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Histochemical Characterization of the Lingual Salivary Glands of the House Gecko, *Ptyodactylus hasselquistii* (Squamata: Gekkonidae)

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Abstract. - Histochemical investigations of the lingual salivary glands of the house gecko, *Ptyodactylus hasselquistii* have been conducted. The glands are comprised of mucous and mucoserous cells. Mucous cells secrete or elaborate neutral mucosubstances, neuraminidase sensitive carboxylated mucins, hyaluronidase resistant sulfomucins, but are devoid of proteins. The mucoserous cells secrete and elaborate neutral mucosubstances and glycoproteins but are devoid of sialomucins and sulfomucins. The results are discussed in the context of the feeding habits and phylogeny of reptiles.

Key words. - Histochemistry, lingual, salivary glands, house gecko, *Ptyodactylus hasselquistii*, Gekkonidae.

Introduction

Histochemical studies on the lingual salivary glands of vertebrates have mainly been concerned with mammals, whereas little attention has been paid to the lingual salivary glands of non-mammalian vertebrates. Most studies on the lingual salivary glands of reptiles have focused with morphological and histological aspects while few histochemical studies have been carried on these glands (Raynaud, 1961; Gabe and Saint-Girons, 1969; Lopes et al., 1982; Taib and Jarrar, 1985a; 1985b; 1985c; 1986; Taib, 1986, Asgah et al., 1990). Nevertheles, the literature on the lingual secretions of lizards is rather scanty and their consitituents have yet to be determined.

The present study is a detailed histochemical characterization of the lingual salivary glands of the house gecko, *Ptyodactylus hasselquistii*.

Materials and Methods

Twenty adults of each male and female house gecko *Ptyodactylus hasselquistii* were trapped from different houses in Riyadh city, Saudi Arabia. They were killed by etherization and the whole tongue was removed from each animal and quickly immersed for 24 hrs in one of the following fixatives: neutral buffered formalin, Bouin's fluid and Gendre's fluid. They were then thoroughly washed in running water, processed for serial sectioning at 4-5 µm thickness and the sections were stained with haematoxylin-eosin or with Mallory trichrome for histological examination, whereas the secretary cells of the glands were characterized by the criterion of Gabe and Saint-Girons (1969). Other 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 alpha-amylase digestion (Luna, 1968), PAS after acetylation blockade (McManus and Cason, 1950), PAS after acetylation-saponification (Oxello 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 neurtal 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 fuchsion (AF) and AF-AB, pH 2.5 (Spicer and Meyer, 1960); weak (25ºC, 16 hr), mild (37ºC, 4hr) and strong (60ºC h hr) methylation-saponification- AB (PH 2.5) (Spicer, et al., 1967); toluidine blue (TB) buffered at pH 1.7 and 3.4 (Landsmeer, 1951), critical electrolyte concentration (CEC) technique for extinction of alcianophilia at pH 5.6 in the presence of gradual concentation of Mg^{2+} (Scott and Dorling, 1965).

Enzyme digestion tests. - Diastase-PAS technique (McManus and Mowry, 1964); neuraminidase (Sialidase, Vibrio chlolerae, type V)-AB (pH 2.5) (Spicer and Warren, 1960); hyaluronidase (testicular)-AB (pH 2.5) (Spicer et al., 1967). Ribonuclease digestion (Love and Rabotli 1963); neuraminidase-TB (pH 3.7), hyaluronidase-TB (pH 2.0) were employed. In each case control sections were incubated for the same length of time at the same temperature in buffer solutions without the enzyme.

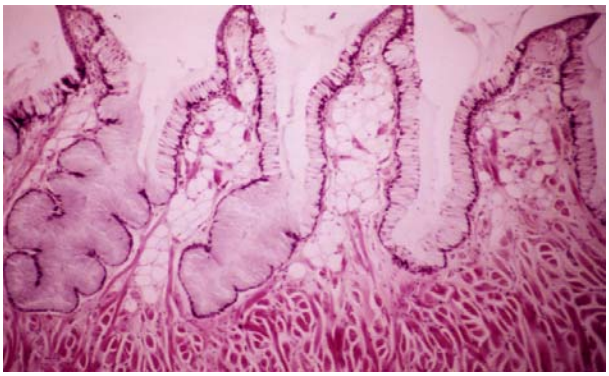

Figure 1. Lingual glands of *P. hasselquistii* after staining with haematoxylin-eosin. Note that the mucous cells of the lingual glands are located in the papillar space of the papillae. x950.

