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HISTOCHEMICAL STUDIES ON THE LINGUAL SALIVARY GLANDS OF THE SPINY-TAILED LIZARD *UROMASTYX MICROLEPIS* (BLANDFORD)

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Noory T. Taib and Bashir M. Jarrar (1985) Histochemical studies on the lingual salivary glands of the spiny-tailed lizard *Uromastyx microlepis* (Blandford). *Bull. Inst. Zool., Academia Sinica* **24**(2): 203-212. Histochemical studies have demonstrated that the posterior mucous lingual glands are the only glandular structure in the tongue of *Uromastyx microlepis*. These glands are tubulo-alveolar where the cells of the mucous acini show neutral mucosubstances, sialidase labile carboxylated mucins and hyaluronidase resistant sulfomucins. The lingual secretions of this species are compared with those reported for other vertebrates and the significance of these secretions to the phylogeny and feeding habits of different vertebrate species is discussed.

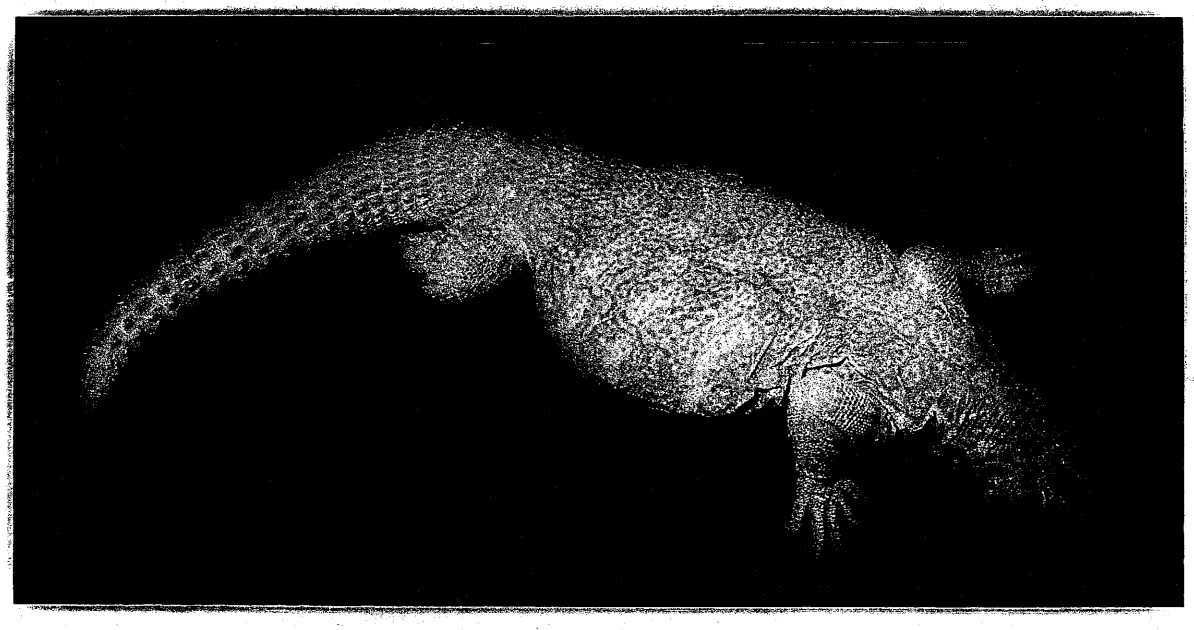
The lingual secretions of reptiles facilitate the feeding process and assist in the lubrication of the sensory organs and taste buds. They are involved in the induction of taste response and in the detection of food contaminants as well as in furnishing the aqueous medium necessary for the ion exchange needed for synaptic and nerve impulses transmission (Castejon, 1970; Garcia-Segura *et al.*, 1978). While in marine reptiles, such secretions are believed to stabilize the serum electrolytes level in the hyperosmotic environment (Burns and Pickwell, 1972).

