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Gel electrophoretic studies with reference to functional morphology of the salivary glands of *Acanthaspis pedestris* Stal. (Insecta: Heteroptera: Reduviidae)

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Abstract. Histological profile of the anterior and posterior lobes of the principal salivary and accessory glands of the reduviid *Acanthaspis pedestris* Stal. has been discussed in relation to enzyme pattern of the posterior lobe and the zootoxic substances of the anterior lobe as well as the protein fractions of the salivary gland lobes and the haemolymph.

Keywords. Reduviidae; principal gland; accessory gland; hilus; zootoxic; watery saliva; protein fraction; R_m value.

1. Introduction

The structure and functional diversity of the salivary glands in the different groups of Hemiptera were analysed by Baptist (1941) and the importance of cecidogenetic and disease transmitting ability of the phytophagous Heteroptera in relation to the structure and function of the salivary system have been highlighted by Bronskill *et al* (1958), Miles (1959, 1964a, b, 1967, 1968, 1972), Salkeld (1960), Miyamoto (1961), Saxena (1963) and Hori (1969). Wigglesworth's (1943) description of the morphology of the salivary gland of Rhodnius suggesting the presence of haemalbumen like pigments, was followed by an analysis by Edwards (1961) of the functions and biochemical components of the salivary gland in *Platymeris rhadamanthus* Gaerst. The variation in salivary gland morphology of 9 sub families of Reduviidae established the inter-species diversity among Reduvoidea (Louis and Kumar 1973). Haridass and Ananthakrishnan (1981) illustrated the structure and functional morphology of the salivary glands of the subfamilies Piratinae, Echtrichodinae and Triatominae (Reduviidae). The present paper highlights the functional morphology of the different lobes of salivary gland along with histological details and electrophoretic analysis of diverse proteins involved in the salivary system of the predatory bug *Acanthaspis pedestris* Stal.

2. Materials and methods

Adults and nymphs of *A. pedestris* were collected from the semiarid zones of Kanyakumari district (Tamil Nadu) and a culture was maintained successfully under laboratory conditions. The adult insects were separated from the mass culture and utilized for the following experiments. The starved insects were etherized and the salivary system was carefully dissected out using insect ringer, fixed in alcoholic Bouin's solution and subsequently dehydrated through the alcohol series for embedding. Sections were made (7 μ) and stained with Delafields haematoxylin and eosin.

Toxic effects of secretion from different lobes of the salivary system were tested. Starved insects were etherized and dissected to expose the salivary glands. Using a graduated needle like glass capillary tube, 25 μl of the secretions were drawn directly and separately from the anterior, posterior and accessory lobes. These secretions were gradually injected into the thorax in the region of the pleural membrane of the hind coxal base of the test insect, *Oxya nitiduala* (Walker) (Acrididae). Using a stop watch, the time required for the arrest of the movements of the legs, tarsal segments, wings, respiratory movements of the abdomen and the total paralysis of the test insects was calculated. Twenty-five μl of diluted (G D water 1:2) salivary extracts obtained separately from known weight of the different salivary lobes dissected out from etherized insects were also tested in a similar way. Salivary secretions extracted in a similar manner, diluted with distilled water, but boiled at 50°C for 10 min in a water bath were used as control. The qualitative protein profiles of salivary glands and haemolymph, through polyacrylamide gel electrophoresis were identified (Davis 1964) on 7·5% tube gels of 11 cm by adjusting the current to 2·5 mA, per tube for about 3 h at 4–10°C, until the tracer dye migrated to a distance of about 10 cm. The samples (50 μl) were injected carefully into the gel tube using Finn pippete digital (Finland). The gels were removed and stained with 0·02% Coomassie brilliant blue in a mixture of methanol, acetic acid and water (25:7:68) for 24 h and destained for 48 h. The gels were stored in 7% acetic acid and were scanned by LKB 2202 ultroscan laser densitometer (Sweden) and the electropherograms were recorded by using the recording integrater LKB 2220 Bromma (Sweden). The different protein fractions were identified by computing the R_m values of the proteins using the following formula:

$$R_m \text{ value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the dye}}$$

