General morphology of the oral cavity of the Nile crocodile, *Crocodylus niloticus* (Laurenti, 1768). II. The tongue

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ABSTRACT

PUTTERILL, J.F. & SOLEY, J.T. 2004. General morphology of the oral cavity of the Nile crocodile, C*rocodylus niloticus* (Laurenti, 1768). II. Tongue. *Onderstepoort Journal of Veterinary Research*, 71: 263–277

The heads of nine 2.5 to 3-year-old Nile crocodiles (*Crocodylus niloticus*) were obtained from a commercial farm where crocodiles are raised for their skins and meat. The animals from which these specimens were obtained appeared clinically healthy at the time they were slaughtered. A description of the macroscopic and microscopic features of the tongue of the Nile crocodile is presented and the results are compared with published information on this species and other Crocodylia. The histological features are supplemented by information supplied by scanning electron microscopy. Macroscopic features of interest were the dome shaped structures grouped in a triangular formation on the posterior two-thirds of the dorsum of the tongue. These structures were identified by light microscopy to contain well-developed branched, coiled tubular glands and associated lymphoid tissue. Other histological features included a lightly keratinised stratified squamous surface epithelium supported by a thick layer of irregular dense fibrous connective tissue. Deep to this region was a clearly demarcated adipose tissue core with a dense mass of striated lingual musculature. Localised thickenings were present in the epithelium which were associated with ellipsoid intra-epithelial structures resembling taste buds.

Keywords: *Crocodylus niloticus*, histology, morphology, Nile crocodile, oral cavity, scanning electron microscopy, tongue

INTRODUCTION

The tongue of Crocodylia has been discussed in a number of publications, mainly in regard to the presence of lingual glands and their participation in salt secretion (Dunson 1976, 1982; Kochva 1978; Taplin & Grigg 1981; Taplin, Grigg, Harlow, Ellis & Dunson 1982; Mazzotti & Dunson 1984; Taplin 1984, 1988; Taplin, Grigg & Beard 1985; Taplin & Loveridge 1988; Chen, Tang, Wei & Zhang 1989; Steel 1989; Taylor, Franklin & Grigg 1995). These papers, however, give very little information on the basic structure of the tongue of crocodylids and are concerned primarily with physiological studies regarding salt balance, tolerance to saline conditions and salt secretion. Although some morphological information has been presented (Gaupp 1888, cited by Röse 1893; Reese 1913; Taguchi 1920; Gabe & Saint Girons 1969; Luppa 1977; Taplin & Grigg 1981; Minnich 1982; Grigg & Gans 1993), only Reese (1913) and Taguchi (1920) provide any

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meaningful histological descriptions of the crocodilian tongue in general. Some detail of the lingual glands of *Crocodylus porosus* and *Alligator mississippiensis*, including ultrastructural features, has also been presented (Taplin & Grigg 1981; Minnich 1982; Taplin 1988; Grigg & Gans 1993).

In view of the lack of information regarding the general histological features of the crocodilian tongue, and considering the economic importance of the Nile crocodile as a farmed animal in Southern Africa, this paper details the morphological features of the tongue of the Nile crocodile (*Crocodylus niloticus*) and compares the findings with published information on other crocodylids.

MATERIALS AND METHODS

Experimental animals

The heads of nine 2.5 to 3-year-old Nile crocodiles (*Crocodylus niloticus*) were obtained from a commercial farm where crocodiles are raised for their skins and meat. The lengths of the animals sampled ranged from 1.2–1.5 m and the animals were clinically healthy at the time they were slaughtered. They were killed by shooting them in the brain at close range using a .22 calibre rifle. After the carcasses had been skinned and eviscerated the heads were removed and immersion-fixed in a large volume of 10 % phosphate-buffered formalin in plastic buckets for 2 h after which the tongues were removed from six of the heads and processed for light microscopy as detailed below. The remaining three heads with tongues *in situ* were further immersion-fixed for a minimum period of 48 h. Care was taken to avoid trapping air in the oral cavity by wedging a small block of wood in the angle of the mouth prior to immersion in the fixative.

Topography

The three formalin-fixed heads with tongues *in situ* were used for a description of the gross anatomical features and topographical relationships of the structures in the oral cavity. Macrophotographs were recorded digitally using a Nikon Coolpix 995 (Nikon, Tokyo, Japan) digital camera or on 35 mm film using a Chinon X-7 (Chinon, Tokyo, Japan) single lens reflex camera. The tongues of these heads were also examined and micrographed using a stereomicroscope (Wild M-400 Photomakroskop, Heerbrugg, Switzerland) to obtain higher magnification micrographs of specific topographical features. A dried skull from a 5-year-old (approximate age) specimen was used to provide supporting evidence for the anatomical description.

Light microscopy

As noted above, the tongues were removed from six heads after a short fixation period of 2 h. This pre-fixation was deemed necessary to firm the relatively soft tissue of the organ prior to it being cut into the various segments for light microscopy and scanning electron microscopy. These tongues were freed from the mandible by cutting through the peripheral membrane and the tissue mass at the base of the tongue. The ventral half of the tongue was removed and discarded and the remaining dorsal surface was divided longitudinally into left and right halves down the midline. Identical transverse sections from the regions indicated in Fig. 1 were taken from each half of the tongue for light microscopy (LM) and scanning electron microscopy (SEM), respectively. Both sets of samples were fixed for a minimum period of 48 h in fresh 10 % buffered formalin. LM samples were dehydrated through 70, 80, 96 and 2X 100 % ethanol and further processed through 50 : 50 ethanol : xylol, 2X xylol and 2X paraffin wax (60–120 min per step) using a Shandon model 2LE Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). Tissue samples were finally embedded manually into paraffin wax in brass moulds. Sections were cut at 4–6 μm, stained with haematoxylin and eosin (H&E) (Luna 1968) or periodic acid-Schiff (PAS) (Pearse 1985) and viewed and micrographed using a Reichert Polyvar (Reichert, Austria) compound light microscope fitted with a differential interference contrast (DIC) prism.

Scanning electron microscopy

The samples of the tongue obtained as indicated above and which had been fixed in 10 % phosphate-buffered formalin for a minimum of 48 h, were rinsed for several hours in water to remove traces of phosphate buffer. These samples were routinely dehydrated through an ascending ethanol series (50, 70, 90, 95 and 3X 100 %, 60 min per step) and critical point dried from 100 % ethanol through liquid-CO_2 in a Polaron Critical Point Drier (Polaron, Watford, England). The samples were then mounted onto brass or aluminium viewing stubs (to expose the epithelial surface) with a conductive paste (carbon dag) and sputter coated with gold using a Balzers 020 Sputter Coater (Balzers Union, Liechtenstein). Specimens were viewed and microtographed using a Hitachi S-2500 scanning electron microscope (Hitachi, Tokyo, Japan) operated at 8 kV.

RESULTS

Macroscopic features

The floor of the oral cavity was formed by the tongue and a wide, rostral mucosal plate continuous with the gingiva. The relatively long tongue was roughly triangular in shape, being much broader caudally than at its tip (Fig. 1). It occupied the greater part of the floor of the oral cavity (apart from the rostral plate over the symphysis of the dentary bones) and was bordered peripherally by a loose, highly folded, continuous, fibrous membrane. This membrane attached the ventro-lateral aspect of the tongue to the ventro-medial aspect of the mandible. The membrane was more complexly folded around the rostral tip of the tongue. In the specimens examined the tip of the tongue was always pulled away (in a caudal direction) from the apex formed by the rostral fusion of the paired dentary bones, revealing the highly folded membrane attaching the tongue to the mandible (Putterill & Soley 2003). Pigmentation varied amongst specimens examined and where present, occurred peripherally in the loosely folded membrane and on occasion, superficially on the surface of the tongue (Fig. 1 and 2). Pigmentation was also often evident at the angle of the tongue and the oral surface of the ventral fold of the gular valve which also formed the ventro-caudal border of the oral cavity. The dorsal surface of the tongue displayed numbers of large, dome-shaped structures which were concentrated in a triangular formation on the posterior two-thirds of the dorsum of the tongue (Fig. 2). Each of these elevated structures revealed a centrally positioned, darkly pigmented opening (Fig. 4) which on histology proved to represent the duct opening of large branched coiled tubular glands situated deeply below the tongue surface (Fig. 13). Stereomicroscopy revealed small, nipple-like surface extensions scattered between the duct openings (Fig. 4). Smaller, more discrete, non-pigmented units were observed on the lateral and rostral aspects of the tongue surface (Fig. 2). Two isolated concentrations of similar structures were situated at the caudo-lateral aspect of the tongue, at the base of the ventral fold of the gular valve (Fig. 3).