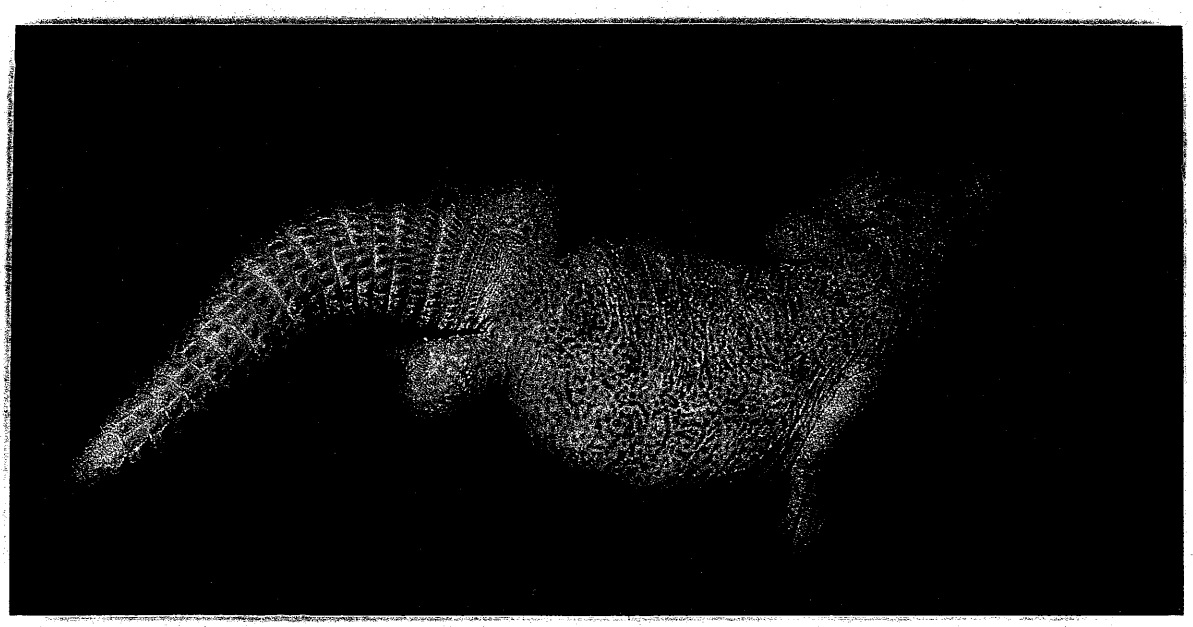
The histochemical characterization of mammalian lingual glands is well decumented in comparison to non-mammalian vertebrates specially reptiles where most studies were concerned with morphological and histological aspects, while histochemical studies were considerably meagre (Lopes *et al.*, 1974; Nalvade and Varute, 1976; Lopes *et al.*, 1982; Taib and Jarrar, 1984, a, b).

In the present study a detailed histochemical characterization of the lingual salivary glands of the herbivorous desert, spiny-tailed lizard, *Uromastyx microlepis* is undertaken.

MATERIALS AND METHODS

Twenty one adult spiny-tailed lizards *U. microlepis* of both sexes were collected from various regions of Saudi Arabia. They were killed by decapitation and the whole tongues were removed and quickly immersed in cold (4°C) solution of 2% calcium acetate in 10% formalin and in buffered formalin (pH 7.0) for 24 hrs. They were then thoroughly washed in running water, followed by routine processing and sectioned at 5-6 μm thickness. The sections were stained with haematoxylin-eosin and with Mallory trichrome stain for histological examination. Paraffin, as well as fresh unfixed frozen sections were used for the following histochemical reactions:





Neutral mucosubstances

Periodic acid Schiff (PAS) technique (Gurr, 1962), PAS after diastase digestion (McManus and Mowry, 1964), 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, AB at pH 1.0, AB at pH 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 (0.1 N HCl, 60°C, 4 hr)-AB (pH 2.5) (Spicer *et al.*, 1967); 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). Histochemical controls consisted of *Uromastyx microlepis* sublingual gland for sialomucins while the control for sulfomucins was provided by the mast cells population in the sections examined.

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). 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).

RESULTS

Sections of the tongue of *U. microlepis* show only Weber's mucous glands localized in the root of the tongue (Figs. 1 & 2). Von Ebner's glands as well as the anterior lingual glands (glands of Nuhn) which were described in the tongues of several mammals are absent in the tongue of *U. microlepis*. On the basis of the histochemical results and in view of the criterion of Gabe and ST. Girons (1969), the ligual glands of *U. microlepis* are of mucous type that contain mainly mucous cells which show slight doubtful reactions for proteins. The glands are small tubulo-alveolar with alveolar cytoplasm where the nuclei are basally positioned. The cells rest on a delicate basement membrane and their granular secretion stain blue in Mallory trichrome stain and bluish purple in haematoxylin-eosin stained sections.

