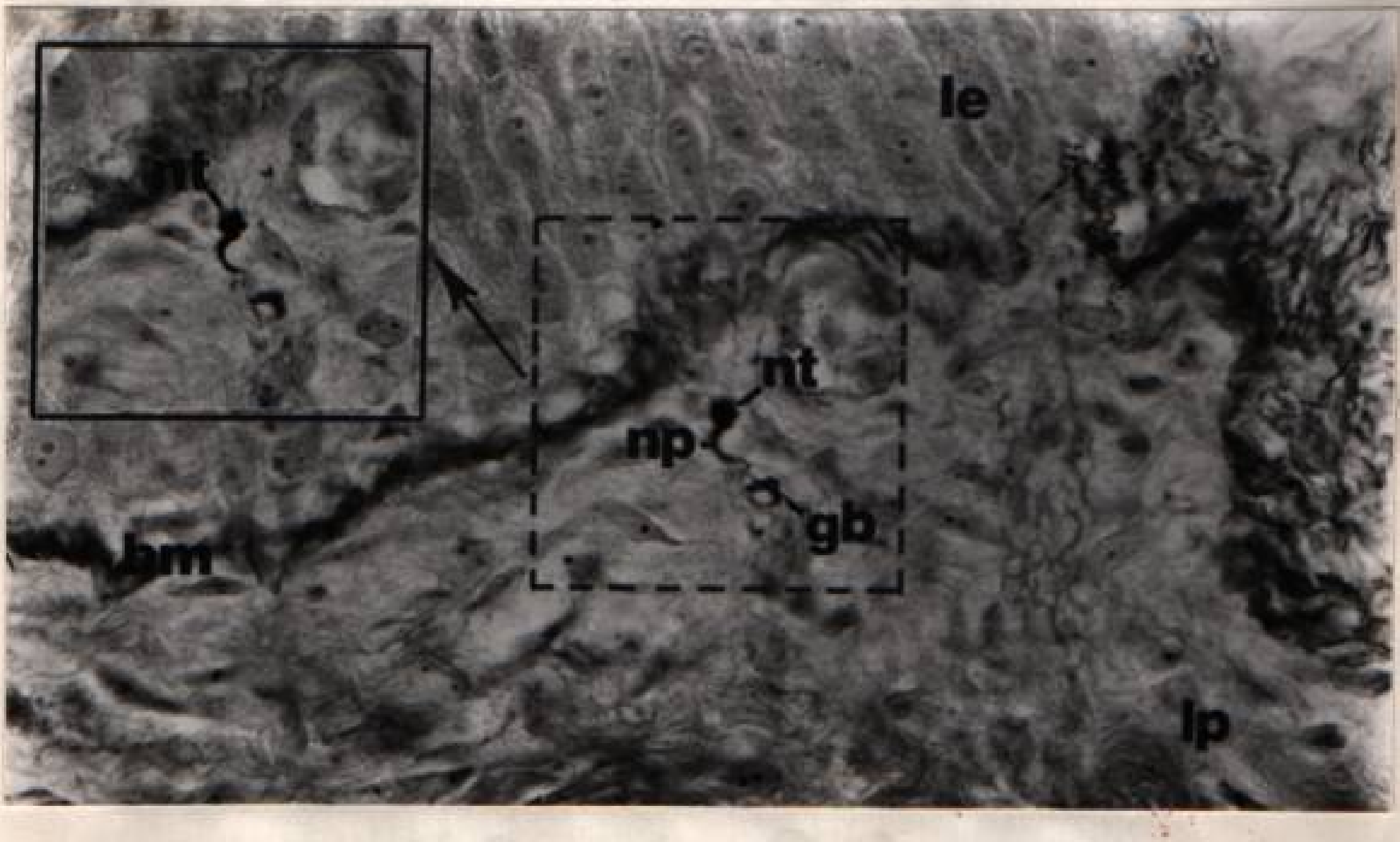

Figure 22. Glomerular bulb along a nerve process leading to a terminal in the lamina propria. Silver, hematoxylin and eosin stain. 780x Abbreviations; gb, glomerular bulb; np, nerve process; nt, nerve terminal; le, lamina epithelialis; bm, basement membrane; lp, lamina propria. Inset: nerve terminal photographed at different focal plane.

