

MACRO- AND MICROMORPHOLOGICAL STUDIES ON THE LARYNGEAL MOUND OF TURKEY

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ABSTRACT

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Ten turkey birds were used to study the morphological features of the laryngeal mound grossly, histologically and by scanning electron microscopy. The laryngeal mound appeared to consist of two adjoining, raised; quadrilateral mucosal plates located in the floor of the pharynx. It divided into two parts by laryngeal inlet which was V- shaped. The length of the mound and its inlet measured about 20.45 mm and 13.6 mm respectively. By SEM, the dorsal surface of the laryngeal mound appeared scaly and carried few small conical papillae at its caudal part. At the caudal end of mound, the laryngeal papillae arranged into two rows of the papillae; each row included 20 – 24 conical papillae. The rostral row consisted of long spinous conical papillae which carried numerous fine elongated papillae toward their bases and displayed two large gaint papillae on the midline. The caudal row had short conical papillae, their bases were surrounded by numerous thin thron-like papillae. Microscopically, the laryngeal mound covered with stratified squamous epithelium and gradually transformed to pseudostratified ciliated columnar epithelium (respiratory epithelium) at the laryngeal inlet. The cricoarytenoid salivary glands were presented in the lamina propria of the lateral part of the laryngeal mound. Their ducts penetrated the mucosa to open into the pharyngeal cavity. Each gland was formed from a varying number of units, each unit comprising many tubules, opening into a common cavity and possessing a common duct. The glandular tubules lined by mucous secreting cells showing strong PAS positive reaction.

Key words: *laryngeal mound, turkey, cricoarytenoid salivary, laryngeal papillae*

INTRODUCTION

The domesticated turkey is a large poultry bird. The modern domesticated form descends from the wild turkey (*Meleagris gallopavo*), one of the two species of turkey (genus *Meleagris*). It was domesticated by the indigenous peoples of Mesoamerica at least 2,000 years ago. In all the birds, the respiratory system continued with a unique choana into the larynx after the nasal cavity. The anatomy, physiology, and mechanics of avian larynx were distinctly different from that of mammals (Reece 2005 and Nash, 2007). The morphology of the larynx in different avian species was studied by several authors (King, and Roberts, 1965; White, 1975; King and McLelland, 1984; Pesek, 2000; Kabak *et al.*, 2007 and AL-Mussawy, 2011). However, the on the morphological studies of the mound of the turkey laryngeal seems to be less sufficient. So this study aims to describe the

morphological features of the laryngeal mound and the entrance of the larynx of the turkey, and to compare the obtained findings with those of other birds in the available literatures.

MATERIALS and METHODS

Ten healthy adult turkeys obtained from Assiut governorate farms were used. Specimens were prepared by bleeding of birds, then heads were separated and the oropharynx was opened. Each specimen of laryngeal mound was dissected and the gross features, position and shape was described. The different dimensions including the length of pharyngeal floor and laryngeal mound as well as the length and width of the laryngeal inlet were measured. Histological observations were made on tissue samples from the different parts of the larynx and laryngeal mound. Sample were fixed in Bouin's fluid and processed for paraffin sections 5-7 4µm

thick. Sections were stained with Haematoxylin and Eosin and Crossman's Trichrome for general histological observation. A modified periodic acid-Schiff (PAS) technique was used to detect the carbohydrates; control sections for glycogen were treated with 0.1 % malt diastase for 30 minutes at 37 °C. For Scanning electron microscopy (SEM), specimens were immersed in 5% glutaraldehyde, post-fixed in 1% Osmium tetroxide, dehydrated in alcohol followed by amyl acetate and critical point dried using liquid CO₂ and mounted on specimen stubs, sputter-coated with gold and examined by a JEOL 5400 LV scanning electron microscope. All fixation and stain techniques were adopted after (Bancroft and Gamble, 2002).