As summarized in Table I, the lingual glands show an intense PAS reaction (Fig. 3) which is not sensitive to diastase digestion and phenylhydrazine treatment while the PAS reaction is abolished by acetylation but is partially restored by sequential acetylation-saponification-PAS techniques. This indicates that the PAS activity is due to neutral and acidic mucosubstances. The glands exhibit strong alcianophilia reaction with AB at pH 2.5 and 1.0 (Figs. 4 & 5) but a comparatively lesser response to AB at pH 0.4. In the sequential AB (pH 1.0)-PAS and AB (pH 2.5)-PAS staining procedure, a bluish purple colouration is formed indicating the presence of vicinal glycol groups together with carboxyl and sulfate groups in the glandular secretion (Figs. 6 & 7). In the AF (Fig. 8) and AF-AB sequential staining procedures the glands react with both stains at pH 2.5 and 1.0. Few cells of the glands react with PAS only giving pink colour and other few cells are AB positive giving the blue colour. This confirms the presence of sulfomucins together with neutral and other acidic mucosubstanecs. Weak and mild methylations partially blocked alcianophilia at pH 2.5, which is completely abolished

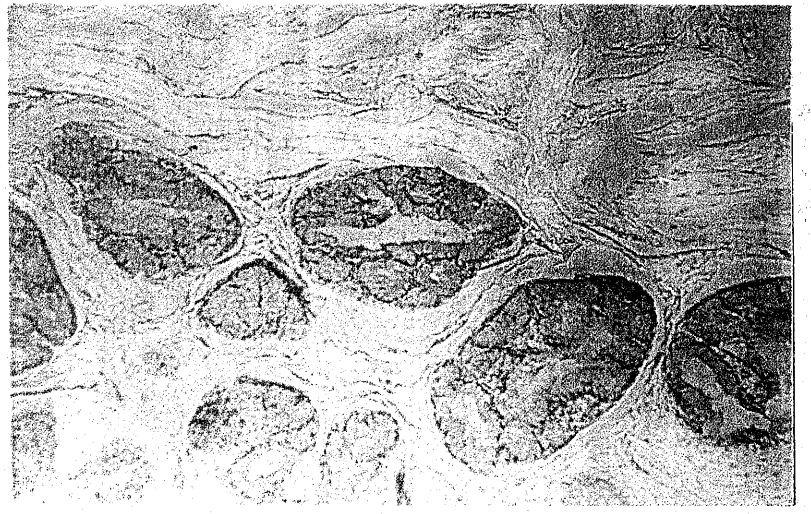


Fig. 1. Lingual glands of *U. microlepis* stained with haematoxylin-eosin. ×430.

Fig 2 Lingual glands of *U. microlepis* stained with haematoxylin-eosin. ×550.

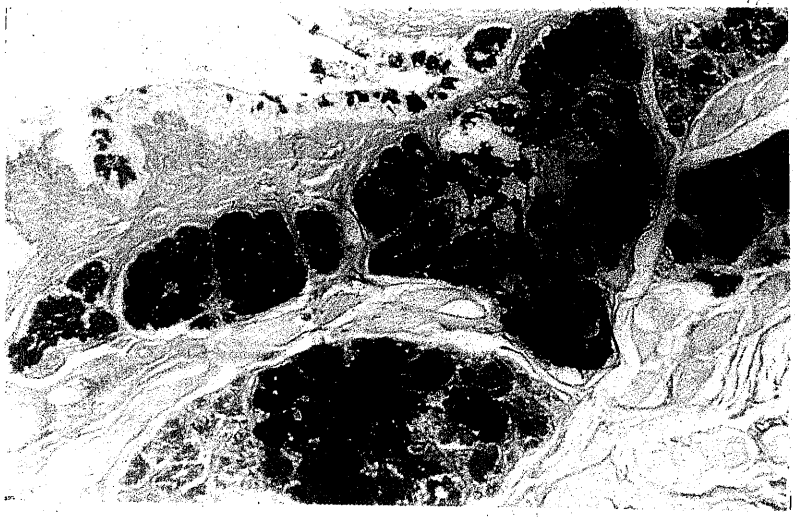


Fig. 3. Lingual glands of *U. microlepis* stained with PAS. ×430.