The non-pigmented units differed from the more obvious pigmented units in that they did not display a duct opening. The centrally positioned structure typical of these units represented a flattened disc of thickened epithelium which on LM was observed to house structures resembling taste buds (see below).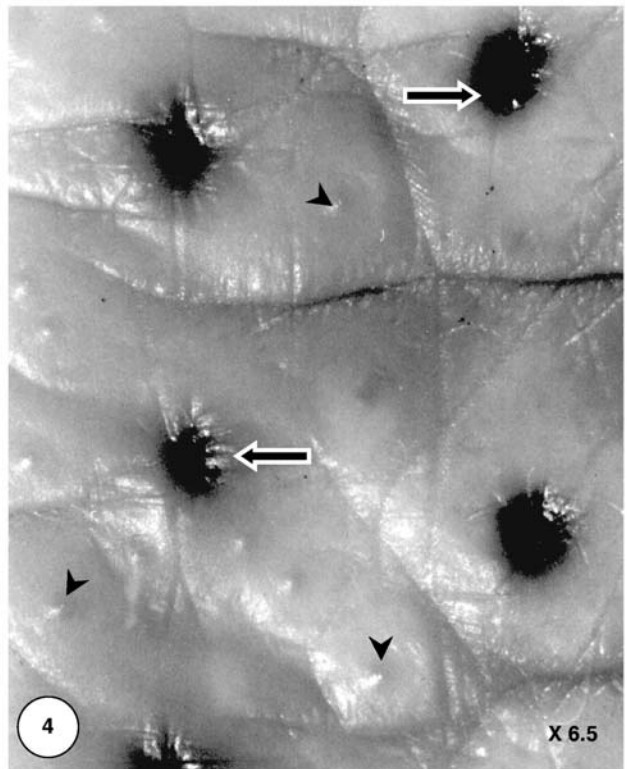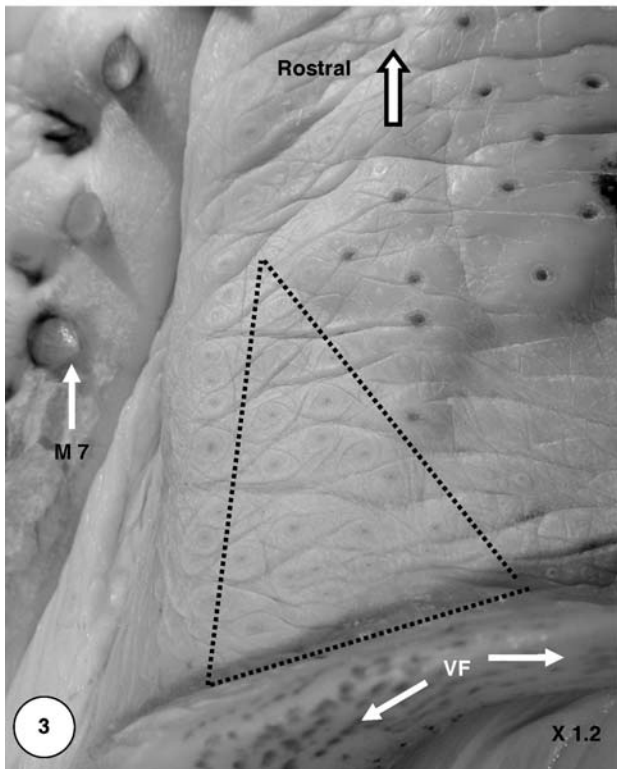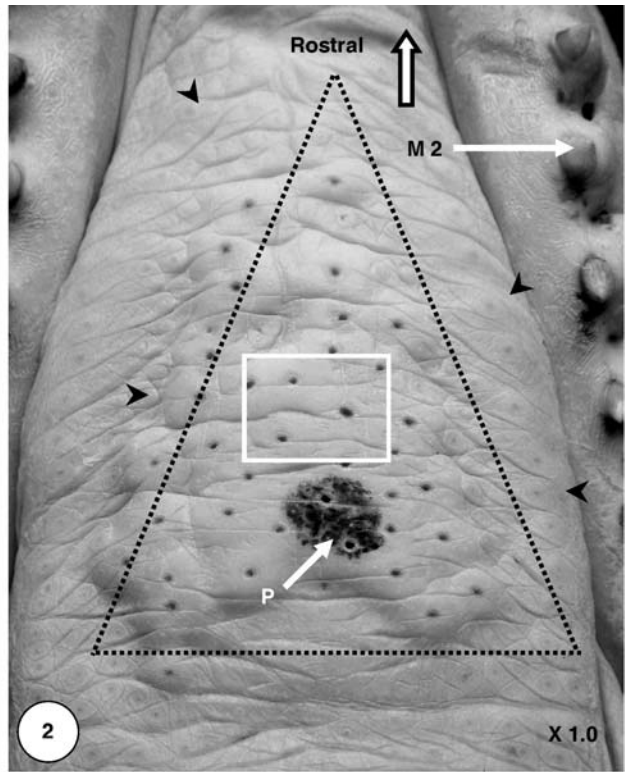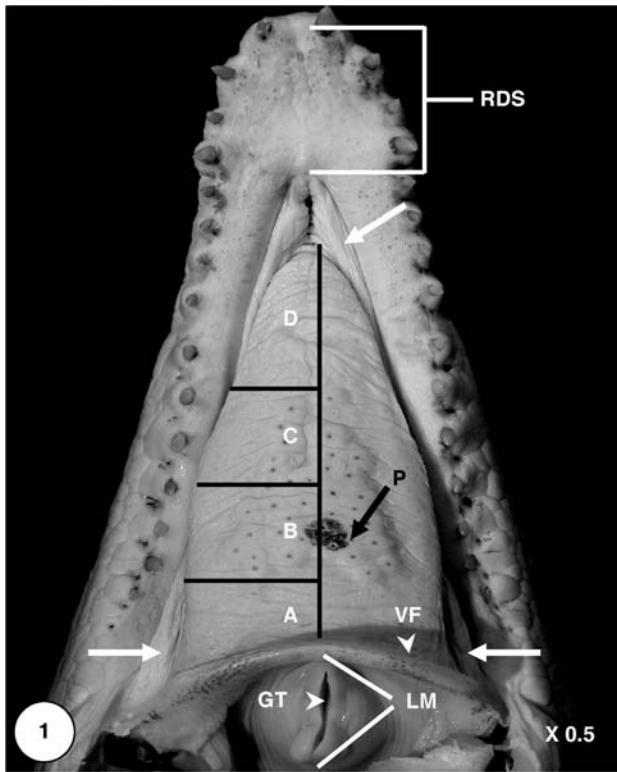 The tip of the tongue displayed deep transverse and oblique grooves which possibly represented a shrinkage or fixation artefact (Fig. 1). The surface of the tongue was generally creamy-yellow in colour and had a slightly spongy texture, even in the fixed specimens.

Light microscopy

Cross-sections of the rostral region of the tongue revealed a relatively thin, lightly keratinised stratified squamous epithelium supported by a thick layer of irregular dense fibrous connective tissue (Fig. 5). The epithelium on the dorsum of the tongue displayed a few shallow folds, but these rapidly increased in number and complexity towards the lateral borders of the tongue. The epithelial folds were supported by the underlying connective tissue and primary, secondary and occasionally tertiary folds could be distinguished. The epithelium in the highly folded lateral zone appeared more heavily keratinised and a distinct layer of desquamated cells (stratum disjunctum) was generally evident (Fig. 10). At the ventro-lateral aspect of the tongue the epithelium, supported by a substantial layer of more loosely arranged irregular dense connective tissue, was reflected laterally towards the medial surface of the mandible with which it was continuous. The loosely arranged, highly folded membrane thus formed, connected the ventro-lateral aspects of the tongue to the mandible. Deep to the connective tissue layer was a clearly demarcated adipose tissue core containing large adipocytes, strands of fibrous connective tissue and variably sized bundles of striated muscle fibres (Fig. 5 and 9).