RESULTS

Anatomically (Table 1 and Figs. 1 & 2): The laryngeal mound was a musculoskeletal prominence projected within the floor of the pharyngeal cavity. It measured about 20.45 mm long and consisted of two adjoining, raised, quadrilateral plates. It was separated from the base of the tongue by transverse gap measured about 6.5 mm long and extended to the first tracheal ring and esophageal entrance caudally. The laryngeal mound bore V - shaped laryngeal inlet which laid caudal to the level of the angle of the mouth by about 10 mm. The inlet had rostral wide and caudal acute narrow commissures. The inlet was limited by laryngeal arytenoid cartilages and measured 13.6 ± 0.26 mm long and 3.2 ± 0.01 mm wide. The rim of the laryngeal inlet appeared smooth. The caudal commissures of the laryngeal inlet continued caudally by laryngeal sulcus till termination of the laryngeal mound. The laryngeal mound was marked caudally by two transverse rows of conical caudally directed papillae. Each row comprised 20 – 24 in number. The rostral row consisted of long spinous conical papillae and displayed two large gaint papillae on the midline. These centrally positioned papillae were bounded the laryngeal sulcus, while the caudal row had short conical.

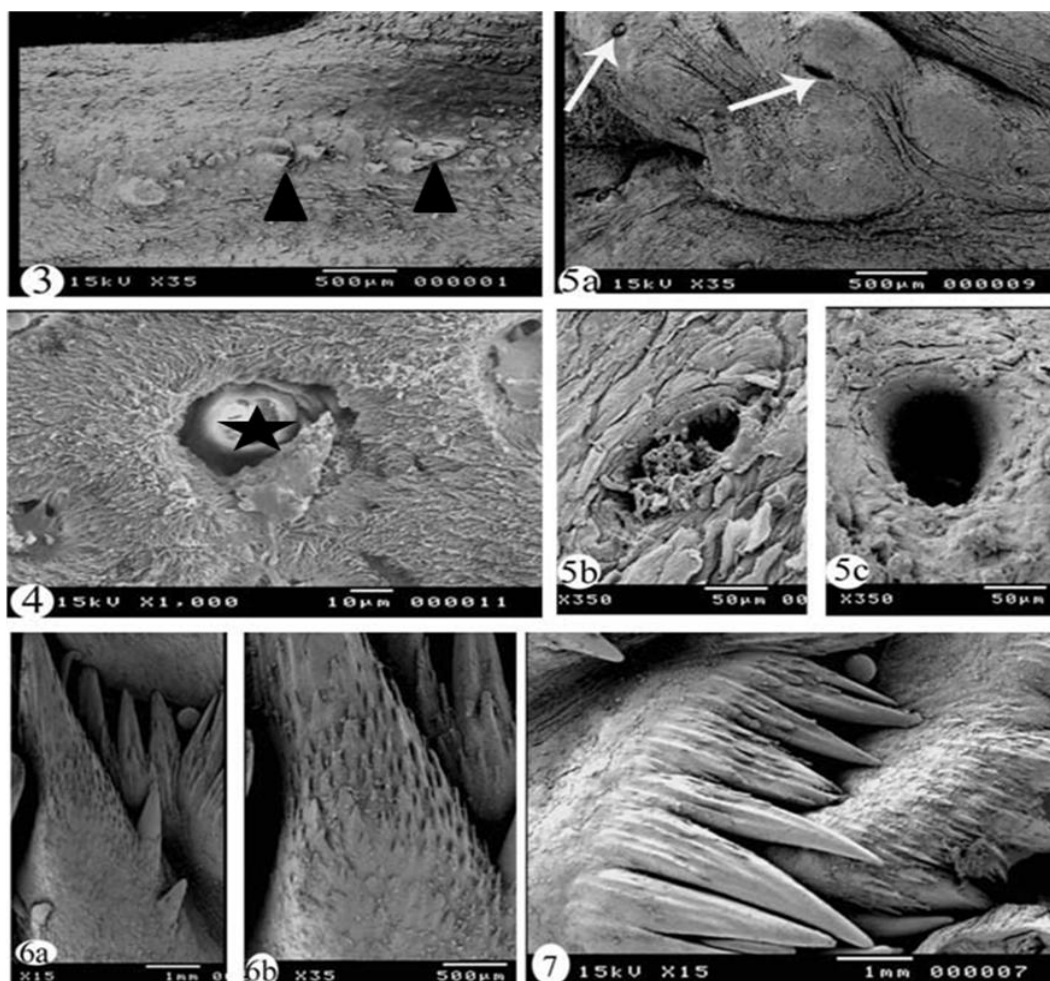
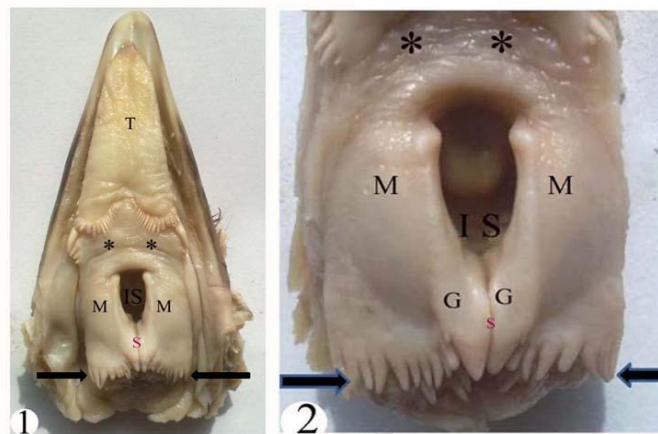
By scanning electron microscope (Figs. 3 - 7): The mucosal surface of the laryngeal mound mostly appeared scaly and carried few small conical papillae scattered on the caudal part. The rim of the laryngeal inlet appeared free from these papillae. The rostral transverse row of the laryngeal papillae consisted of long spinous conical papillae pointed caudally which carried numerous fine elongated papillae toward their bases. The median giant papillae were thick and conical in shape. They carried secondary small conical papillae at their bases and numerous fine

