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Histochemical Characterization of the Lingual Salivary Glands of the Eurasian Collared Dove, *Streptopelia decaocta*

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Abstract: Histochemical characterization of the lingual salivary glands of the collared dove *Streptopelia decaocta* has been conducted. The glands are composed of two entities at the dorsal second half of the free part of the tongue and in the dorsal part of its base. The lingual glands are made of mucous cells that elaborate neutral mucosubstances together with sialomucins and sulfomucins liable to neuraminidase and hyaluronidase respectively, but are devoid of proteins. The results of the present study are discussed in context of the feeding habits of the bird.

Key words: Salivary glands, lingual, histochemistry, collared dove, *Streptopelia decaocta*

Introduction

Histochemical investigation on the secretions of the lingual salivary glands of vertebrates are mainly concentrated on mammals, with little attention being paid to non-mammalian vertebrates, especially a birds (Fujii and Tamura, 1966; Jerrett and Goodge, 1973; Taib and Jarrar, 1998). Salivary glands are absent in some birds and present in others. They are the best developed and quite functional in seed and insect eaters and least developed in birds that eat soft diet (King and McLelland , 1984; Blanks, 1993). Saliva in birds is primarily a lubricant or a sticky coat to tongue to trap insects and seeds and in some birds are used also in gluing together the ingredients used in building the nest.

The present study was performed to characterize histochemically the secretions of the lingual salivary glands of the collared dove *Streptopelia decaocta* , a common bird in many cities and towns of Saudi Arabia and shows increasing presence from the Indian subcontinent to Arctic Circle in Scandinavia.

Materials and Methods

Twelve adults of both sexes collared dove *Streptopelia decaocta* were killed by decapitation. The whole tongue with the surrounding tissues was removed from each bird and quickly immersed in one of the following fixatives: neutral buffered formalin, Bouin's fluid and Gendre's fluid.

Fixed materials were then thoroughly washed in running water, sectioned at 4-5 μm thickness and stained with hematoxylin-eosin and Masson trichrome stains for histological examination. Other paraffin sections were then utilized in the following histochemical reactions:

Neutral mucosubstances: Periodic acid-Schiff (PAS) technique (Gurr, 1962), PAS after diastase digestion (McManus and Mowry, 1964), PAS after α-amylase digestion (Luna, 1968), PAS after acetylation blockade (McManus and Cason, 1950), PAS after acetylation-saponification (Ozello *et al*., 1958), and PAS after phenylhydrazine treatment (Spicer *et al*., 1967).

Acid mucosubstances: Alcian blue (AB) at pH 2.5 and 1.0 (Mowry, 1956; Luna, 1968).

Distinction between acidic and neutral mucosubstances: AB (pH 2.5)-PAS (Mowry and Winkler, 1956) and AB (PH 1.0)-PAS (Spicer *et al*., 1967).

Distinction between sulfomucins and sialomucins: Aldehyde fuchsin (AF) and AF-AB, pH 2.5 (Spicer and Meyer, 1960); weak (25 °C, 16 hr), mild (37 °C, 4hr) or strong (60 °C, 4hr) methylation-saponification-AB, pH 2.5 (Quintarelli *et al*., 1961); acid hydrolysis (0.1 N HCl, 60 °C, 4hr)-AB (pH 2.5) (Spicer *et al*., 1967); toluidine blue (TB) buffered at pH 1.7 and 3.4 (Landsmeer, 1953); Critical electrolyte concentration (CEC) technique for extinction of alcianophilia at pH 5.6 in the presence of gradual concentration of Mg^{+2} (Scott and Dorling, 1965).

Enzyme digestion tests: Diastase-PAS technique (McManus and Mowry, 1964); neuraminidase (Sialidase, Vibrio cholerae, type V)-AB at pH 2.5 (Spicer and Warren, 1960); hyaluronidase (testicular)-AB pH 2.5 (Spicer *et al*., 1967), neuraminidase-TB (pH 3.7) and hyaluronidase-TB pH 2.0 (Pearse, 1972). Control sections were incubated in the buffer solutions without enzymes.

Protein tests: Mercuric bromophenol blue (Mazia *et al*., 1953); ninhydrin-Schiff (Yasuma and Itchikawa, 1953) and chloramine T-Schiff (Pearse, 1972) methods were used for detection of proteins. Photographs were taken with a 35mm Zeiss Ikon camera on Kodacolor NR 100 film.