A large plexus of blood and lymphatic vessels was situated between the irregular dense connective tissue and the adipose tissue core. A large vascular and lymphatic plexus was also situated at the angle of the tongue and the ventral fold of the gular valve. These vessels were sandwiched between the thick sub-epithelial connective tissue layer and the adipose tissue core (Fig. 9). More caudal cross-sections of the tongue displayed a similar arrangement of the basic tissue layers outlined above. In addition, however, a dense mass of striated lingual musculature was evident ventral to the adipose core, and large branched coiled tubular glands associated with prominent duct openings characterised the dorsal sub-epithelial layer of connective tissue (Fig. 14).

The lightly keratinised stratified squamous epithelium varied in thickness (3–6 cell layers) and, in PAS-stained sections, was seen to rest on a promi-

nent basement membrane. The shape, appearance and orientation of the cells and their nuclei were similar to that described previously for the palate and gingivae (Putterill & Soley 2003). Although the epithelium was relatively thin, it displayed the features typical of stratified squamous epithelia in general. The stratum spinosum was poorly developed in the thinner regions of the epithelium and was

FIG. 1 Macrophotograph of the mandible with the tongue *in situ* showing the sampling sites for histology of the tongue (A–D). The fibrous membrane attaching the tongue to the mandible is also obvious in places (white arrows). The rostral dentary shelf (RDS) is indicated in the region above the symphysis of the dentary bones. The glottis (GT) and laryngeal mound (LM) are shown *in situ* on the floor of the pharyngeal cavity and the ventral fold (VF) of the gular valve is seen separating the ventral aspects of the oral and pharyngeal cavities. P = Pigmented area. Formalin fixed specimen. X 0.5

FIG. 2 Macrophotograph of the surface of the tongue showing the triangular arrangement of the glandular region (indicated by the dotted line) situated in the posterior two-thirds of the tongue and stretching from approximately M 2 to M 6/7 (not indicated in photograph). The pigmented gland openings are clearly visible. Note the less conspicuous, low-profiled units (black arrowheads) situated laterally to the glandular region. The rectangular area is enlarged in Fig. 4. P = Pigmented area. Formalin-fixed specimen. X 1

FIG. 3 Macrophotograph of the caudo-lateral region of the tongue showing a discrete, triangular region of low-profiled surface units at the base of the ventral fold (VF) of the gular valve. M 7 = Molar 7. Formalin-fixed specimen. X 1.2

FIG. 4 Stereomicrograph of the darkly pigmented openings to salivary glands (block arrows) on the surface of the tongue. Small nipple-like surface extensions (arrowheads) are scattered between the openings. Formalin-fixed specimen. X 6.5

only clearly defined in the areas of localised epithelial thickening associated with glandular openings and specialised sensory structures (Fig. 6, 7, 11–13).

In addition to ubiquitous epithelial folds, the epithelial lining of the tongue demonstrated randomly distributed localised thickenings associated with ellipsoid intra-epithelial structures resembling taste buds (Fig. 7). The epithelial specialisations were positioned level with, or slightly elevated above, the adjoining epithelial surface, the increase in thickness being accommodated at the expense of the underlying connective tissue layer (Fig. 6 and 7). The most noticeable features of the specialisations were an increase in the number of layers of the stratum spinosum and the vertical orientation of cells towards the centre of the epithelial concentration. The vertically positioned cells formed structures with features characteristic of taste buds (Fig. 7) similar to those observed in the palate and gingivae (Putterill & Soley 2003). The underlying connective tissue contained conspicuously less collagen fibres and formed a conical or cup-shaped zone associated with medullated nerve fibres. In some instances the modified connective tissue zone revealed a centrally positioned cell-rich mass. Some of the cells morphologically resembled fibroblasts while others were large, round or oval cells with a spherical nucleus. In a few areas the round cells appeared swollen and were arranged in poorly defined vertical rows. Small lymphocytic aggregations were often associated with the epithelial specialisations (Fig. 6 and 11). The taste receptors appeared to be most numerous on the dorso-lateral aspects of the tongue and also in the epithelium lining the lateral wall of the organ. Not all sections of the epithelial thickenings, however, revealed the intraepithelial, ellipsoid structures and Pacinian-like

corpuscles were occasionally seen in the vicinity of the modified connective tissue core, particularly towards the tip of the tongue. This may indicate that some of the specialisations represent touch or pressure receptors in addition to the more commonly encountered taste receptors (see SEM results below). It is also possible that individual sections did not always pass through the centrally positioned taste bud, creating the erroneous impression that it was absent.

Immediately beneath the epithelium was a narrow layer of fine connective tissue followed by a diffuse yet prominent layer containing melanin pigment (Fig. 12 and 13). The melanin appeared to be concentrated around capillaries present in this region and was also observed in the deeper parts of the connective tissue stroma in the vicinity of larger blood vessels and nerves. The sub-epithelial capillary supply appeared relatively sparse. However, in addition to the vascular and lymphatic plexuses mentioned above, numerous large blood and lymphatic vessels and attendant medullated and non-medullated nerves were observed throughout the deeper lying layer of irregular dense connective tissue.

The caudal two-thirds of the tongue was dominated by the presence of large collections of glandular tissue (lingual salivary glands) and masses of associated lymphoid tissue (Fig. 13–16). The more centrally positioned glands were generally larger and more complex than those situated more laterally. In oblique or transverse sections the glandular tissue exhibited features typical of compound tubular glands and appeared to be composed of lobes and lobules separated by tracts of connective tissue (Fig. 15 and 16). When sectioned in the longitudinal plane, however, it was obvious that the glands were branched, coiled tubular in nature with each branch