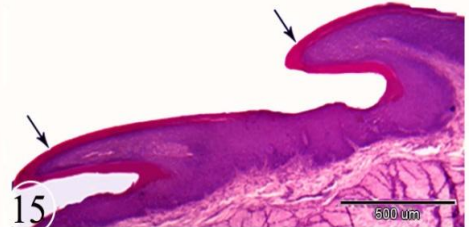
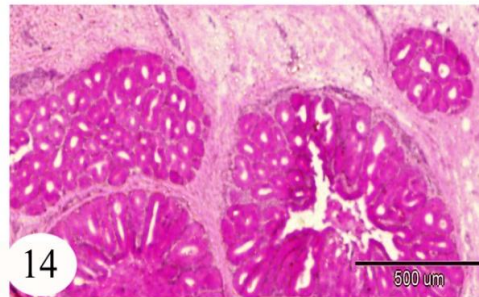
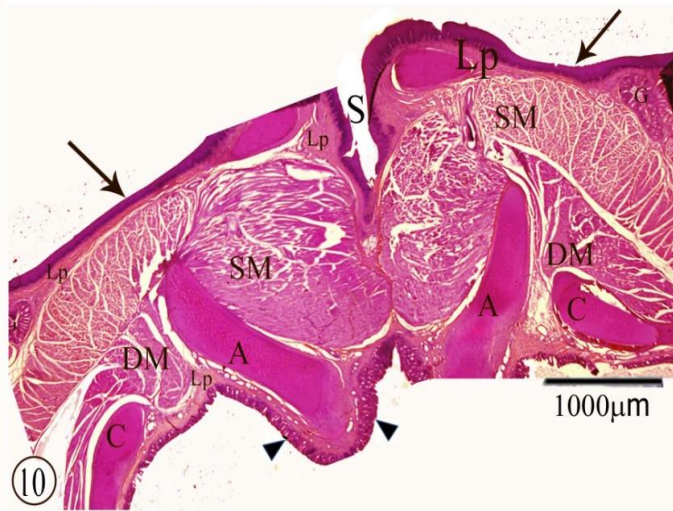
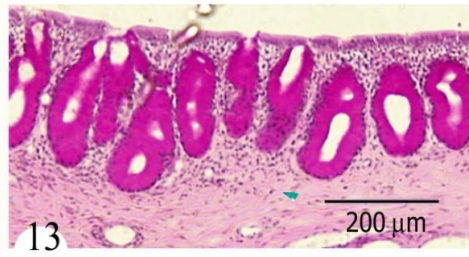
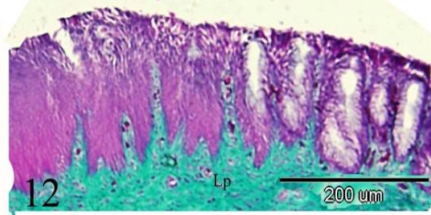
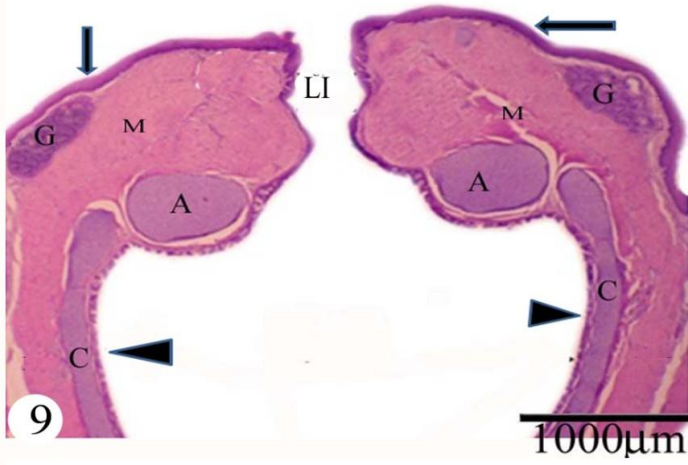
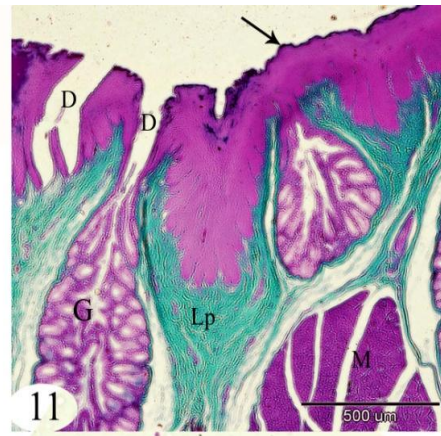
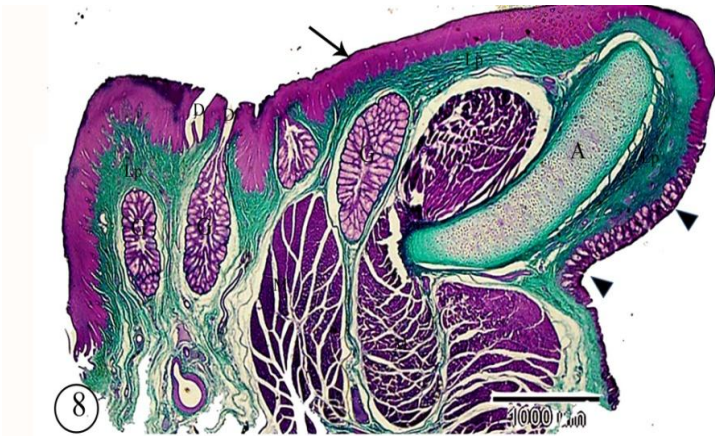
papillae direct toward their apices. The caudal transverse row had short conical papillae. Their bases were surrounded by numerous thin thorn-like papillae. The openings of the salivary glands were demonstrated in two groups; the rostral one was represented by three mucosal elevations arranged nearly in a longitudinal row on both sides of the rostral part of the laryngeal inlet. These elevations contained oval or slit-like openings. The caudal group were scattered on the mucosal laryngeal mound near the papillae. The ventral mucosal surface of the laryngeal mound and laryngeal cavity was covered by dense long cilla interrupted by the openings of the mucous glands.