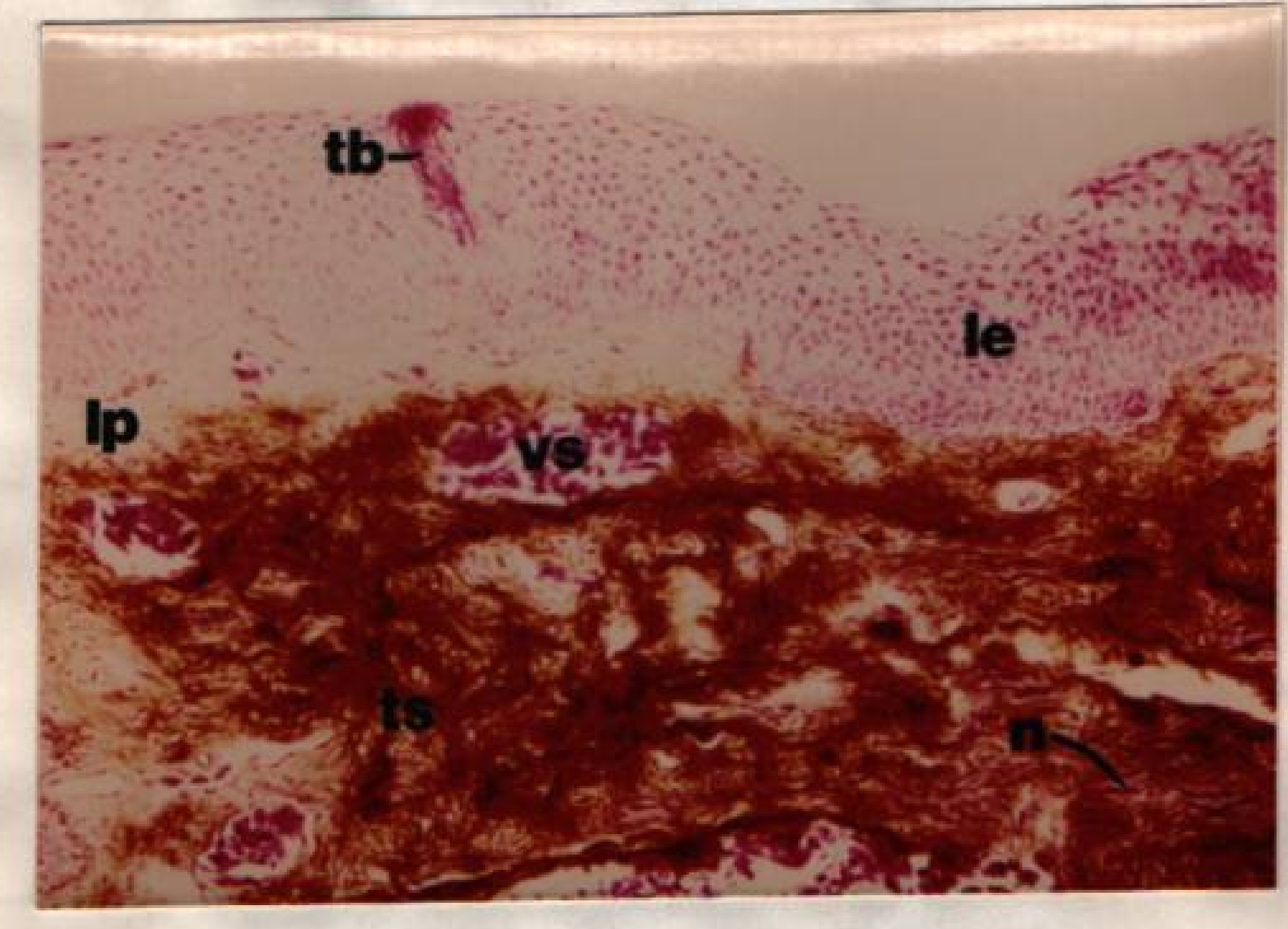

Figure 23. Taste bud. Silver, hematoxylin and eosin stain. 125x Abbreviations: tb, taste bud; le, lamina epithelialis; lp, lamina propria; ts, tunica submucosa, vs, venous sinus; n, nerve.