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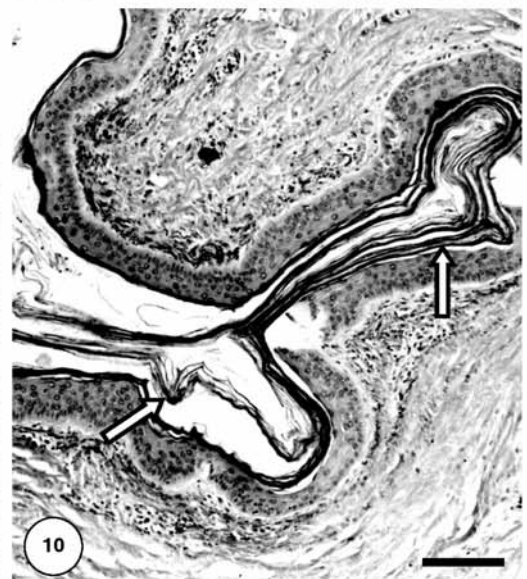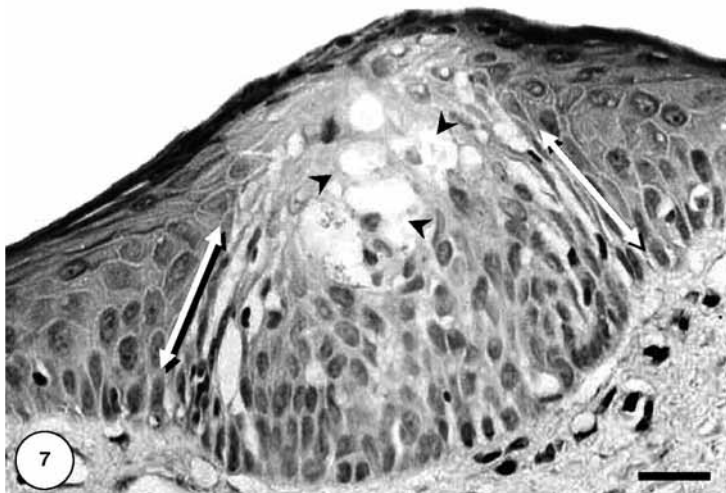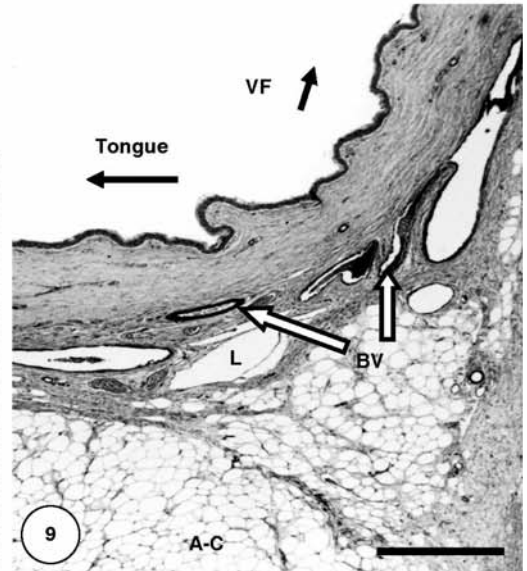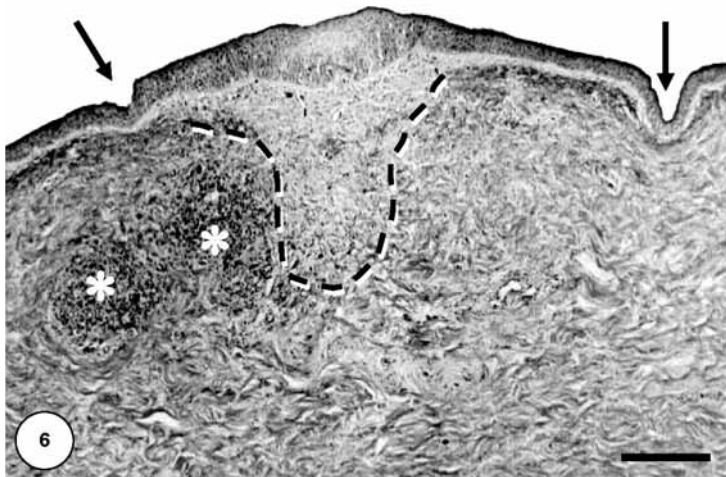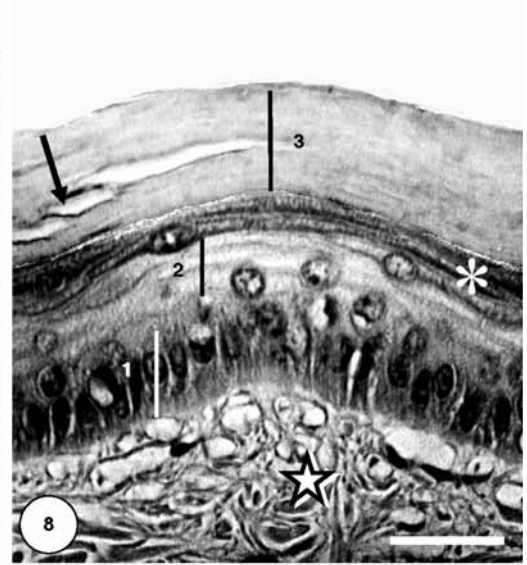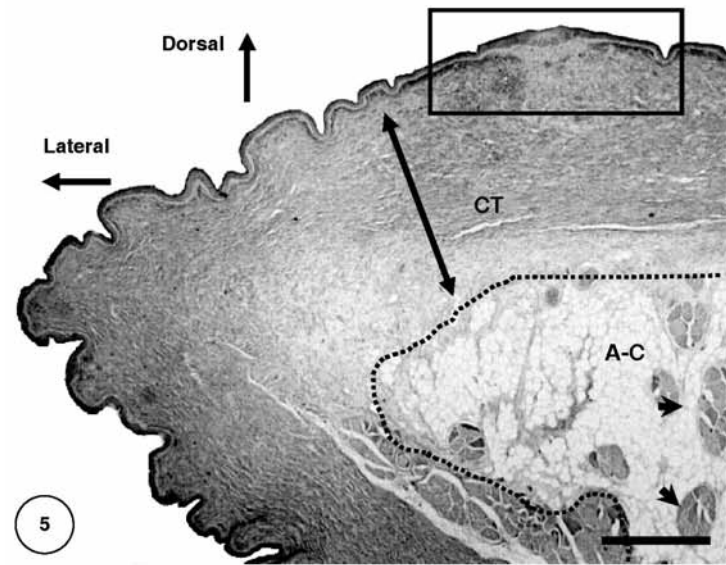
FIG. 5   Cross-section of the rostral part of the tongue illustrating the lateral aspect. Note the increase in the number of folds on the lateral surface, the thick layer of irregular dense subepithelial connective tissue (CT) and the adipose tissue core (A-C) (approximately following the dotted outline) containing scattered bundles of striated muscle fibres (arrowheads). The blocked area shows an epithelial specialisation enlarged in Fig. 6. H&E stain. Bar = 2 mm

FIG. 6   An enlargement of the epithelial specialisation blocked in Fig. 5. Note the thickened epithelium, the characteristic, pale, conical zone of modified subepithelial connective tissue (outlined) and the closely associated lymphocytic aggregations (asterisks). The arrows indicate the groove/trough often seen surrounding these structures. H&E stain. Bar = 500 μm

FIG. 7   Modification of the thickened epithelium of a specialisation to form an enlarged oval core reminiscent of a taste bud. Note the vertical orientation of the core cells (arrows) and the more substantial stratum spinosum surrounding the taste bud. Vacuolation is apparent in the superficial region of the receptor (arrowheads). H&E stain. Bar = 50 μm

FIG. 8   Enlargement of the epithelial covering of the tongue surface showing the single layer of vertically oriented cells forming the stratum basale (1), the narrow stratum spinosum (2), the darkly staining stratum granulosum (asterisk) and the superficial stratum corneum (3) which shows signs of desquamation (arrow). The epithelium is supported by irregular dense connective tissue (star). H&E stain. Bar = 50 μm

FIG. 9   Caudal limit of the tongue where it abuts the ventral fold (VF) of the gular valve. Note the large lymphatic (L) and vascular (BV) plexus in this region as well as the underlying adipose tissue core (A-C) of the tongue. H&E stain. Bar = 1 mm

FIG.10   Lateral wall of the tongue illustrating the highly folded nature of the surface and the greater degree of desquamation of the surface layers of the epithelium in this region (arrows). H&E stain. Bar = 250 μm
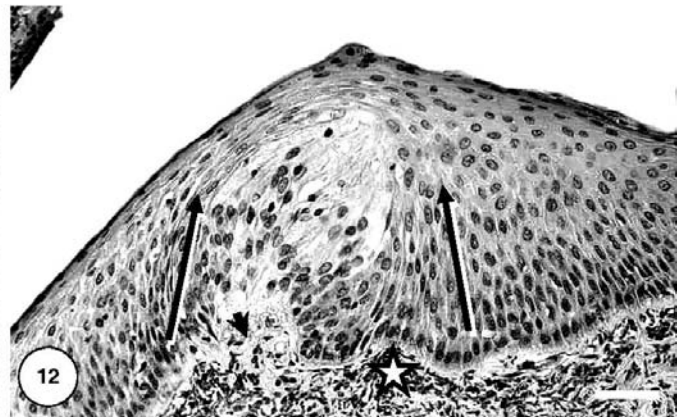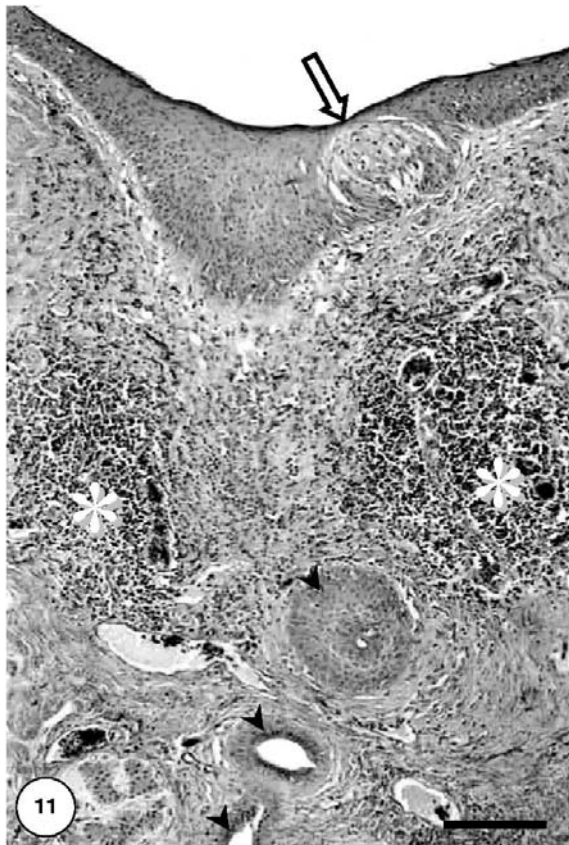