Histologically (Figs 8 – 15): The laryngeal mound constituted epithelium, lamina propria, hyaline cartilage and muscular layer. The paharyngeal surface of the laryngeal mound was covered by keratinized stratified squamous epithelium which was gradually transformed into respiratory epithelium at the edges of the laryngeal inlet. The whole laryngeal cavity was lined by pseudostratified ciliated columnar epithelium (respiratory epithelium). The lamina propria of the respiratory epithelium was formed of highly cellular connective tissue, rich in collagenous fibers and contained simple tubular mucous glands. These glands were lined by single layer of columnar mucous secreting cells with small flattened, basally located nuclei and faintly marked borders. These cells had foamy, vacuolated, faintly stained basophilic cytoplasm and strongly PAS positive material. Beneath this epithelium, there is a thick connective tissue layer of the lamina propria submucosa contained group of the laryngeal glands. Their ducts penetrated the mucosa to open into the pharyngeal cavity. Each gland was formed from a varying number of units, each unit comprising many branche tubuloalveolar mucous glands opened into a common cavity and possessing a common duct. These glands were lined by columnar secretory cells with small flattened basally located nuclei and their cytoplasm reacted strongly positive with the PAS stain. The cartilaginous layer was consisted of hyaline covered dorsally with the muscular layer and mucosa but at the rostral part of inlet edges was covered only by the mucosa. The muscular layer consisted of skeletal muscle layer laid under the mucosa of the dorsal surface of the laryngeal mound. It gave the shape appearance of laryngeal mound. The deep intrinsic muscles were thick in the laryngeal mound and thin in the lateral wall of the larynx. The laryngeal papillae were appeared a cornified projections consisting of connective tissue core capped by highly keratinized epithelium.