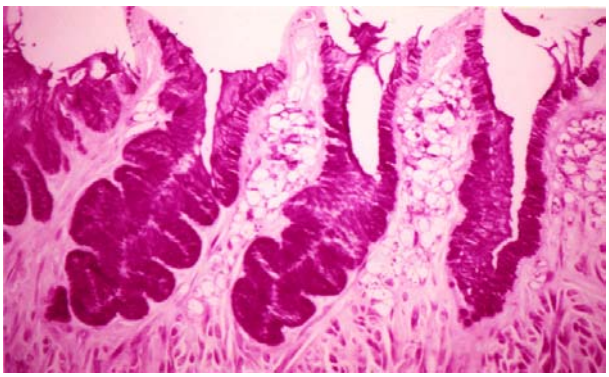

Figure 2. Lingual glands of *P. hasselquistii* after staining with PAS. The reactivity of the glands confirms the presence of the neutral mucosubstances. x950.

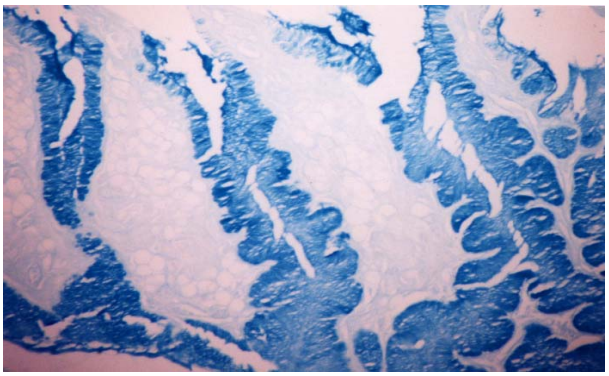

Figure 3. Lingual glands of *P. hasselquistii* after staining with AB (2.5), confirming the presence of sialomucins and sulfomucins. x950.

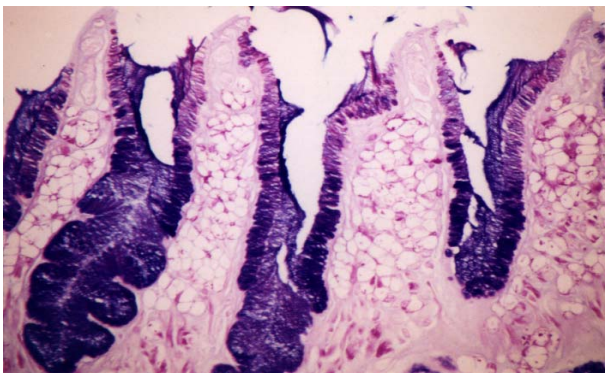

Figure 4. Lingual glands of *P. hasselquistii* after staining with AB (1.0)-PAS. The bluish purple color indicates the presence of neutral and sulfated mucosubstances simultaneously. x950.

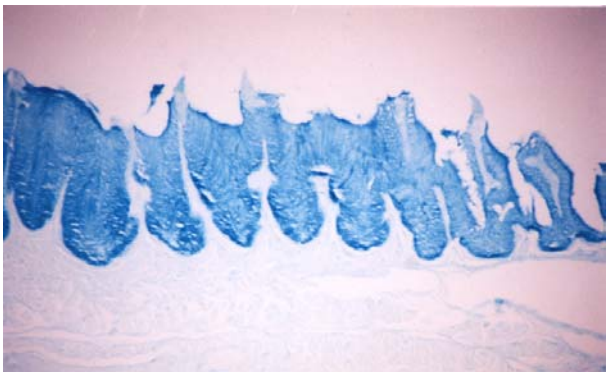

Figure 5. Lingual glands of *P. hasselquistii* after staining with CEC at 0.3M Mg^{++}., confirming that the mucosubstances produced by the glands contain carboxyl and sulfated groups. x950.

