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THE HISTOCHEMISTRY OF THE LINGUAL SALIVARY GLANDS OF THE LIZARD *Acanthodactylus schmidti* (WIEGMANN) (REPTILIA, LACERTILIA, LACERTIDAE)*

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Abstract

The lingual salivary glands of *Acanthodactylus schmidti* were investigated histochemically and were observed to secrete and elaborate neutral mucosubstances, sialidase labil carboxylated mucosubstances and hyaluronidase resistant sulfomucins but no glycoproteins. These results are discussed in the context of the feeding habits and phylogeny of reptiles.

Introduction

Histochemical studies on lingual salivary glands of vertebrates are mainly concerned with mammals while little attention is being paid to the lingual glands of non-mammalian vertebrates. Few studies have been carried on the lingual salivary glands of turtles (Carmignani and Zaccone, 1975; Nalvade and Varute, 1976; Taib and Jarrar, 1985a) and some lizards (Raynaud, 1961; Gabe and ST. Girons, 1969; Taib and Jarrar, 1985b,c).

In the present study, histochemical characterization of the lingual salivary glands of the diurnal, insectivorous lizard, *Acanthodactylus schmidti* (Wiegmann) was undertaken.

Materials and Methods

Fifteen adult, male and female *Acanthodactylus schmidti*, 12.5-16.7 cm in length and 8.75-24.35 gm in weight were trapped from Riyadh Region, Saudi Arabia. They were killed by etherization and the whole tongue was removed from each animal and quickly immersed in cold (4°C) 2% calcium acetate in 10% buffered formalin (pH 7.0) and alcohol fixatives for 24 hrs. They were then thoroughly washed in running water, processed for sectioning at 4-5 µm thickness and the sections were stained with haematoxylin-eosin Masson trichrome stain for histological examination. Paraffin, as well as unfixed frozen sections were then utilized in the following histochemical reactions.

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Neutral Mucosubstances: Periodic and 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, 1968), PAS after acetylation-saponification (Ozello et al., 1958), PAS after phenylhydrazine treatment (Spicer et al., 1967) and PAS after treatment with chloroform and methanol.

Acid mucosubstances: Alcian blue (AB) at pH 2.5, 1.0, and 0.4 (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, 4 hr) and strong (60°C, 4 hr) methylation-saponification-AB, pH 2.5 (Quintarelli et al., 1961); acid hydrolysis (0.1N HCl, 60°C, 4 hr)-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^{++} (Scott and Dorling, 1965). Sections of the lingual glands of the lizard *Uromastyx microlepis* were used as controls for sialomucins (Taib and Jarrar, 1985b) and the mast cells population in the tongue of the species under study were used as controls for sulfomucins.

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

Proteins: Mercuric bromophenol blue method (Mazia et al., 1953); ninhydrin-Schiff (Yasuma and Itchikawa, 1953), chloramine-T-Schiff (Pearse, 1972).

Photographs were taken with a 35 mm. Zeiss Ikon camera on kodacolor NR 100 film.

Results

The tongue of *A. schmidti* is lined with backward pointing filiform papillae in the dorsal epithelium. The lingual glands comprise goblet cells occurring in the papillar invaginations of the posterior third of the dorsal surface and the lateral sides of the tongue (Fig. 1); other parts of the organ are almost devoid of any glandular structure. Goblet cells gradually increase inside the crypts towards the larynx area where they become larger and more closely packed. A well developed forward-pointing fold containing mainly mucous cells is found at the most posterior part of the dorsum. The glands cells have flattened, basally located nuclei with clear apical ends and are surrounded by a delicate basement membrane.