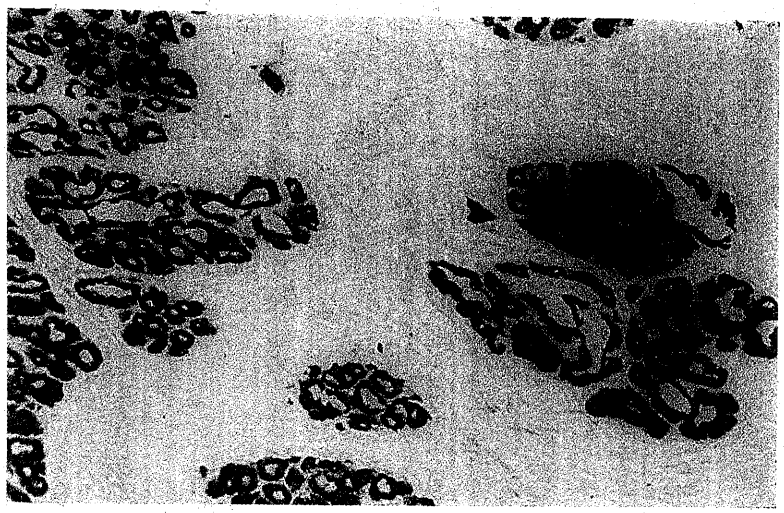


Fig. 4. Lingual glands of *U. microlepis* stained with AB (pH 2.5). ×430.

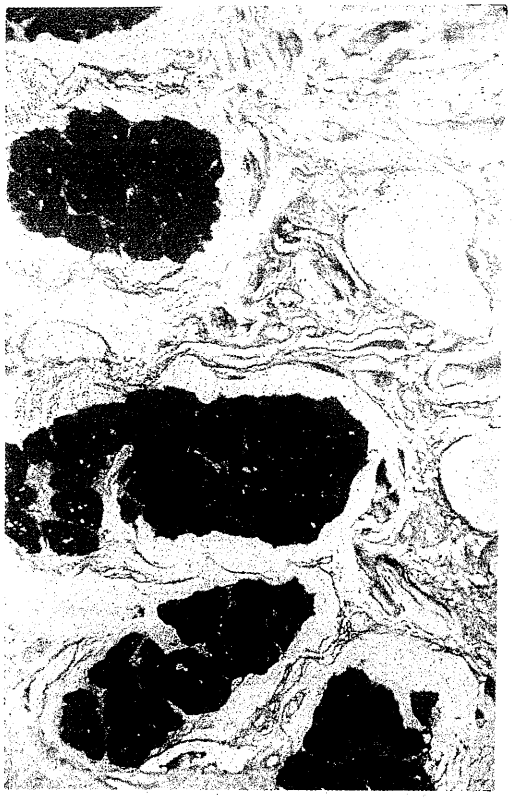


Fig. 6. Lingual glands of *U. microlepis* stained with AB (pH 2.5)-PAS. ×620.

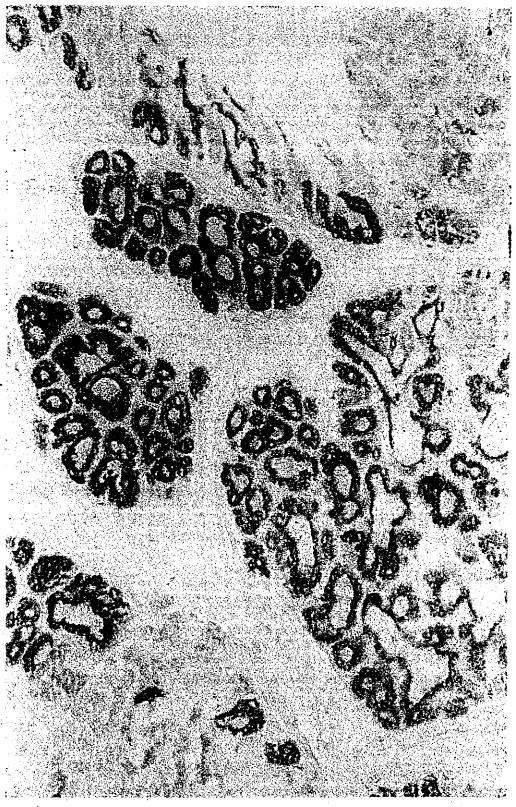


Fig. 8. Lingual glands of *U. microlepis* stained with AF. ×430.

Fig. 5. Lingual glands of *U. microlepis* stained with AB (pH 1.0). ×620.