Figure 24. Taste bud. Silver, hematoxylin and eosin stain. 700x Abbreviations: tb, taste bud; gr, granules; le, lamina epithelialis; bm, basement membrane; np, nerve process; lp, lamina propria; rbc, red blood cell.

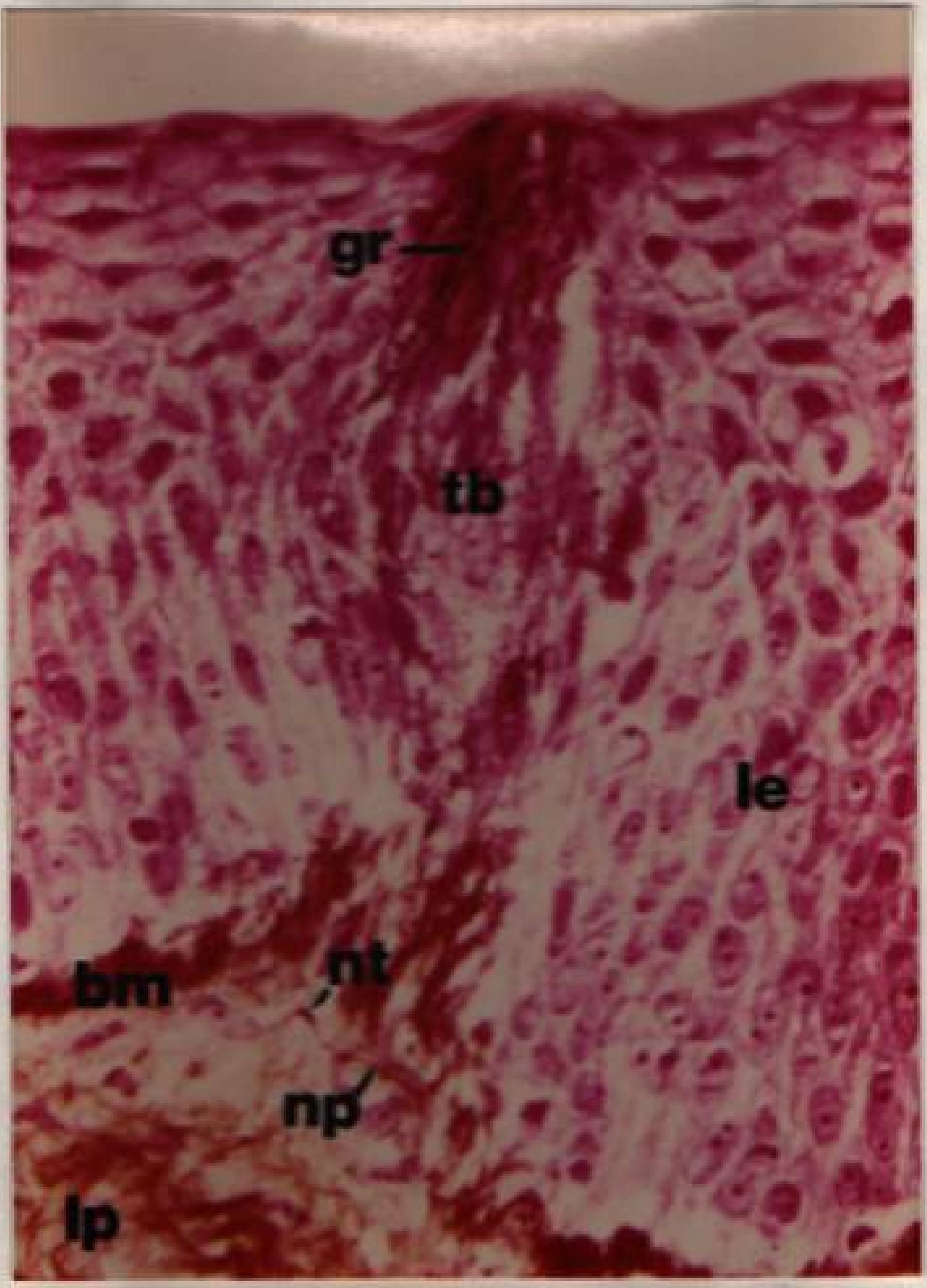

Figure 25. Taste bud. Silver, hematoxylin and eosin stain. 700x Abbreviations: tb, taste bud; gr, granules; le, lamina epithelialis; bm, basement membrane; np, nerve process; nt, nerve terminal; lp, lamina propria.