Results

The lingual salivary glands of the collared dove are located in the laminia propria of the second half of the free part of the tongue and in the dorsal part of its base (Fig. 1), while the

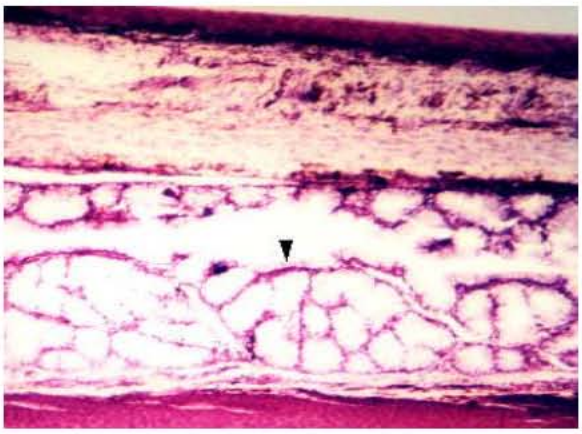

Fig. 1: Lingual salivary glands of *S. decaocta* stained with hematoxylin-eosin. x 450.

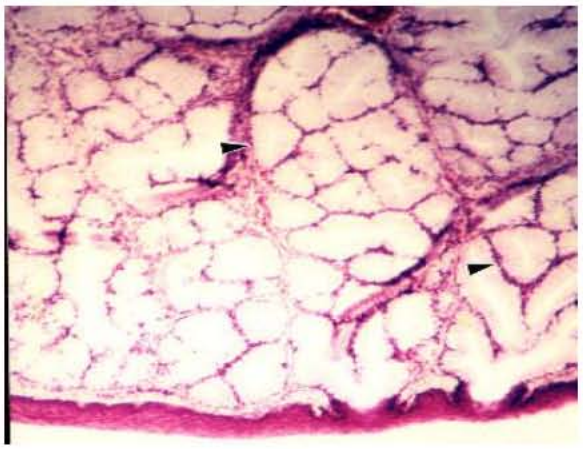

Fig. 2: Lingual salivary glands of *S. decaocta* stained with hematoxylin-eosin. x850.

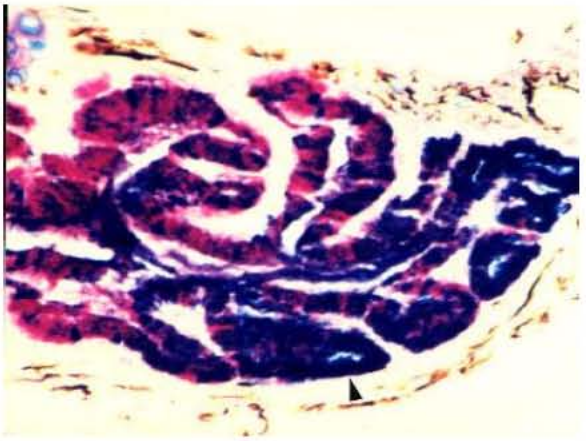

Fig. 5: Lingual salivary glands of *S. decaocta* stained with AB (pH 2.5)-PAS.x850.

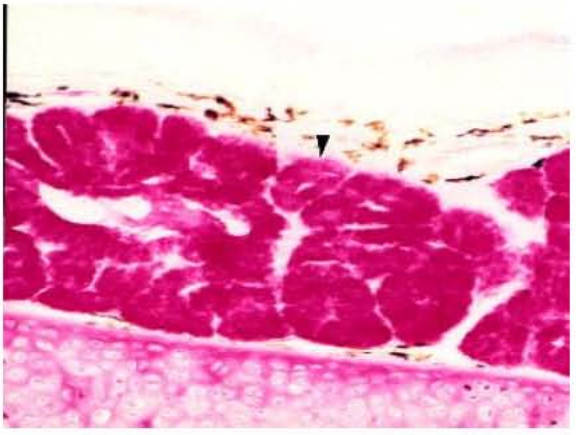

Fig. 3: Lingual salivary glands of *S. decaocta* stained with PAS, x 450.