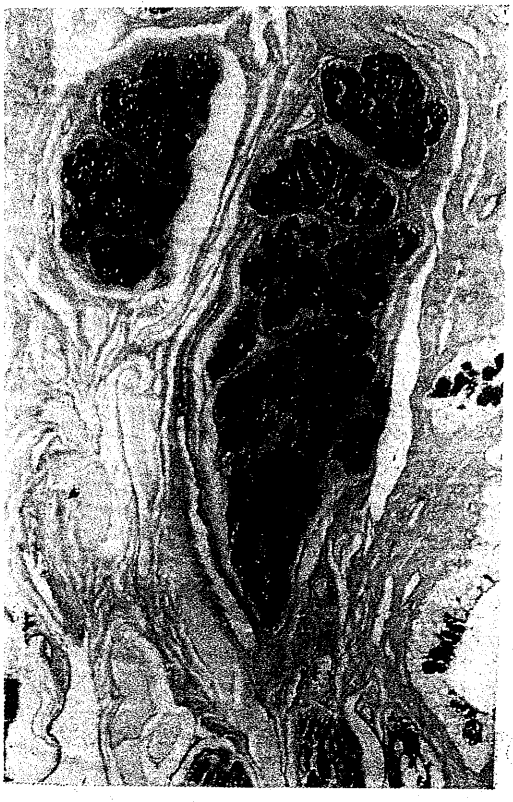


Fig. 7. Lingual glands of *U. microlepis* stained with AB (pH 1.0)-PAS. ×430.

TABLE 1
The histochemical reactions in the lingual salivary glands of *U. microlepis*

Histochemical reaction	Results	Histochemical reaction	Results
PAS	++, P	M. methylation-AB (pH 2.5)	±, Pb
Diastase digestion-PAS	Nb	M. methylation-Saponification-AB (pH 2.5)	+, B
Acetylation-PAS	Cb	S. methylation-AB (pH 2.5)	Cb
Acetylation-Saponification-PAS	+, P	S. methylation-Saponification-AB (pH 2.5)	±, B
Phenylhydrazine-PAS	Nb	CEC (AB, 0.1 M)	+, B
AB (pH 0.4)	+±, B	CEC (AB, 0.3 M)	±, B
AB (pH 1.0)	++, B	CEC (AB, 0.5 M)	+, B
AB (pH 2.5)	++, B	CEC (AB, 0.8 M)	±, B
AB (pH 1.0)-PAS	++, BP	CEC (AB, 1.0 M)	−
AB (pH 2.5)-PAS	++, BP	Neuraminidase-AB (pH 2.5)	+, Pb
AF	+, P	Hyaluronidase-AB (pH 2.5)	Nb
AF- (AB pH 1.0)	+, Bp, B, P	Ninhydrin-Schiff	±?
AF- (AB pH 2.5)	+, BP, B, P	Hg-bromophenol blue	±?
Acid hydrolysis-AB (pH 2.5)	++, B, Pb	Chloramine T-Schiff	±?
W. methylation-AB (2.5)	+, Pb		
W. methylation-Saponification-AB (pH 2.4)	+±, B		

Reactions: −, negative; ±, weak; +, moderately positve; ++, intensely positve; Cb, complete blockade; M, mild; Pb, Partial blockade; Nb, no blockade; S, strong; W, weak.
Colours: B, blue; Bp, bluish purple; P, pink;

by strong methylation and partially restored by methylation-saponification sequential technique which confirms the presence of sialomucins. The alcianophilia is also partially abolished by acid hydrolysis and neuraminidase digestion indicating the presence of sialic acid. The glands demonstrated alcianophilia at 0.1 up to 0.8 M $MgCl_2$. There is no change in the alcianophilic reaction after treatment with hyaluronidase. This confirms the co-existence of sialomucins with hyaluronidase resistant sulfomucins.

The glands show a dubious slight reaction to the three different techniques employed for proteins detection. There is no difference between the sexes in lingual secrction.

DISCUSSION

Neutral mucosubstances are PAS positive, diastase resistant, as well as unstainable by cationic dyes. Acetylation produces derivatives of primary and secondary amines which prevent 1, 2 glycol groups, from reacting with PAS indicating the presence of neutral mucosubstances or sialic acid, separately or simultaneously. Alcian blue is generally considered as being specific for identifying acid mucosubstances where alcianophilia at pH 2.5 and 1.0 is specific for sialomucins and sulfomucins respectively (Mowry and Winkler, 1956). In the combined aldehyde fuchsin-alcian blue sequential techniques, sulfomucins stain purple blue and sialomucins blue (Spicer and Meyer, 1960). Sialomucins can be identified by alcianophilia at pH 2.5 which is partially lost following acid hydrolysis and completely removed after neuraminidase digestion, but neuraminidase will not affect the staining of sulfated mucosubstances. A loss of alcianophilia after hyaluronidase digestion is due to the removal of hyaluronic acid and chondroitin sulfates. Methylation blocks subsequential staining of simple mucosubstances by esterification of carboxyl groups and complex sulfated mucosubstances by desulphation. Subsequent treatment with potassium hydroxide