FIG. 11   LM micrograph of an oblique section through the secretory duct of an underlying salivary gland. Note the taste bud (block arrow) situated in the wall of the pore opening onto the tongue surface. Oblique sections of the duct (arrowheads) can be seen. Lymphocytic aggregations (asterisks) are closely associated with the wall of the secretory duct and the secretory pore (see also Fig. 13). H&E stain. Bar = 500 μm

FIG. 12   Enlargement of a typical taste bud located in the duct of a salivary gland. Note the vertical orientation of the epithelial cells (arrows) and the more substantial stratum spinosum surrounding the taste bud. At the base of the specialisation an apparent nerve plexus (arrowhead) is seen to penetrate below the epithelium into the melanin layer (star). H&E stain. Bar = 100 μm

leading to an elaborately coiled secretory endpiece. Each secretory endpiece formed a distinct glandular unit. The various branches were linked to a single secretory duct which opened onto the dorsum of the tongue. The main secretory duct was lined by a thick stratified squamous epithelium originating from the stratum germinativum of the surface epithelium with which it was continuous (Fig. 13). The epithe-lial cells, however, were vertically oriented with the final layer lining the duct appearing typically columnar in nature (Fig. 17). The epithelium of the main duct thinned rapidly to form a relatively narrow tube lined by three to four layers of cells before dividing from the surface inwards into a number of smaller branches. These branches were lined by a simple columnar epithelium which was continuous with the
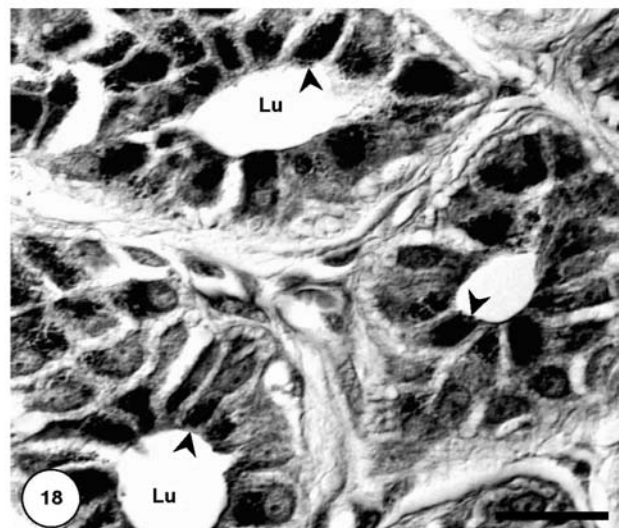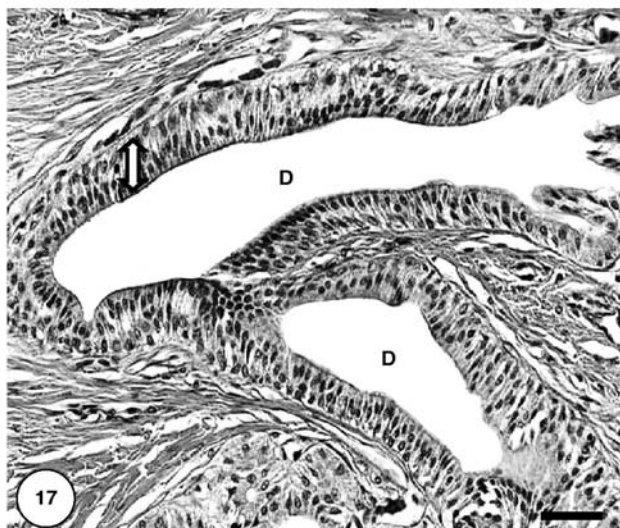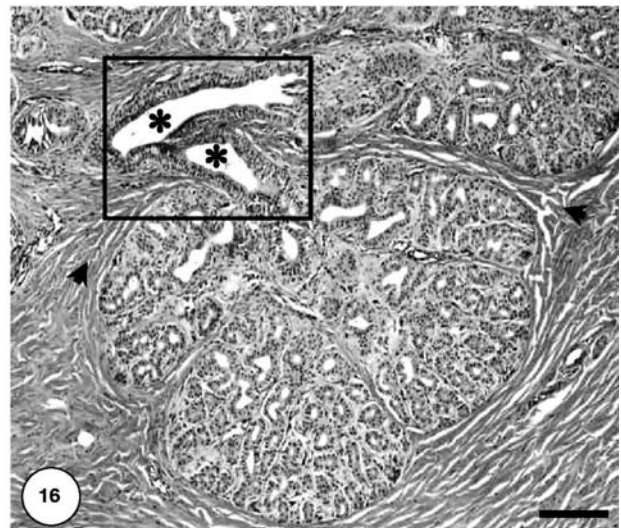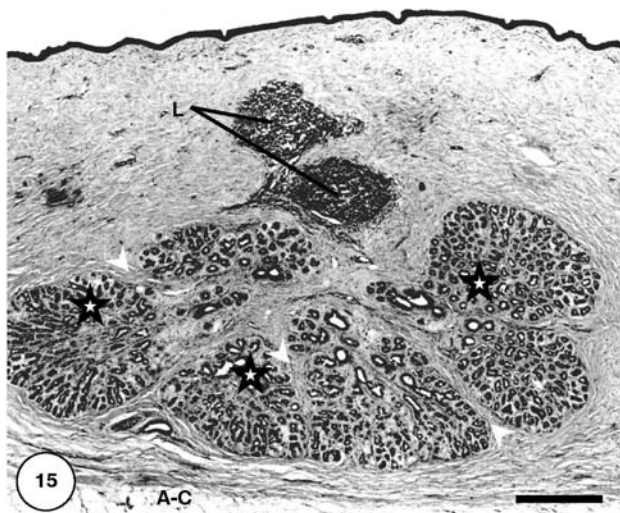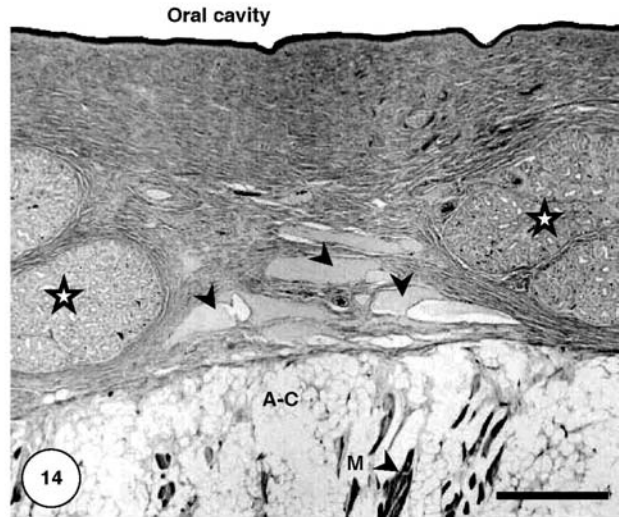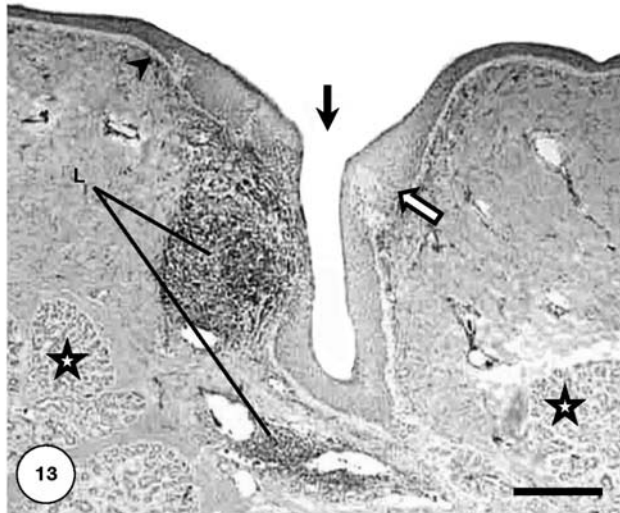
FIG. 13   Low magnification of the secretory duct of a deeper lying salivary gland. The duct opens onto the tongue surface by means of a pore (arrow). The duct is not entirely visible due to the slightly oblique plane of the section towards its base. Note the lymphoid aggregation (L) associated with the duct. A taste bud (block arrow) is observed towards the opening of the duct (see also Fig. 12). Note the thin, but prominent layer of melanin pigmentation (arrowhead) immediately below the epithelium. A portion of the secretory units (stars) of a salivary gland are also visible in the micrograph. H&E stain. Bar = 500 μm

FIG. 14   Secretory units (stars) of two lingual salivary glands separated by a well-developed vascular and lymphatic plexus (arrowheads). The glandular tissue is positioned deep within the subepithelial connective tissue, close to the adipose tissue core (A-C). Striated lingual muscle fibres (M). H&E stain. Bar = 2 mm.