Table 1: The dimensions (mm) of the laryngeal mound.

Laryngeal mound dimensions	mm
-Length of laryngeal mound	20.45 ± 0.72
- Ratio (%) of laryngeal mound to pharyngeal floor	73.62 ± 0.55%
- Length of laryngeal inlet (glottis)	13.6 ± 0.26
- width of laryngeal inlet (glottis)	3.2 ± 0.1
-Distance between tongue and laryngeal mound	6.5 ± 0.51





LEGENDS

- Figs. (1 & 2):** Photograph, showing the dorsal view of the floor of the oropharynx entry: tongue (T), the laryngeal mound (M), laryngeal inlet (IS), transverse gap between the tongue and larynx (*), caudal laryngeal papillae (Arrows), laryngeal sulcus (S).
- Fig. (3):** Scanning electron micrograph of the right caudal part of laryngeal mound. Note the dorsal surface was scaly and carried few small conical papillae (Arrowheads).
- Fig. (4):** Scanning electron micrograph of the rostral part of laryngeal cavity, showing the openings of the mucous glands (star) surrounded by ciliated epithelium.
- Fig. (5a):** Scanning electron micrograph of the lateral part of laryngeal mound. Showing the openings of cricoarytenoid glands (arrows).
- Figs. (5b & c):** Scanning electron micrographs of higher magnification of the openings of cricoarytenoid glands (Arrows).
- Fig. (6a & b):** Scanning electron micrographs of the medial giant conical papillae carried smaller secondary papillae at their bases (6a) and numerous fine papillae towards its apex (6b).
- Fig. (7):** Scanning electron micrograph showing the laryngeal papillae were arranged on two transverse rows papillae; the rostral one consisted of long spinous conical caudally pointed papillae which carried numerous fine elongated papillae at their bases.
- Fig. (8):** Photomicrograph of the rostral part of the laryngeal mound, showing arytenoid cartilages (A), covering epithelium (arrows) is transformed into respiratory epithelium (arrow heads) in the laryngeal cavity, cricoarytenoid glands (G) and their ducts (D), muscular layer (m), Lamina propria (Lp). Crossmon's Trichrom stain.
- Fig. (9):** Photomicrograph of the middle part of laryngeal mound, the arytenoid cartilages (A) is separated from the covering (arrows) by thick muscular layer (M), laryngeal inlet (LI), respiratory epithelium (arrow heads), cricoarytenoid glands (G), cirocoid cartilage (C) lamina propria (Lp). H& E stain.
- Fig. (10):** Photomicrograph at the caudal commisure of laryngeal mound, the arytenoid cartilages (A), covering epithelium (arrows), muscular layer (M), respiratory epithelium (arrow heads), cricoarytenoid glands (G), cirocoid cartilage (C), lamina propria (Lp). H& E stain.
- Fig. (11):** Higher magnification of the laryngeal mound, showing epithelium covered with thin keratin (arrow), lamina propria and papillary invagination (LP), Two of cricoarytenoid glands and their cavities (G), openings and ducts (D), (M) muscular layer. Trichrom stain.
- Fig. (12):** Higher magnification, showing the transformation of the epithelium at the largngeal inlet. Trichrom stain.
- Fig. (13 & 14):** The mucosa of the laryngeal cavity and cricoarytenoid glands was reacted strongly with PAS. PAS stain.
- Fig. (15):** Photomicrograph showing highly cornified epithelium covering the laryngeal papillae (arrows). H& E stain.