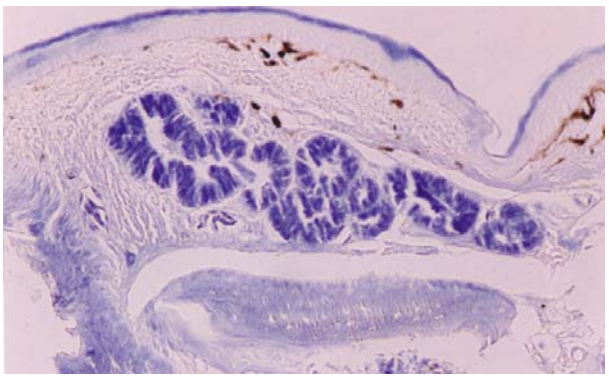

Figure 6. Lingual glands of *P. hasselquistii* after staining with MBPB, indicating the protein contents of the glands secretion. x700.

Proteins. - Mercuric bromophenol blue method (Mazia et al., 1953); ninhydrin-Schiff (Yasuma and Itchikawa, 1953), mercuric-bromophenol blue (MBPB) and PAS after trypsin digestion (Pearse, 1972).

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

Results

The lingual salivary glands of the house gecko, *Ptyodactylus hasselquistii* occupy the papillar invagination of the posterior two-thirds of the dorsal surface together with the lateral sides of the tongue. The anterior part of the tongue is devoid of any glandular structure and covered by keratinized squamous epithelium. These glands are made of mucous cells located in the inner papillar space of the filiform papillae (Fig. 1) together with simple tubular structures made of mucoserous cells seen at the most posterior part of the dorsum. The mucous cells have an alveolar cytoplasm and flattened, basally located nuclei with clear apical ends resting on a delicate basement membrane.

As summarized in Table 1, the mucous cells of the lingual glands of *P. hasselquistii* exhibited strong PAS reactivity (Fig. 2) which was neither labile to alpha-amylase nor to saliva digestion but completely lost by phenylhydrazane treatment. However, this reactivity was completely blocked by acetylation and was partly restored by deacetylation-PAS sequential techniques. They showed marked alcianophilia at both pH 2.5 (Fig. 3) and 1.0 but to lesser extent at pH 0.4. They also reacted with both PAS and AB and stained bluish purple with AB (2.5)- PAS and AB (1.0) PAS (Fig. 4). These glands also reacted with AF as well as with AF-AB (2.5) and AF-AB (1.0). The alcianophilia of the glands was partly lost at pH 2.5 with acid hydrolysis and weak methylation and there after restored by saponificiation techniques. They demonstrated alcianophilia with the CEC techniques at 0.1M, 0.2M, and to some extent at 0.3M Mg^{2+} (Fig. 5) and showed metachromasia at pH 3.4 and 1.7 but reacted negatively to all protein detection tests.

The mucoserous cells of the glands showed PAS reaction, exhibited no alcianophilia at pH 2.5 and 1.0 and were orthochromatic at pH 3.4 and 1.7 but reacted positively to all protein detection tests (Fig. 6). No sexual dimorphism was observed in the lingual secretion of the species under study.