(Saponification) after methylation will restore the staining of carboxyl groups (Drury *et al.*, 1967). The mucosubstances that are stained at 0.1 M $MgCl_2$ in the CEC reaction, but not at 0.2 M $MgCl_2$ are believed to contain carboxyl groups and no sulfate groups. Sulfated mucosubstances, on the other hand, stain strongly and selectively at 0.2 Mg^{++} but lose their alcianophilia at different levels with increasing $MgCl_2$ concentration (Spicer and Lillie, 1960). Accordingly, the lingual salivary glands of *U. microlepis* contain neutral mucosubstances, sialadase labile carboxylated mucosubstances and hyaluronidase resistant sulfomucins.

Among reptiles, the lingual secretions in turtles are produced by three types of goblet cells (Nalvade and varute, 1976; Taib and Jarrar, 1984a), whilst, lizards and snakes have both unicellular and multicellular lingual glands (Nalvade and Varute, 1976; Shevliuk, 1976), but some lizards such as *Scincus mitranus* and *Acanthodactylus schmidti* possess mainly goblet cells and some simple glandular acini as the sole glandular structure in their tongues (unpublished data). The occurrence of goblet cells as the sole glandular structure in fishes, amphibians, turtles and some lizards may be regarded as a primitive developmental stage towards the formation of definitive lingual glands of higher vertebrates (Shevliuk, 1976).

The lingual glands of *U. microlepis*, which is a purely herbivorous lizard, morphologically resemble those of the insectivore *Agama blandfordi* but differ in the absence of sulfomucins in the secretion of the posterior lingual glands of the later species (Taib and Jarrar, 1984b). Sulfomucins are also absent from the lingual secretions of other insectivorous lizards such as *Scincus mitranus* and *Acanthodactylus schmidti* (unpublished data) as well as from secretions of the posterior mucous lingual glands of insectivorous bats but not from those of frugivorous ones (Nalvade and Varute, 1972a). However, sulfomucins occur with more frequency in mucous cells of the posterior lingual glands than in mucous ones of the major salivary glands, while sialomucins are present in the major as well as in the minor salivary glands (Nogueira and Carvalho, 1973). Sulfated mucosubstances are highly charged compounds which might be involved in the distribution of cations which is needed in the conduction of nerve impulses. Sulfomucins were found in the posterior mucous lingual glands of carnivorous amphibians and mammals (Carvalho and Nogueira, 1967; Nalvade and Varute, 1971; Nalvade and Varute, 1972b), but those of non-carnivorous vertebrates such as some rodents and chickens secrete only sulfomucins (Fuji and Tamura, 1966). On the other hand, neutral mucins were present in the lingual glands of almost all species studied except the rabbit in spite of the differences in their feeding habits (Nogueira and Carvalho, 1973).

The lingual glands of reptiles show developmental stages from non-glandular tongues in varanidae, Amphisbaenia, Serpentes and the turtle *Chelonia mydas* through a unicellular lingual glands in Testudinidae and Chelydridae to a well developed branched tubular polystomatic and monostomatic glands in Agamidae, Iguania, Gekkota, Angiuoidea and Chamaeleonidae (Kochva, 1978). The heterogeneous histochemical reactivity of the lingual glands does not seem to be related to the phylogeny of the species, but the differentiation of their secretory products might be appeared in the evolutionary lines of reptiles and higher vertebrates to meet the different changes in the feeding habits of the different species. The reactivity of the lingual glands of *Testudo uromastyx*, Iguania and some tortoises has been atributed to their herbivorous diet (Fava de Moraes *et al.*, 1966). The development of the lingual glands among reptiles and their reactivity do not imply real phylogenetic relationship, but a point to be considered in the reptilian evolution. More work is needed to elucidate this point.

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刺尾蜥蜴 *Uromastyx microlepis* (Blandford) 其舌唾腺之組織化學研究

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由組織化學的研究已知後黏液舌腺是 *Uromastyx microlepis* 舌部唯一的腺體。本文討論該腺體之分泌物性質及與其他脊椎動物的比較。此外也討論該分泌物對於不同脊椎動物之種系發育和攝食習性的重要性。