3. Results

3.1 *Histological profile*

The small caplike anterior lobe and a long tubular posterior lobe of the principal salivary gland of *A. pedestris* is separated by a distinct hilus (figures 1A and C). The accessory glands attached to the lateral sides of the first midgut appear triangular with a tubular appendix opening into the common salivary duct (figure 1G). Both the lobes of the principal salivary gland comprise of single layer of oval shaped cells. The uninucleate anterior lobe cells are devoid of vacuoles having a few granular materials with less viscous cytoplasm, whereas the posterior lobe cells have two nuclei with highly viscous granular cytoplasm (figure 1B, D, E). The holocrine salivary secretion of both anterior and posterior lobes (figure 1B, F), is separately stored in the spacious lumen. The anterior part of both lobes reveal increased secretory activities. The salivary lobes open out individually by a small pore, guarded by thick circular muscles, into a compartmentalised hilus (figure 1C). The accessory gland cells are uninucleate with less viscous cytoplasm and secretes a watery fluid which is stored in its flattened lumen (figure 1H). A well developed,

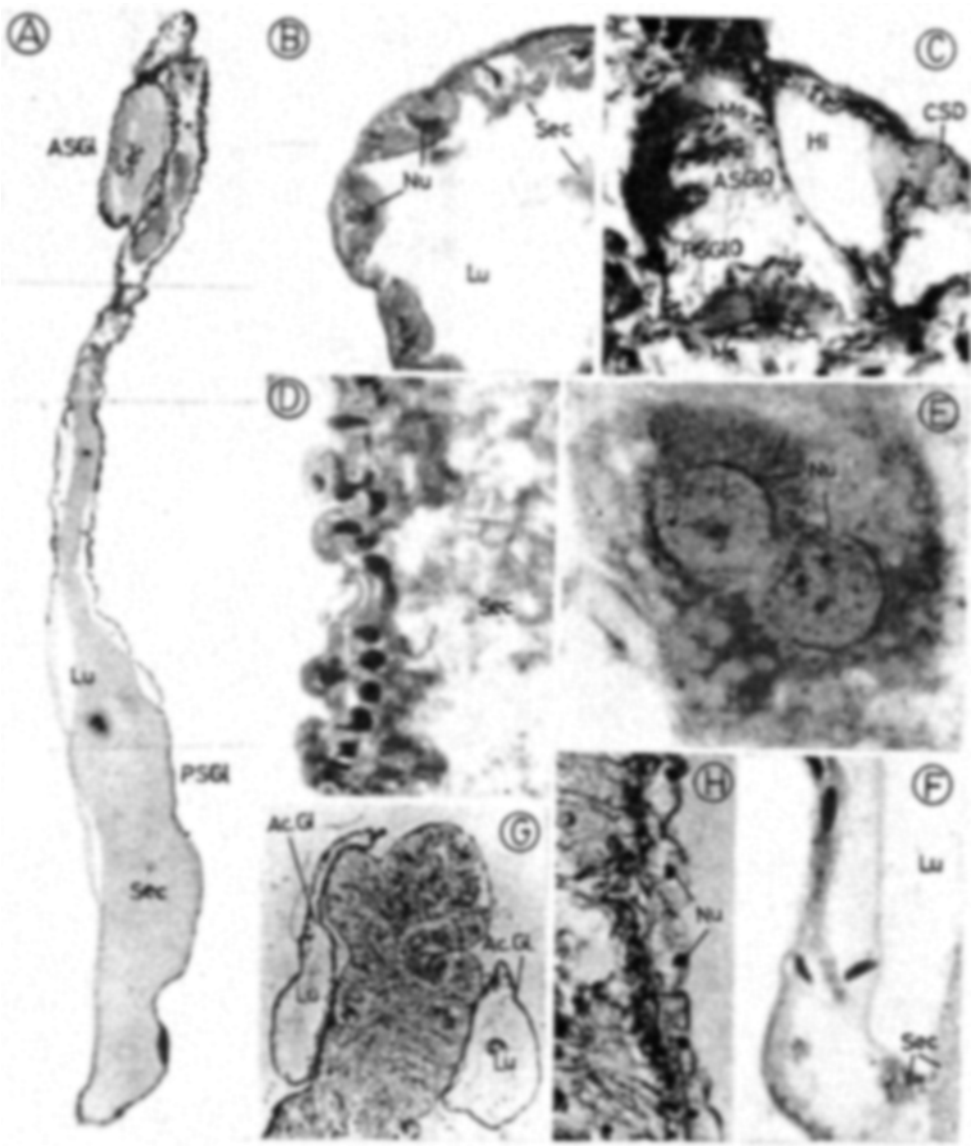

Figure 1. Histology of the salivary system in *A. pedestris*. **A.** LS of the principal salivary gland (× 22). **B.** Uninucleate cells of anterior salivary lobe (× 222). **C.** LS of hilus of salivary lobes (× 200). **D.** Posterior salivary lobe (portion enlarged) (× 262). **E.** Binucleate cells of posterior lobe (× 782). **F.** Posterior salivary gland showing holocrine secretion (× 225). **G.** Accessory salivary gland (× 24). **H.** Uninucleate cells of accessory gland (× 175).