Fig. 1

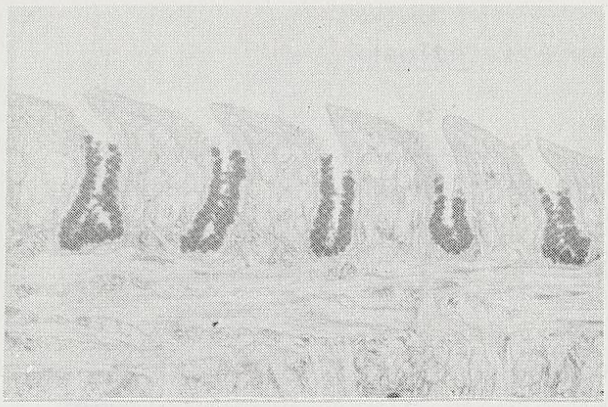

Fig. 2

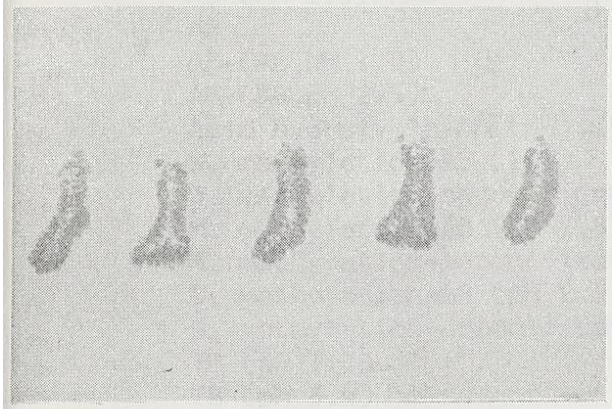

Fig. 3

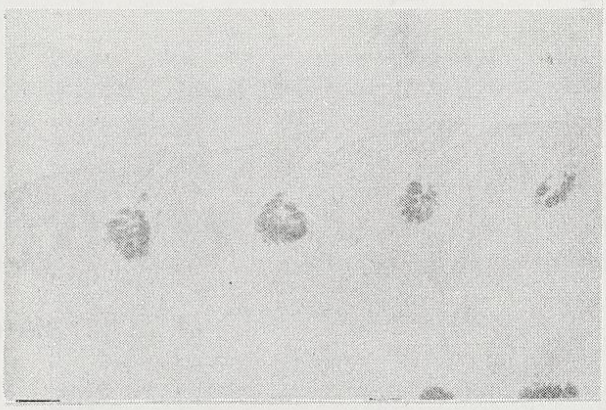

Fig. 4

Fig. 1 Lingual glands of *A. schmidti* after staining with haematoxylin-eosin. X800.

Fig. 2 Lingual glands of *A. schmidti* after staining with PAS. X420.

Fig. 3 Lingual glands of *A. schmidti* after staining with AB(pH 2.5). X420.

Fig. 4 Lingual glands of *A. schmidti* after staining with AB(pH 1.0). X370.

Fig.5

Fig.6

Fig.7

Fig. 5 Lingual glands of *A. schmidti* after staining with AB(pH 1.0)-PAS. X370.

Fig. 6 Lingual glands of *A. schmidti* after staining with AF. X450.

Fig. 7 Lingual glands of *A. schmidti* after staining with AB(pH 2.5). X370.

Table 1. The histochemical reactions in the lingual salivary glands of *Acanthodactylus schmidti*.

Histochemical Reaction	Results
PAS	+++,P
Diastase digestion-PAS	Nb;+++P
α-amylase-PAS	Nb;+++P
Acetylation-PAS	Cb
Acetylation-deacetylation-PAS	+,P
Phenylhydrazine-PAS	Nb;++P
AB (pH 0.4)	+±,B
AB (pH 1.0)	+++,B
AB (pH 2.5)	+++,B
AB (pH 1.0)-PAS	++,BP
AB (PH 2.5)-PAS	++,BP
AF	++,P
AF-(AB pH 1.0)	++,Bp,B,P
AF-(AB pH 2.5)	++,BP,B,P
Acid hydrolysis-AB (pH 2.5)	±+,B,Pb
W. methylation-AB (2.5)	±+,Pb
W. methylation-saponification-AB (pH 2.5)	+±,B
M. methylation-AB (pH 2.5)	±,Pb
M. methylation-saponification-AB (pH 2.5)	+,B
S. methylation-AB (pH 2.5)	Cb
S. methylation-saponification-AB (pH 2.5)	±,B
TB (pH 1.7)	±
TB (pH 3.4)	+
CEC (AB, 0.1 M)	+±,B
CEC (AB, 0.2 M)	++,B
CEC (AB, 0.4 M)	+,B
CEC (AB, 0.5 M)	±,B
CEC (AB, 0.6 M)	-
Neuraminidase-AB (pH 2.5)	++,Pb
Neuraminidase-TB (pH 3.7)	Pb;+B
Hyaluronidase-AB (pH 2.5)	Nb,+++B
Hyaluronidase-TB (pH 2.0)	Nb;±,B
Ninhydrin-Schiff	-
Hg-bromophenol blue	-
Chloramine T-Schiff	-
Trypsin digestion-PAS	Nb;+++,P
(Chloroform + methanol)-PAS	Nb;+++,P

Reactions: -, negative; ±, very weak; +, feeble; ++, moderate; +++, intensely positive; Cb, complete blocking; M, mild; Pb, partial blockade; Nb, no blockade; S, strong; TB, toluidine blue; W, weak.

Colours: B, blue; Bp, bluish purple; P, pink.