Discussion

The lingual salivary glands present great diversity in morphology amongst the various groups of reptiles. These glands are entirely absent from Varanidae, Amphisbaenia, Ophidia and some species of Chelonia such as *Chelonia mydas* (Kochva, 1978). On the other hand, these glands are simple consisting of three different types of goblet cells in most species of Testudinidae (Nalvade and Varute, 1976; Taib and Jarrar, 1984). Some lizard possess mainly goblet cells together with simple tubular glandular structures in their tongues (Nalvade and Varute, 1976; Shevliuk, 1976; Taib and Jarrar 1985 b; 1985c and 1986), while others have more developed lingual salivary glands as seen in some Agamidae, Iguanidae, Gekkonidae, Anguidae and Chamaleonidae (Gabe and Saint-Girons, 1969; Kochva, 1978; Asgah et al., 1990). On the bases of the results of the present study and in view of the criterion of Gab and Saint-Girons (1969), the lingual salivary glands of *Ptyodactylus hasselquistii* are made of unicellular mucous goblet cells lining the dorsal epithelium of the tongue with mucoserous simple tubular glandular apparatus at the base of the tongue. The structure of the lingual salivary glands of *P. hasselquistii* is different from those of *Tupinambis teguixin*, *Agama blandfordi*, *Uromastyx microlepis*, *Acanthodactylus schmidti* and *Scincus mitranus*, which have only mucous cells in their lingual glands (Lopes et al, 1974; Taib and Jarrar, 1985b; 1985c; 1986; Taib, 1986). According to Gabe and Saint-Girons (1969), the lingual glands are mucous in Gekkonidae, mucoserous on Sphenodontidae, Anguidae and Pygopodidae, but seromucous in Chamaleonidae and serous in some speices of Iguanidae and Agamidae. The grading from non-glandular tongues through unicellular with or without simple tubular glandular structure to only simple tubular and to then tubulo-alveolar ones may reflect developmental stages towards the definitive lingual glands of higher vertebrates (Shevliuk, 1976; Kochva, 1978).

A tentative interpretation of the types of mucosubstances in the lingual glands of *P. hasselquistii* can be made from the results of the different histochemical reactions used in the present investigation and from the classification of mucosubstances proposed by Mowry and Winkler, 1956; Spicer and Meyer, 1960; Scott and Dorlling, 1965; Pearse, 1972). 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 sulformucins 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).

Table 1. The histochemical reactions in the lingual salivary glands of *Ptyodactylus hasselquistii*.

Histochemical reaction	Results	
	MC	MSC
PAS	++,P	++,P
Diastase digestion -PAS	Nb	Nb
Acetylation-deacetylation-PAS	++,p	++,p
Phenylhdrazine-PAS	Cb	Cb
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	-
AF- (AB pH 2.5)	++,Bp	-
Acid hydrolysis- AB (pH 2.5).	Pb	-
W. methylation - AB (2.5)	Pb	-
W. methylation-saponification - AB (pH 2.5)	++,Bp	-
M. methylation-AB (pH 2.5)	Cb	-
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)	+	-
CEC (AB, 0.2 M)	+	-
CEC (AB, 0.3 M)	±	-
CEC (AB, 0.5 M)	-	-
Neuraminidase-AB (pH 2.5)	+B,pb	-
Hyaluronidase-AB (pH 2.5)	++B,Nb	-
Ninhydrin-Schiff	-	+
Hg- bromophenol blue	-	+
Chloramine T-Schiff	-	+
Trypsin digestion-PAS	Nb	-
(Chloroform + methanol)-PAS	Nb	-

Reactions: - negative; ± weak; +, moderately positive; ++, intensely positive; Cb, complete blockade; M, mild; Pb, partial blockade; Nb, no blockade; S, strong; TB, toluidine blue; W, weak.
Colors: B, blue; Bp, bluish purple; P, pink.
Glands: MC, mucous cells; MSC, mucoserous cells.

Sialomucins can be identified by alcianophilia at pH 2.5 which is partially lost following acid hydrolysis and completely removed after neuraminidase digestion, but neuroaminidase did 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 stainig of simple mucosubstances by esterification of carboxyl groups and complex sulfated mucosubstances 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 0.1M $MgCl_2$ in the CEC reaction, but not at 0.2M $MgCl_2$ are believed to contain carboxyl group and no sulfate groups. Sulfated mucosubstances, on the other hand, stain strongly and selectively at 0.2M Mg^{2+} but lose their alcianophilia at different levels with increasing $MgCl_2$ concentration (Spicer and Lillie, 1960). The lingual glands of the species under study resisted trypsin digestion and the action of (chloroform-methanol) which excludes the possibility of lipids and proteins. Accordingly, the lingual salivary glands of of the house gecko, *Ptyodactylus hasselquistii* contain neutral mucosubstances, sialadase labile carboxylated mucosubstances and hyaluronidase resistant sulfomucins and glycoproteins.