DISCUSSION

The laryngeal mound was a musculoskeletal prominence projected within the caudal part of the floor of the pharyngeal cavity and consisted of two adjoining, raised, quadrilateral plates. These results were fully confirmed to that was reported in nm the duck, goose chicken, turkey, and long-legged buzzard finding (White, 1975; Bacha and Bacha, 2006; Kabak *et al.*, 2007 and AL-Mussawy *et al.*, 2011), but uncorresponding with Lbe *et al.* (2008) who described the laryngeal mound as roughly triangular in shape in the west african guinea fowl. The present work revealed that the laryngealmound divided into two halves by V- shaped laryngeal inlet which was limited by the arytenoid cartilages. These findings consequences agreed with Lbe *et al.* (2008) and

Tadjalli *et al.* (2008) in the West African guinea fowl and ostriches. In domestic fowl and geese, the largngeal inlet was slit like opening (King and Mclelland, 1984 and Alsayed, 2010). The mean length of glottis in the examined turkey was about 13.6 mm nearly corresponded with that reported by White, (1975) who mentioned that the length of the glottis was about 11 mm in the chicken, 15 mm in turkey and 13 mm in duck and goose. Kabak *et al.* (2007) referred that the length of the glottis was 9 mm in long legged buzzard. In addition, Tadjalli *et al.* (2008) who stated that the glottis in the ostriches was very large and its mean length was 33.3. The width of the turkey glottis was 3.2 mm; this unconsequence agreement with White, (1975) who said that the width of glottis was 5.0 mm, 3.0 mm, and 4.0 cm in turkey, duck, and goose respectively. In legged buzzard the

width of the glottis measured about 1.86 mm (Kabak *et al.*, 2007). Functionally, the turkey glottis was unlike that of mammals, it was not covered by an epiglottis and there was no soft palate so the laryngeal inlet (glottis) regulates the passage of air by a dilator and constrictor muscle that prevents aspiration of food material. The larynx plays no role in sound production (King and Mclelland, 1984). It could be thought as a valve atop the lungs which can prevented air flow out of the lungs or the inward flow of foreign matter like food or water into the lungs (Fitch, 1994; Tadjalli *et al.*, 2008 and SmallWood, 2010).

The present work indicated that the laryngeal papillae were arranged in two transverse rows at the caudal end of the mound. The rostral row consisted of long spinous conical papillae which carried numerous thin thorns like papillae at their bases and displayed two large gaint papillae in the midline. These consequences corresponding with (White, 1975) in turkey, and incongruity with Hassouna, (2002) who mentioned the presence of 5-7 transverse rows of thin, medium-sized caudally directed papillae laid in ducks and Kabak *et al.* (2007) who described two sagittal rows of 5-6 small papillae run parallel with the rims of the inlet and dorsal furrow chicken and long legged buzzard. In cage and aviary birds, a few filiform papillae are distributed over the laryngeal prominence and caudal floor of the pharynx (Evans, 1996). While in ostrich the papillae were not visualized on the larynx (Tadjalli *et al.*, 2008). The backward-pointing cornified papillae of the mound assisted in the ingestion of solid particles and helped in the raking movement of the larynx during swallowing (White, 1975; King and Mclelland, 1984 and Fitch, 1994).