FIG. 15   An example of a typical lingual salivary gland. Note the separation of the glandular tissue into lobules/secretory units (stars) by wide tracts of connective tissue (arrowheads). The main secretory duct does not appear in the section although some lymphoid tissue (L) is visible. A-C = Adipose tissue core. PAS-stain. Bar = 1 mm.

FIG.16   A lobule/secretory unit of a lingual salivary gland. Secondary or tertiary branches (asterisks) of the main secretory duct, displaying a wide lumen, can be observed in the rectangle (enlarged in Fig. 17). Wide connective tissue tracts (arrowheads) can be seen between the lobules of the salivary gland. H&E stain. Bar = 500 μm

FIG.17   Higher magnification of the rectangle in Fig. 16 showing the double columnar nature of the duct epithelium (double-headed arrow) and the wide lumen of the ducts (D). H&E stain. Bar = 1 mm

FIG.18   Higher magnification of a group of secretory tubules of the branched coiled tubular units of the gland demonstrating the PAS-positive granules (arrowheads) observed in the apical cytoplasm of the cells. However, this was not a consistent feature. Note the wide lumen (Lu) of the tubules. PAS-stain. Bar = 50 μm

coiled secretory endpieces of the gland. The point of exit of the secretory duct onto the surface of the tongue was marked by a distinct depression or pore. Intra-epithelial structures resembling taste buds were often located within the wall of the pore (Fig. 11–13).

The secretory portions of the glands were coiled tubular in nature and lined by a simple columnar epithelium. The nuclei of the secretory cells were generally round or oval and although situated towards the base of the cell, were not compressed. PAS-positive granules were identified in cells lining some tubular profiles, generally towards the periphery of the glands, while most of the secretory tubules showed a light staining reaction or none at all. The tubular lumen was wide and individual secretory profiles were separated by delicate bands of connective tissue (Fig. 18). Each individual secretory unit was separated from neighbouring units by relatively wide tracts of connective tissue (Fig. 15 and 16).

The individual units of the lingual salivary glands were generally situated in the deeper region of the sub-epithelial connective tissue, close to the border of the adipose tissue core (Fig. 14 and 15). Between the glandular tissue and the surface of the tongue, and initially associated with the secretory duct, were large aggregations of lymphoid tissue which formed lingual tonsils (Fig. 11 and 13). Although the lymphoid masses displayed diffuse infiltration into the thickened stratified epithelium of the secretory duct, obliteration of the epithelium was not observed (Fig. 13). Loosely arranged collections of lymphoid tissue were also sometimes associated with the glandular tissue itself.

The glandular tissue was richly supplied with blood vessels and the vascular and lymphatic plexuses present at the junction of the irregular dense connective tissue and adipose tissue layers were often positioned close to the glandular units (Fig. 14). Non-medullated nerves were associated with the glandular tissue.

Scanning electron microscopy

Scanning electron microscopy of the tongue surface confirmed the LM observation that the dorsum of the tongue was partially composed of a number of clearly demarcated round to angular units. The various units appeared to be elevated and were surrounded by deep, sometimes wide, clefts which accentuated individual units. The exaggerated division of the units was attributed to shrinkage caused by the drying process for SEM. The round to polygonal-shaped units occupying the triangular glandular region situated in the posterior two-thirds of the tongue (Fig. 2) exhibited a relatively low profile and smooth surface features. A centrally positioned pore which was frequently filled with debris/glandular secretions, and which was shown by LM to represent the opening of the main secretory duct of the underlying lingual salivary glands, was the most prominent feature. A number of grooves (probably representing epithelial folds) radiated outwards from the pore. The grooves varied in length and occasionally extended the entire diameter of the unit (Fig. 19 and inset Fig. 19). Situated towards the periphery of the units were small, spherical, low-profiled, dome-shaped protrusions (Fig. 19 and inset Fig. 19) which appeared to represent the nipple-like sur-
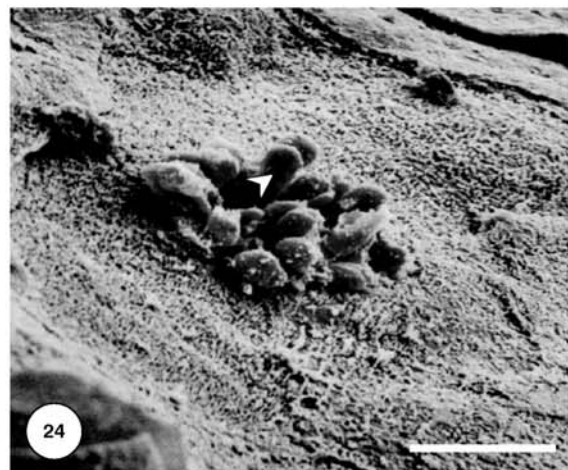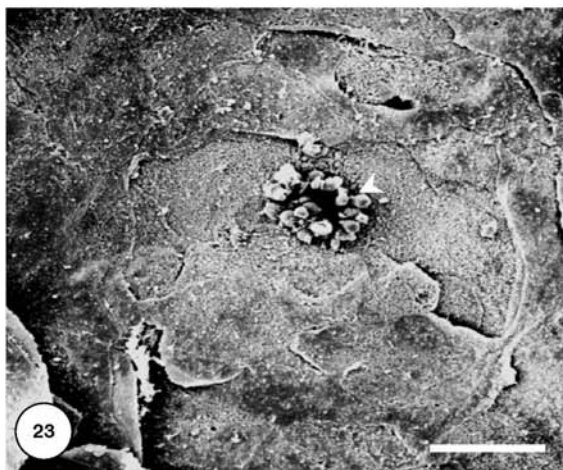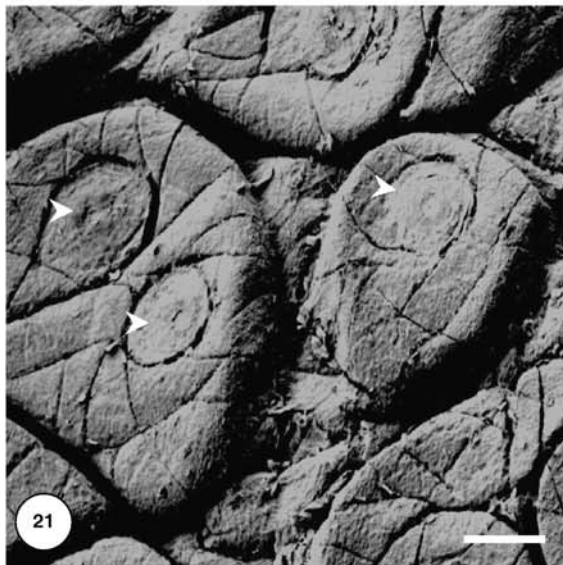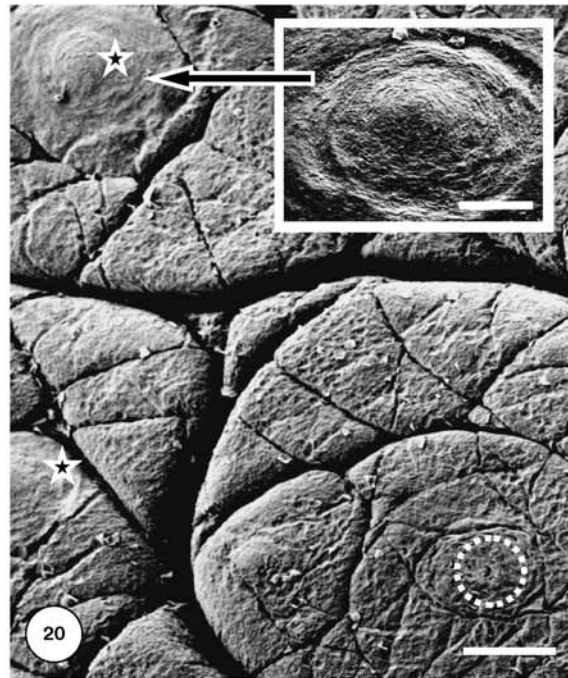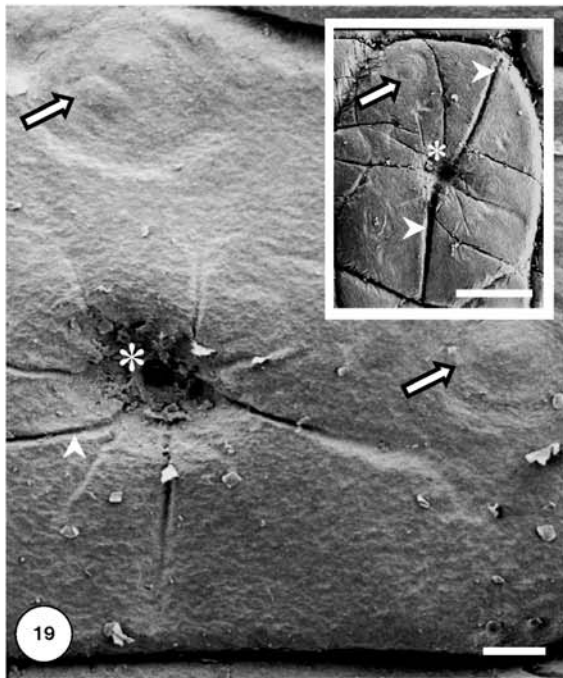
FIG. 19 A surface unit found on the glandular area of the tongue. Note the large, centrally positioned gland opening (asterisk), the radiating epithelial grooves (arrowhead) and the dome-shaped structures (block arrows). Bar = 100 μm