The lingual secretions of *P. hasselquistii* are different from those of some lizards such as *Tupinambis teguixin* and *Agama blandfordii* which contain neutral

mucosbstances and sialomucins but no sulfomucius (Lopes et al., 1974; Taib and Jarrar, 1985c). They also differ from the secretions of the lingual glands of *Uromastyx microlepis*, *Acanthodactylus schmiditi*, *Scincus mitranus* and *Stenodactylus slevini* which contain neutral mucosubstances, sialomucins and sulformucins but no glycoprotein (Taib and Jarrar, 1985b, 1986; Taib, 1986; Asgah et al., 1990). Neutral mucosubstances have been demonstrated in the secretions of all studied reptiles that possess salivary glands while phylogenetically, the absence of sulfomucins in the lingual glands would favour the concept that sialomucins secretive cells are more primitive than sulfated mucosubstances secretive ones. In addition, the heterogenous histochemical recactivity of the lingual glands might have appeared in the evolutionary lines of reptiles to meet the different changes in the feeding habits of various species. Neutral mucins were present in the lingual glands of almost all studied reptile species while sialomucins together with neutral mucosubastances were identified in the lingual glands of all insectivorous reptiles so far studied (Nalvade and Varute, 1972; Taib and Jarrar, 1985c, 1986; Taib, 1986). The lingual glands of all insectivorous and carnivorous reptiles studied thusfar exhibited sulfomucins. More work is needed to elucidate whether the lingual secretion diversity of reptiles imply phylogenetic relationships or different feeding habits.

Literature Cited

Asgah, N. A., N. T. Taib, and B. M. Jarrar 1990. Morphology, histology and histochemistry of the cephatic glands of Slevin's ground gecko, *Stenodactylus slevini* Hass, 1957. Tropical Zoology 3:209-217.

Burns, B. and V. G. Pickwell. 1972. Cephalic glands in sea snakes (*Pelamis*, *Hydrolis,* and *Laticauda*). Copeia 1972 (3):547-559.

Carmignani, M. A. and G. Zaccone. 1975. Histochemical distribution of acid mucopolysaccharides in the tongue of reptiles. 1-Chelonia (*Pseudemys scripta* Clark). Annals of Histochemistry 20:77-88.

Drury, A. R., E. A. Wallington, and R. S. Cameron. 1967. Carleton's histological techniques. 4th ed. Oxford University Press, London. 203-204 pp.

Gabe, M. and H. Saint-Girons. 1969. Donnees histologiques sur les glandes salivaires des lepidosauriens. Memoires du Museum National d' Histoire Naturelle 58:1-112.

Gurr, E. 1962. Staining animal tissue: Practical and theoretical. Leonard Hill, London. 631 pp.

Kochva, E. 1978. Oral glands of the reptilia. Pp. 43-161. In C. Gans and F. H. Pough, (eds.), Biology of the Reptilia. Academic Press, New York.

Landsmeer, J. M. F. 1951. Some colloid chemical aspects of metachroasia. Influence of pH and salts in metachromatic phenomena evoked by toluidine blue in animal tissue. Acta Physiologica et Pharmacologica 2:112-128.

Lopes, R. V., V. Valeri, C. Oliveria, G. Compos, and S. Jucif. 1974. Morphological and histological study of the salivary glands of the lizard *Tupinambis teguixin* (Teiidae, Lacertilia). Ciencia Cultura 26:1035-1070.

Lopes, R. A., R. V. Costa, A. M. Piccolo, and S. O. Petnusci. 1982. The salivary glands of *Ameiva ameiva* (Teiidae, Lacertilia). A morphological, morphometric and histochemical study. Anatomischer Anzeiger 151:41-49.

Luna, G. 1968. Manual of histological staining method of the Armed Forces Institute of Pathology. 3rd Ed. McGraw-Hill Book Co., New York. 258 pp.

Mazia, D., P. A. Brewer, and M. Alfert. 1953. The cytocehmical staining and measurement with mercuric bromophenol blue. Biological Bulletin 104:57-67.