Histologically, the present study revealed that the laryngeal mound of the turkey was covered with stratified squamous epithelium that gradually transformed to pseudostratified ciliated columnar epithelium (respiratory epithelium) at the laryngeal inlet. The respiratory mucosa contained many simple tubular mucous glands which also associated with the respiratory epithelium of other birds. This in corresponding with that reported of Samuelson, (2007) in the bird respiratory system. The surface epithelium of turkey laryngeal mound was interrupted by the openings of simple, branched alveolar mucous gland. These findings consequences agreed with Bacha and Bacha, (2006) in other avian species. The mucous glands at the laryngeal mound called cricoarytenoid salivary glands (King and Mclelland, 1984; Liman *et al.*, 2001 and Mohamed, 2004) while, Homberger and Meyers (1989) named these glands as laryngeal glands. As reported by McCallion and Aitken, (1953) in the fowl, the laryngeal glands of turkey were located within the lamina propria and their ducts penetrated the mucosa to open into the

pharyngeal cavity. Each gland was formed from a varying number of units, each unit comprising many tubules which open into a common cavity and possessing a common duct. The glandular tubules lined by mucous secreting cells showing strongly positive PAS reaction. In the birds, the main function of the saliva is to act as lubricant in swallowing (King and Mclelland, 1975). In this study, the strong PAS-positive reaction was observed within the cells lining the laryngeal glands indicating the presence neutral mucopolysaccharides. This observation was similar to those reported in the lingual and laryngeal gland of quails (Liman *et al.*, 2001), lingual glands of the chicken (Gargiulo *et al.*, 1991) and quail (Menghi *et al.*, 1993). Furthermore, sialidase-labile sialomucins (N-acetyl sialomucins) were PAS positive (Bancroft and Cook, 1984). For this reason, the PAS-positive reaction could be revealed the presence of sialidase-labile sialomucins in the secretory parts of these glands as stated in chicken (Suprasert *et al.*, 1986; Gargiulo *et al.*, 1991; Menghi *et al.*, 1993) and in the linguolaryngeal minor salivary glands of penguin (Samar *et al.*, 1999).

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دراسات ماكرو وميكرومورفولوجية على التل الحنجري في الرومي

عبد المهيمن مصطفى محمد صالح

اجريت الدراسة على عدد عشرة من طيور الرومي البالغة السليمة ظاهريا تم جمعها من مزارع محافظة اسيوط. حيث تم دراسة التركيب المورفولوجي للتل الحنجري عينيا وهستولوجيا باستخدام الميكروسكوب الضوئي والماسح الالكتروني. وجد مدخل الحنجرة الذي ظهر على شكل حرف V يقسم تل الحنجرة ينقسم الى جزئين بواسطة . يبلغ متوسط طول تل الحنجرة حوالي 20.45 مم وطول مدخلها حوالي 13.6 مم على التوالي. باستخدام الماسح الالكتروني، لوحظ ان السطح الظهري لتل الحنجرة يغطي بخلايا حرشفية كما ظهرت بعض حلمات مخروطية صغيرة في الجزء الخلفي. كما لوحظ وجود الحلمات الحنجرية في الطرف الخلفي لتل الحنجرة مرتبة في صفين؛ يتألف كل صف منها من 20-24 من الحلمات المخروطية. ويتكون الصف الامامي من حلمات مخروطية شوكية التي تحمل في اتجاه قواعدها العديد من الحلمات الطولية الدقيقة. ويتوسط هذا الصف اثنان من الحلمات العملاقة. بينما يتكون الصف الخلفي على حلمات مخروطية قصيرة يحاط قواعدها بالعديد من الحلمات الدقيقة. مجهريا، تبين ان التل الحنجري مغطى بطلائية حرشفية تحولت تدريجيا إلى طلائية عمودية كاذبة مهدبة (طلائية الجهاز التنفسي) في مدخل الحنجرة. وجدت الغدد اللعابية الحلقية الطرجهالية في صفيحة النسيج الضام على جانبي التل الحنجري حيث اخترقت قنواتها الغشاء المخاطي لتفتح في التجويف البلعومي. تكونت كل غدة من اعداد مختلفة من الوحدات الغددية، كل وحدة تضم العديد من الأنابيب تفتح في تجويف مشترك يؤدي الى قناة عامة للغده. بطنت الأنابيب الغددية بخلايا مخاطية كانت إيجابية التفاعل مع صبغة PAS .