Inset: Overview of another surface unit demonstrating longer epithelial grooves (arrowheads) and similar features (gland opening—asterisk; dome-shaped structure—block arrow) to those seen in the main micrograph. Bar = 500 μm

FIG. 20 Typical surface units found in the mid-lateral region of the tongue showing a mosaic pattern of grooves. A flat, disc-shaped structure (possible taste receptor—encircled) and raised, dome-shaped structures (possible pressure receptors—stars) are present. Bar = 250 μm

Inset: Enlargement of the dome-shaped structure indicated by the outlined arrow. Bar = 100 μm

FIG. 21 A group of caudo-laterally situated surface units (see Fig. 3) showing flat disc-shaped structures (arrowheads). Two of these discs are situated on the same unit. Bar = 250 μm

FIG. 22 Enlargement of a disc-shaped structure from the caudo-lateral aspect of the tongue showing the central pore (arrow) and the concentric rings of loosened, desquamating surface epithelial cells (arrowheads). Bar = 100 μm

FIG. 23 Higher magnification of the central pore to show the protruding cellular processes (arrowhead). Note the spongy appearance of the surface of the surrounding epithelial cells. Bar = 10 μm

FIG. 24 An oblique view of clavate cellular processes (arrowhead) which protrude from the pore for only a short distance above the surface. The surrounding epithelial cells display a complex arrangement of microridges. Bar = 5 μm

face extensions seen by stereomicroscopy (Fig. 4). Occasional flattened discs (see below) with a random distribution were also observed. More discrete surface units were observed laterally on the tongue surface (Fig. 2 and 20) and also in the smaller paired triangular areas situated caudo-laterally at the base of the tongue (Fig. 3 and 21). The surface of the discrete units presented a rough, corrugated appearance with epithelial folds of variable depth creating a mosaic-like pattern (Fig. 21). Centrally, and occasionally eccentrically, positioned on each unit were clearly defined, flattened, disc-shaped structures. More than one disc-shaped structure was seen on some units (Fig. 19 and 21). At the centre of each disc was a small pore, which in many cases was obscured by cell debris and/or bacteria. In pores not filled with debris, a variable number of short processes were observed to protrude from the opening. The processes often displayed clavate tips (Fig. 23 and 24). Based on LM observations, the pores with their processes would appear to represent the superficial component of the taste buds located within localised epithelial specialisations (Fig. 7, 11–13). The pore itself was surrounded by concentric arrangements of desquamating surface cells (Fig. 22 and 23). Individual cells were polygonal in shape and displayed a complicated array of microridges, giving the surface a sponge-like appearance (Fig. 23 and 24). Occasional dome-shaped structures similar to those described in the glandular area were sometimes observed.

DISCUSSION

The present study confirmed the basic histological features reported by both Taguchi (1920) in *Alligator sinensis*, *Crocodylus vulgaris*, which represents *C.*

niloticus (King & Burke 1997) and *C. porosus*, and Reese (1913) in the "Florida alligator" (*A. mississippiensis*). Reese (1910) noted that the dorsum of the tongue is covered with small, evenly distributed papillae. This observation was confirmed by Shimada, Sato, Yokoi, Kitagawa, Tezuka, & Ishii (1990) in *A. mississippiensis* who described cone-shaped filiform papillae spread across the dorsal surface of the tongue. The small, nipple-like surface projections (Fig. 4) seen in the Nile crocodile probably represent the papillae observed in the American alligator. The folded nature of the tongue surface revealed in this study was also confirmed in *A. mississippiensis* (Reese 1913) and in the species studied by Taguchi (1920).

Both Reese (1913) and Taguchi (1920) described the covering of the tongue as a keratinised stratified squamous epithelium which thickened appreciably towards the body and base of the tongue. In the specimens examined during the present study the epithelium, although typically keratinised stratified squamous in structure, appeared thin and uniform in thickness throughout all regions of the tongue except for localised thickenings containing taste buds and at the mouths of the ducts of the lingual salivary glands. To what extent the diet of commercial pellets on which the specimens of *C. niloticus* were raised may have influenced the thickness of the epithelial lining of the tongue could not be determined.

The sequence of supporting connective tissue layers observed in the tongue of *C. niloticus*, namely, the thick layer of irregular dense fibrous connective tissue and the deeply positioned adipose tissue core was also confirmed in the study of Taguchi (1920). According to Taguchi the layer of fibrous

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connective tissue contained numerous muscle fibres which extended close to the surface of the tongue as well as into the core of adipose tissue. In the Nile crocodile the muscle fibres were confined to the adipose tissue core. It is of interest that Reese (1913) makes mention of neither an adipose tissue core nor the muscle fibres in *A. mississippiensis*. However, Taguchi (1920) did note that the layer of adipose tissue was practically absent in *C. porosus*. The muscle tissue housed within the tongue is described by Taguchi (1920) as the Musculus hyoglossus. Gnanamuthu (1937) supports this observation and notes that the "M. hyoglossus of *Crocodilus* [*palustris*] is peculiar in being the sole muscle of the tongue". The findings of Gnanamuthu (1937) also support the observation in the Nile crocodile that the muscle fibres decrease in number towards the tip of the tongue.

Taguchi (1920) observed that mucosal folds divide the surface of the tongue into small, demarcated fields or units. This was confirmed in the present study both macroscopically (Fig. 2) and more graphically by SEM (Fig. 19 to 22). Based on surface details provided by SEM, different types of units could be identified. The large units typically situated on the posterior two-thirds of the tongue displayed a large central pore, radiating surface grooves and scattered, peripherally situated dome-shaped protrusions. The more discrete surface units occupying the lateral and caudo-lateral regions of the tongue were characterised by the presence of numerous flattened, disc-shaped structures at the centre of which was a small pore. When correlated with LM observations, these structures appear to represent the superficial component of specialised taste receptors. These observations have not previously been reported.

Taguchi (1920) in his study of three crocodilian species, mentions the presence of lingual taste buds but provides no further detail other than that they are situated between the epithelial cells in the stratum spinosum. Taste buds in the Nile crocodile are associated with localised epithelial thickenings located throughout the tongue, particularly in the lateral and caudo-lateral aspects of the organ. These epithelial thickenings appear to represent the flattened, disc-shaped structures seen by SEM. The presence of short processes seen emanating from the central pore of these structures in SEM preparations lends further support to this observation. Taste buds were also seen to be concentrated in the thickened epithelium forming the surface openings of the lingual salivary glands. The morphology of the lingual taste buds was similar to that reported for taste buds in the palate and gingivae of the Nile crocodile (Putterill & Soley 2003) and reflects the features previously described by Bath (1905, 1906) and Taguchi (1920) in these regions of the oral cavity.