Table 1 summarizes the results of histochemical reactions of these glands which exhibit strong PAS reactivity (Fig. 2), neither labile to α-amylase nor to saliva digestion or to prior phenylhydrazine treatment. This reactivity is completely blocked by acetylation but is partly restored by deacetylation-PAS sequential techniques. The mucous cells of the glands exhibit marked alcianophilia at pH 2.5 and 1.0 but to lesser extent at pH 0.4 (Figs. 3 & 4). The glands react with both PAS and AB and stain bluish purple with AB(2.5)-PAS and AB(1.0)-PAS (Fig. 5). The glands react with AF (Fig. 6) as well as with AF-AB(2.5) and AF-AB(1.0). The alcianophilia of the glands is partly lost at pH 2.5 with acid hydrolysis and weak methylation and thereafter restored by saponification techniques. The glands react moderately with critical electrolyte concentration technique at 0.1M $MgCl_2$ and more intense colour is obtained at 0.2M $MgCl_2$ up to 0.6M where the complete disappearance of alcianophilia. Metachromatic reaction was observed with buffered toluidine blue at pH 1.7 and 4.5, but neither the alcianophilia at pH 1.0, nor the metachromatic at pH 2.0 were affected by hyaluronidase digestion. However, both at, respectively, pH 2.5 and pH 3.7 were partially lost by neuraminidase digestion. Moreover, these glands reacted negatively with ninhydrin-Schiff, mercuric bromophenol blue and chloreamine-T Schiff for protein detection and no sexual dimorphosim was observed in their secretions.

Discussion

The lingual glands are entirely absent from the Varanidae, Amphisbaenia, Ophidia and some species of Chelonia such as *Chelonia mydas* (Kochva, 1978). On the other hand, there is great diversity both in morphology and reactivity of these glands in the reptiles that have them extending from primitive glands with three types of goblet cells (similar to those in fishes) as in the terrapin, *Mauremys caspica* (Taib and Jarrar, 1985a) through goblet cells and simple tubular glands of the turtle *Pseudemys scripta* (Carmignani and Zaccone, 1975) to more developed glands in lizards (Raynaud, 1961; Dornesco and Andri, 1966; Gabe and ST. Girons, 1969; Taib and Jarrar, 1985b,c). The lingual glands of lizards vary from mucous cells together with simple tubular structures as in *Anguis fragilis*, *Eremias arguta*, *Ablepharuns kilaibeli* and *Scincus mitranus* (Raynaud, 1961; Dornesco and Andri, 1966; Taib, 1985) to only simple tubular ones as in *Agama blandfordi* and to tubulo-alveolar in *Uromastyx microlepis* (Taib and Jarrar, 1985b,c). However the lingual glands of *A. schmidti*, seen in the present study, seem to be amongst the most primitive in lizards.

A tentative interpretation of the types of mucosubstances in the lingual secretion of *A. schmidti* can be made from the results of different histochemical reactions used in the present study and from the classification of mucosubstances proposed by Mowry and Winkler (1956), Spicer and Meyer (1960), Scott and Dorling (1965), Pearse (1972). Accordingly, the lingual salivary glands of *A. schmidti* secrete and elaborate neutral mucosubstances, sialidase labile carboxylated mucosubstances and hyaluronidase resistant sulfated mucosubstances while glycoproteins are absent from their secretions. A variable histochemical characterization of the lingual salivary glands are obtained among different species within a given order of reptiles. Neutral

mucosubstances, sialomucins as well as sulfomucins were identified in the glandular secretions of the terrapin *Mauremys caspica* (Taib and Jarrar, 1985a) and the fresh water turtle *Geoemyda trijuga*, but the later species showed hyaluronic acid and/or chondriotin sulfate A and C (Nalvade and varute, 1976). On the other hand, Carmignani and Zaccone (1975) have reported the existence of sialoglycoproteins as well as hyaluronidase resistant sulfomucins in the glandular secretion of the turtle *Pseudemys scripta*. The lingual glands are mucous in Gekkonidae, Xantusiidae, mucoserous in Sphenodotidae, Anguidae and Pygopodidae, but seromucous in Chamaleonidae and typically serous in some species of Iguanidae and Agamidae (Gabe and ST. Girons, 1969).

The structure and secretions of the lingual glands of the purely insectivorous lizard, *A. schmidti* observed in the present study are, however, different from those of other insectivorous lizards, such as *Agama blandfordi* that has mucous cells in the posterior portion of the tongue which synthesize neutral mucosubstances and sialomucins but no sulfomucins (Taib and Jarrar, 1985c) and from those of *Tupinambis teguixin* whose glands comprise mucous cells that secrete only mucosubstances and sialic acid (Lopes et al., 1974). They also differ, but only in structure from those of the herbivrous lizard *U. microlepis* that has tubulo-alveolar glands with the same type of secretion (Taib and Jarrar, 1985b).

The significance of the histochemical diversity, as well as, the morphology and histological variations in the lingual salivary glands of the various reptiles is not fully understood, but might imply phylogenetic relationships and/or different feeding habits of these animals, a point to be investigated.

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