(ASGl, Anterior salivary gland; PSGl, posterior salivary gland; Ac.Gl, accessory gland; Sec, secretory materials; Lu, lumen; Nu, nucleus; Hi, hilus; Ms, muscle layers; ASGlo, anterior salivary gland opening; PSGlo, posterior salivary gland opening; CSD, common salivary duct).

compartmentalised hilus with muscular valve at the junction of the anterior and posterior lobes of the principal salivary gland communicates with the accessory glands, opening into the common salivary duct.

3.2 *Zootoxic effect*

Table 1 indicates the zootoxic effect of concentrated as well as diluted saliva of anterior lobe, posterior lobe, accessory glands and the control. The anterior lobe secretion immobilized the test insect faster than the posterior lobe secretion whereas the accessory gland secretion did not show any effect.

3.3 *Gel electrophoresis studies*

The zymograms and densitometric electropherograms (figure 2) confirm the total number of protein fractions such as 6, 11, 2 and 9 in the anterior and posterior salivary lobes, accessory gland and haemolymph respectively. The banding pattern

Table 1. Toxicity in the secretion of different lobes of salivary gland of *A. pedestris.*

	Time required to immobilize the acridid *Oxya nitidula* (Walker)	
Organ	Saliva	Dilute saliva
Anterior lobe	69 ± 3 s	532 ± 49 s
Posterior lobe	289 ± 50 s	1591 ± 54 s
Accessory gland	No effect	No effect
Control	No effect	No effect