The concentration of cells observed in the modified connective tissue zone beneath the taste papillae of the tongue has been mentioned in earlier publications. Bath, who identified taste buds in the oral cavity of *C. niloticus*, was of the opinion that these cells represented supportive and protective elements for the nerve fibres supplying the taste buds (Bath 1905, 1906). In contrast, Hulanicka (1913), in a study of the nerve endings in the tongue, palate and the skin of two crocodylids (*C. niloticus* and *A. lucius* [= *M. mississippiensis*]), concluded that these "supportive" cells were in fact more typical of tactile cells. This observation was based on the morphological characteristics and arrangement of the cells and their relationship to the nerve fibres supplying the taste bud. The "tactile" cells were innervated by branches of the fibres supplying the taste bud. Although this study did not examine the distribution of nerve endings in the tongue, the arrangement of the connective tissue elements in the stroma beneath the epithelial specialisations was essentially similar to that described by Hulanicka (1913). This would imply that the epithelial specialisations with their modified connective tissue zone possibly function as both taste and tactile receptors.

Hulanicka (1913) also describes a variety of tactile corpuscles in the crocodilian tongue, including structures similar to the corpuscles of Krause. As this study did not specifically address nerve fibre distribution, the authors cannot confirm the presence of the various types of tactile structures. However, structures reminiscent of Pacinian corpuscles were regularly identified towards the tip of the tongue, often in association with epithelial specialisations apparently devoid of taste buds.

Earlier literature describing the tongue of crocodylids (Röse 1893; Bath 1905, 1906; Reese 1913; Taguchi 1920; Woerdeman 1920; Luppa 1977) mentioned the fact that glandular tissue occurred in the specimens examined. Reese (1913) studied the histology of the enteron of *A. mississippiensis* and described the glandular tissue in the tongue as "... probably mucous or slime secreting" and the structure of the glands as being compound tubular alveolar. Taguchi (1920) made a comparative histological study of the digestive tract, including the tongue, of three crocodilian species (*A. sinensis, C. niloti-*

cus and *C. porosus*) and described lingual glands. These glands, identified by Taguchi (1920) as being of the branched tubular type, appear to be typical of the glandular tissue observed during this study. Although Taguchi (1920) did not specify a mucous secretion as such, he did state that the secretory cells appeared to be filled with fine granular material. None of the earlier studies, however, mentioned the role of lingual glands in salt secretion.

In more recent studies the tongues of the marine or estuarine crocodile (*C. porosus*) and the American crocodile (*Crocodylus acutus*) have received most attention, seemingly due to the animals' salt water or estuarine habitat and the implication of the lingual glands in salt secretion during osmoregulation or electrolyte homeostasis (Taplin & Grigg 1981). Steel (1989) reported that the marine or estuarine crocodile (*C. porosus*) is "quite at home in a marine environment" and "ranges far out into the open ocean." Various authors have noted that the tongue of *C. porosus* contains 40 complex tubular salt glands that secrete a concentrated solution of NaCl when the species is in a saline environment (Taplin & Grigg 1981; Taplin *et al.* 1982; Taplin *et al.* 1985; Taplin 1988; Grigg & Gans 1993). Taplin & Grigg (1981) describe the glands in this species as compound tubular glands having "a broad, shallow duct which leads down to a series of smaller ducts that branch repeatedly into lobules of the gland. The larger branches are lined with columnar to cuboidal epithelium which passes into squamous epithelium in the smaller branches. Each lobule of the gland is densely packed with branching secretory tubules lined almost entirely by columnar epithelial cells." According to Taplin (1988), the ultrastructural features of the salt secreting glands revealed by transmission electron microscopy were the "characteristically complex interdigitations of lateral cell membranes, expanded intercellular spaces, abundant mitochondria, and extensive network of blood vessels and unmyelinated nerve fibres."

Chen *et al.* (1989) examined the lingual glands of the Chinese alligator, *Alligator sinensis*, and reported that they were either simple tubular or complex acinotubular in nature. They concluded that the glands, which number about 100 throughout the tongue, appeared in the posterior two-thirds of the tongue and further stated that they functioned as salt secreting glands as well as serving to lubricate food. This investigation on the Nile crocodile identified glandular tissue in a triangular area occupying the posterior two-thirds of the tongue. Each lingual salivary gland was demarcated by a raised, domed

unit with a centrally positioned, pigmented pore which indicated the opening of the secretory duct (Fig. 1, 2, 4 and 13). There were approximately 40 pores present which is similar in number to those seen in the saltwater crocodile by Taplin & Grigg (1981). However, in species of Alligatorinae, many more pores are present with 100 being reported in *A. sinensis* by Chen *et al.* (1989) and 200 or more in *A. mississippiensis* (Taplin *et al.* 1982).

Taplin & Grigg (1981) and Taplin *et al.* (1985) used methacholine chloride to stimulate lingual gland secretion in various species of Crocodylinae (*Crocodylus acutus, C. johnsoni, C. acutus, C. palustris, C. cataphractus, C. niloticus* and *Osteolaemus tetraspis*), Alligatorinae (*Alligator mississippiensis* and *Caiman crocodilus*) and in *Gavialis gangeticus* (Family Gavialidae), and reported that in all instances the animals possessed functional lingual glands, but that only the Crocodylinae secreted significant concentrations of Na^+, indicating the presence of salt secreting glands. These lingual salt secreting glands open through pores in the posterior region of the tongue and secrete a solution of Na^+ and Cl^- at concentrations similar to that of seawater (Taplin & Grigg 1981). Steel (1989) also confirmed that "alligators and caimans do have minute pores on the back of their tongues and in the palatal epithelia around the buccal valve, but it would seem that these are primarily salivary glands, although some Na^+ and K^+ is excreted at a low rate." Taplin (1988) and Grigg & Gans (1993) note that salt glands have not been found in any members of the Alligatoridae, and a study of captive *A. mississippiensis* suggests that this species cannot maintain homeostasis in hyperosmotic water (Lauren 1985). Some contradictory evidence has been provided by Chen *et al.* (1989) who noted that the lingual glands of *A. sinensis* function as salt secreting glands and also serve to lubricate the passage of food. Steel (1989) postulated that "this subfamily (Crocodylinae) originated in a marine environment and subsequently colonised a freshwater habitat, or conversely, that all crocodylid subfamilies were of freshwater origin but only the Crocodylinae acquired a saltwater adaption." Bellairs (1989) also suggested that modern crocodylines had ancestors with "markedly marine habits" and cites Taplin (1984). From illustrations presented by Taplin & Grigg (1981), Minnich (1982), Taplin (1988) and Grigg & Gans (1993), it may be deduced that the pores seen by them in the estuarine crocodile represent the region of glandular tissue observed in *C. niloticus* during this study.

No physiological examination of the glands in the

tongue of *C. niloticus* was carried out during this study and it is therefore not possible to unequivocally identify them as "salt secreting". However, the histological structure of the lingual glands observed in the Nile crocodile is similar to that described in *C. porosus*, despite the difference in classification of the glands as "branched coiled tubular" (*C. niloticus*—this study) or "compound tubular glands" (*C. porosus*—Taplin & Grigg 1981). A noteworthy difference was that the ducts connecting the main secretory duct to the secretory units was lined by a simple columnar epithelium in the Nile crocodile as opposed to the simple squamous epithelium identified in *C. porosus* (Taplin & Grigg 1981).

It was noted in the present study that lymphoid tissue was closely associated with the glandular tissue, particularly with the large ducts linking the glands to the surface of the tongue. A similar observation was made by Taguchi (1920) in the three species he examined (*A. sinensis*, *C. niloticus* and *C. porosus*) and he described lymph follicles in close proximity to the secretory ducts of the lingual glands as well as lymphocytic penetration of the epithelial layer of the ducts. These lymphoid aggregations prompted Taguchi (1920) to state that they "remind one of tongue tonsilli of higher animals." Varying degrees of lymphocytic infiltration of the main secretory duct was observed in the present study and the amount and location of the lymphoid tissue would warrant reference to these structures as lingual tonsils.

ACKNOWLEDGEMENTS

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