IV. DISCUSSION

Due to the lack of skeletal muscle within the lingual appendage it appears that most movements of the individual horns are controlled by the muscles acting on the hyoid skeleton and tissue immediately adjacent to the lingual appendage. When luring or assuming a defensive posture, the hyoid skeleton is protracted, which elevates and extends the lingual appendage. The processus lingualis presumably is postioned juxtaposed to, or within the body of, the appendage where it can directly initiate movement of the body and horns resulting from the contraction of one or more of the following muscles: the M. geniohyoideus, the M. branchiohyoideus, the M. genioglossus, the M. hypoglossoglossus, the M. hyoglossus. Further, the processus lingualis may be retracted, deviated laterally and/or depressed by the aforementioned msucles and by the M. hypoglossohyoideus, M. branchiomandibularis visceralis and the M. coracohyoideus.

The large volume of vascular space coupled with the personal observation of numerous flaccid, pale white appendages becoming tumescent, pseudoerect and pinker, suggests that the lingual appendage is a hemodynamic organ. Engorgement with blood presumably enables the amelanistic juvenile appendage to mimic small worms and/or insect larvae. While this engorgment itself does not result in the pseudoerect condition of the lingual appendage, it presumably does confer a certain rigidity to an otherwise intrinsically unsupported structure.

With the added bouyancy of water the appendage does attain a pseudoerect stature. The flexible "erection" of the lingual appendage presumably permits the wriggling motion displayed by the horns, that would not be possible if it were truly erect. This flexibility also may make it more difficult to be grasped by lured-in prey, thus, making it less likely to be injured.

The mucoid substance contained in the goblet cells and on the surface of the lingual appendage may serve two functions. First, as suggested by Nalavalde and Varute (1976), the mucopolysaccarides of the turtle oral cavity may play a role in the initial digestion of food stuffs. Indeed, they suggest that the goblet cells in the lamina epithelialis may be an intermediate step in the evolution of salivary glands. Second, the mucoid substance may function to preserve the integrity of the non-keratinized stratified squamous epithelium of the lingual appendage.

The degree of pigmentation of the lingual appendage increases with age. This may be a result either of maturation of existing melanoblasts present within the appendage or of the slow, ontogenetic migration of melanocytes into the appendage (Burgers, 1965). Increased pigmentation may be an adaptive quality associated with the luring and subsequent ingestion of the greater quantities of prey necessary to maintain larger biomasses of adults. Presumably, the greater melanocyte concentration increases the number of patterns displayed by the lingual appendage, thus increasing the number of potential food items being lured within grasping distance of the jaws. However, no data are available concerning the feeding habits of alligator snappers in the wild (except for mention of fecal contents of a single specimen, one day after capture; Allen and Neill, 1950). Information of this nature would aid in determining the validity of this hypothesis.

The lingual appendage contains many nerve fibers, presumably sensory in function, in its epithelial layer and in its lamina propria. The taste buds presumably serve as chemoreceptors, enabling the turtle to distinguish the quality of the prey encountered. The smaller presumed sensory receptors may be responsible for detection of prey near or within the oral cavity. Drummond (1979), noted that actual contact with the lingual appendage had to occur in order to initiate the "attack phase" of this behavior in hatchlings. His finding implied the presence of touch receptors. Although in the present study nerve fibers were seen terminating in the layers of the tunica mucosa which could subserve this touch function, further studies will have to be conducted to indicate their functional activity. It is difficult, however, to believe such a highly innervated and vascularized structure could remain functional very long under continual attack by prey, unless the snapping response is rapid enough to avoid injury of the appendage. It may be, as Drummond's (1979) behavioral data suggest, that hatchling alligator snapping turtles rapidly learn to attack prior to the actual contact of the lingual appendage by prey, thereby avoiding injury to the appendage.

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