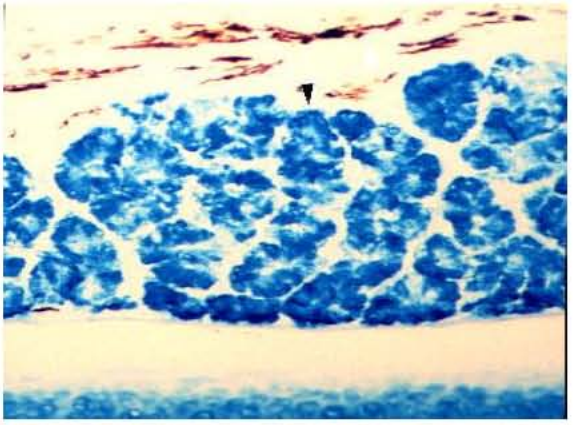

Fig. 6: Lingual salivary glands of *S. decaocta* stained with CEC. x 450.

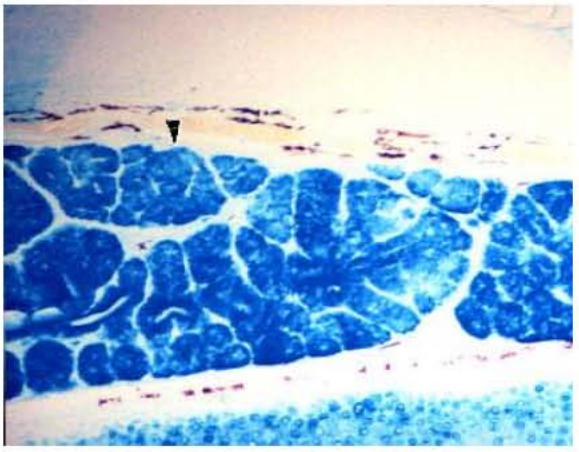

Fig.4: Lingual salivary glands of *S. decaocta* stained with AB (pH 2.5). x450.

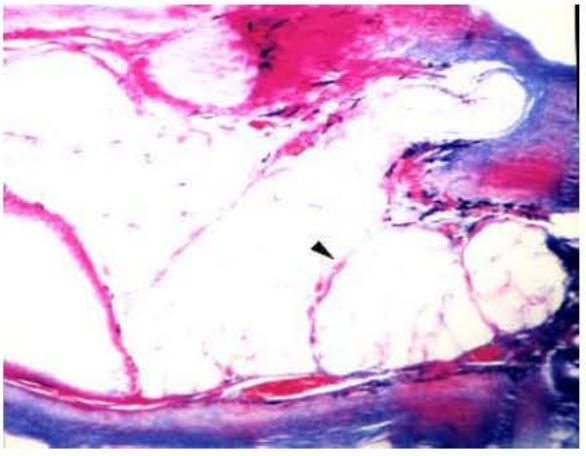

Fig. 7: Lingual salivary glands of *S. decaocta* stained with Ninhydrin- Schiff. x 850.

anterior portion of the tongue is devoid of any glandular structure. The anterior lingual glands are interspersed between the stratified squamous epithelium lining the dorsal and the ventral surface of the tongue while the posterior ones are located between the dorsal mucosa of the tongue and its intrinsic muscle bundles lateral and dorsolateral to the paraglossal bone. The secretory end pieces of the lingual glands consisted of tall columnar mucous cells with flattened nuclei close to the proximal pole of the cells resting at a delicate basement membrane. The cytoplasm was extensively vesicular, and thus stained lighter with H & E (Fig. 2). As summarized in Table 1, the mucous cells of the lingual salivary glands exhibited strong PAS reaction (Fig. 3), that was neither liable to α-amylase, saliva digestion nor was it blocked by prior phenylhydrazine treatment. However, this reactivity was blocked by acetylation, but was partly restored by deacetylation-PAS sequential treatments. The glands also exhibited marked alcianophilia at pH 2.5 (Fig. 4) and at pH 1.0. but to lesser extent at pH 0.4 (Fig. 4) that were liable to neuraminidase and to a lesser extent to hyaluronidase digestion. This alcianophilia was partially blocked by acid hydrolysis and by weak or moderate methylation . It was completely abolished by strong methylation but was partly restored by methylation saponification sequential techniques. Cells of the lingual salivary glands positively stained bluish purple with both AB (PH 2.5)-PAS and AB (PH 1.0)-PAS (Fig. 5). Moreover, they also reacted strongly to critical electrolyte concentration technique at 0.1, 0.2, 0.3, 0.4M $MgCl_2$, but less so at 0.5M $MgCl_2$, and the alcianophilia have disappeared completely at 0.6M $MgCl_2$ (Fig. 6) and showed metachromasia at pH 3.4 and 1.7 but have reacted negatively with all techniques used to detect proteins (Fig. 7). No sexual dimorphorism was observed in the lingual secretion of the species under study.