McManus, J.A. and J. E. Cason. 1950. Carbohydrate histochemistry studies by acetylation techniques. I. Periodic acid method. Journal of Experimental Medicine 91:651-54.

McManus, J. A. and R. W. Mowry. 1964. Staining methods, histological and histochemical. Harper and Row, New York. 423pp.

Mowry, R. W. 1956. Alcain blue techniques for histochchemical study and acidic carbohydrates. Journal of Histochemistry and Cytochemistry 4:407.

Mowry, R. W. and C. H. Winker. 1956. The coloration of acidic carbohydrates of bacteria and fungi in tissue sections with special reference to capsules of *Cryptococcus neoformas* and *Staphylococcus*. American Journal of Pathology 32:628-29.

Nalvade, N. M. and T. A. Varute. 1976. Histochemical studies on the mucins of the vertebrate tongues.

Vlll. Histological analysis of mucosubstances in the tongue of the turtle *Geomyda trijug*a. Folia Histochemica et Cytochemica 14(3):123-133.

Oxello, L., M. Ledding and F. F. Speer. 1958. The ground substances of the central nervous system in man. American Journal of Pathology 34:363-373

Pearse, A. G. E. 1972. Histochemistry: Theoretical and applied. 3rd ed. J. & A. Churchill, London. 1518 pp.

Raynaud, M. J. 1961. Sur la structure des glands salivaries de l'orvet (*Anguis fragilis* Linnaeus, 1758). Bulletin de la Societe Zoologique de France 86:710-713.

Scott, D. E. and J. Dorling. 1965. Differneital staining of acid glycosaminoglycans (Mucopolysaccharides) by alcian blue in salt solutions. Histochemie 5:221-233.

Shevliuk, N. N. 1976. Contribution to histological characterstics of salivary glands of the tongue in certain classes of vertebrates. Anatomia Histologia Embriologia 70(30):58-62.

Spicer, S. S. and D. B. Meyer. 1960. Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsin-alcian blue staining. American Journal of Clinical Pathology 33:433-460.

Spicer, S. S. and R. D. Lillie. 1960. Saponification as a mean of selective reversing the methylation blockade of tissue basophilia. Journal of Histochemistry and Cytochemistry 7:123-125.

Spicer, S. S. and L. Warren. 1960. The histochemistry of sialic acid containing mucoproteins. Journal of Histochemistry and Cytochemistry 8:135-137.

Spicer, S. S., R. G. Horn, and T. J. Leppi. 1967. Histochemistry of connective tissue mucopolysaccharides. pp 251-303. In: The connective tissues. International Academy of Pathology Monograph No.7. Williams and Wikins, Balthimore.

Taib, N. T. 1986. Histochemical observations on the lingul salivary glands of the skink *Scincus mitranus* (Anderson, 1871) (Scindidae, Reptilla): Bulletin of the Chicago Herpetological Society 21(1-2):14-22.

Taib, N. T. and B. M. Jarrar. 1985a. Histochemical characterization of musousbstances in the tongue of the terrapin, *Mauremys caspica* (Gmelin) (Reptilia, Testudins, Emydidae). Jorunal of Biological Sciences Research, Iraq 16(2):239-249.

Taib, N. T. and B. M. Jarrar 1985b. Histochemical studies on the lingual salivary glands of the spiny-tailed lizard *Uromastyx microlepis* (Blandford). Bulletin of the Institute of Zoology, Academia Sinica 24(2):203-212.

Taib, N. T. and B. M. Jarrar. 1985c. Histochemical analysis of mucosubstances in the lingual salivary glands of the lizard *Agama blandfordi* (Agamidae, Reptilia). Sudan Journal of Science 1:97-101.

Taib, N. T. and B. M. Jarrar. 1986. The histochemistr of the lingual salivary glands of the lizard *Acanthodactylus schmidti* (Wiegmann) (Reptilia, Lacertilia, Lacertidae). Bulletin of the Maryland Herpetological Society 22(2):27-36.

Yasuma, A. and T. Itchikawa. 1953. Nihydrin-Schiff and alloxan Schiff staining, A new histochemical method for protein. Journal of the Laboratory Clinical Medicine 41:296-299.