Table 1: The histochemical reactions in the lingual salivary glands of collared dove, *Streptopelia decaocta*

Histochemical reactions *	Results
PAS	+ +
Diastase digestion-PAS	Nb
α-amylase-PAS	Nb
Acetylation-PAS	-
Acetylation-deacetylation-PAS	+ +
Phenylhydrazine-PAS	Cb
AB(pH 1.0)	+ +
AB (pH 2.5)	+ +
AB (pH 1.0)-PAS	+,Bp
AB (pH 2.5)-PAS	+ +,BP
AF	+
AF-(AB pH 1.0)	+,Bp
AF-(AB pH 2.5)	+,B
Acid hydrolysis-AB (pH 2.5)	+B
W. methylation-AB (2.5)	±B
W. methylation-saponification-AB (pH 2.5)	+B,Pb
M. methylation-AB (pH 2.5)	±
M. methylation-saponification-AB (pH 2.5)	+, B
S. methylation-AB (pH 2.5)	-,Cb
S. methylation-saponification-AB (pH 2.5)	+,B
CEC (AB, 0.1M)	+ +
CEC (AB, 0.2M)	+ ±
CEC (AB, 0.4M)	+
CEC (AB, 0.5M)	-
Neuraminidase-AB (pH 2.5)	-
Neuraminidase-TB (pH 3.7)	+, Pb
Hyaluronidase-AB (pH 2.5)	+, B
Hyaluronidase-TB (pH 2.0)	Pb
Ninhydrin-Schiff	-
Hg-bromophenol blue	-
Chloramine T-Schiff	-

***Reactions:** -, negative; ±, very weak; +, feeble; + +, moderate.
Blocking: Cb, complete blockade; Pb, partial blockade; Nb, no blockade
Colours: B, blue; Bp, bluish purple.

Discussion

On the basis of the histochemical results and in view of the criterion of Gabe and Saint-Girons (1969), the lingual salivary glands of the collared dove, *S. decaocta* are of the mucous type that have reacted negatively to proteins, but positively to mucosubstances. A tentative interpretation of the type of those mucosubstances could be made according to the results of the present study, and on the basis of the classification of mucosubstances proposed by other workers such as Mowry and Winkler (1956); Pearse (1972); Scott and Dorling, (1965) and Spicer and Meyer (1960). Thus, the lingual salivary glands of *S. decaocta* found in this study to be PAS positive, diastase resistant as well as unstainable by cationic dyes are considered as glands that secrete neutral mucosubstances . On the other hand, lingual salivary glands were positive to the specific technique of alcian blue (Mowry and Winkler, 1956), the aldehyde fuchsin-alcian blue technique of Spicer and Meyer (1960), and the methods of Drury *et al.* (1967) are considered as glands that secrete acid mucosubstances. The lingual glands on the other hand, were found to contain neutral mucosubstances, sialidase-liable sialomucins and hyaluronidase-resistant sulphomucins.

The results of the present study clearly demonstrate that the secretory products elaborated by the lingual salivary glands of *S. decaocta* are different from those secreted by the similar glands in birds such as the white Leghorn breed of chicken and the quil, *Coturnix coturnix*. In the chicken the lingual glands were reported to be only of mucous type and the anterior lingual glands elaborate mainly nonsulphated mucosubstances while the posterior ones consist mainly of sulphated mucopolysaccharides (Fujii and Tamura, 1966). In the quil, *C. coturnix*, the lingual glands are of serous type that have reacted positively to proteins but negatively to mucosubstances and mucous type that have reacted negatively to proteins and positively to neutral and acid mucosubstances (Taib and Jarrar, 1997). The secretory products of the lingual salivary glands of *S. decaocta* form a blend of mucous saliva which may lubricate the ingested food and facilitate the swallowing. Moreover, secretions of the mucous lingual glands of the collar dove may act as a protective covering for the surface of the mucous membrane of the upper digestive tract .

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