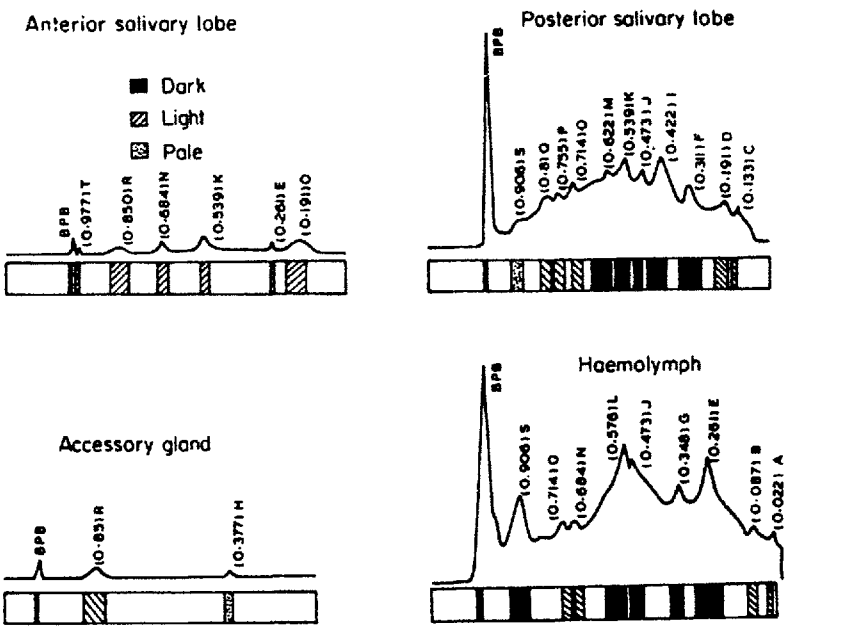

Figure 2. Densitometric scan of protein fractions in salivary system and Haemolymph of *A. pedestris.*

of the histogram as well as the peak obtained in the chromatograms indicate the protein concentration, with most of the bands relating to the haemolymph and posterior salivary lobe being dark, while those of the anterior lobe are light, and those of the accessory gland being pale. Table 2 illustrates the percentage composition of the protein fractions in the salivary system and the haemolymph. The protein fractions 'E' and 'N' in the anterior lobe as well as 'J', 'O' and 'S' in the posterior lobe are found to be similar to the haemolymph proteins, while the other protein fractions differ from each other. In the anterior and posterior salivary lobes the proteins 'K' and 'D' appear to be identical.

4. Discussion

The salivary systems of Reduviids with diverse morphology are found in the different sub-families. *A. pedestris* have a bilobed principal gland and elongated accessory gland as those of the members of the sub-families Harpactorinae, Piratinae, Rhaphidosomatinae and Salyavatinae (Haridass and Ananthakrishnan 1981). Despite their similarity in the morphology there are histological and functional variations in the salivary gland of *A. pedestris*. While Haridass and Ananthakrishnan (1981) earlier indicated binucleate nature of the cells with apocrine and merocrine in anterior and posterior lobes respectively, present observations indicate uninucleate cells with merocrine nature of the secretion in the anterior lobe and the binucleated cells with holocrine nature of secretion in the

Table 2. Electrophoretic analysis of protein profiles of Haemolymph and salivary glands of *A. pedestris*.

Protein (R_m value)		Haemolymph	Salivary apparatus		
			Anterior lobe	Posterior lobe	Accessory gland
(0·022)	A	0·752	—	—	—
(0·087)	B	3·312	—	—	—
(0·133)	C	—	—	0·427	—
(0·191)	D	—	46·453	3·655	—
(0·261)	E	18·572	3·449	—	—
(0·311)	F	—	—	6·936	—
(0·348)	G	14·515	—	—	—
(0·377)	H	—	—	—	9·816
(0·422)	I	—	—	15·872	—
(0·473)	J	17·439	—	10·268	—
(0·539)	K	—	5·434	20·72	—
(0·576)	L	31·301	—	—	—
(0·622)	M	—	—	23·194	—
(0·684)	N	3·42	16·114	—	—
(0·714)	O	2·024	—	6·733	—
(0·755)	P	—	—	4·345	—
(0·8)	Q	—	—	6·963	—
(0·85)	R	—	25·339	—	90·183
(0·906)	S	8·67	—	0·889	—
(0·977)	T	—	3·212	—	—

Values represent area percentage.

posterior lobe of the principal salivary gland. Observations from the zootoxicity experiments and the diverse functions of the secretions were further confirmed by the presence of the different protein fractions in the anterior, posterior and accessory glands of the salivary system. This result deviates from the earlier analysis made by Edwards (1961) in the different lobes of salivary gland of the reduviid, *Platymeris rhodhamanthus* Gaerst., wherein he confirmed the presence of zootoxic substances both in the anterior as well as posterior lobes besides the digestive enzyme secreted by both the lobes. The accessory glands differ histologically from the lobes of the principal gland and secretes watery saliva, which has less protein fraction than the other lobes. Similar results were highlighted recently in pentatomid and coreid bugs (Miles and Slowiak 1976) and in assassin bugs (Haridass and Ananthakrishnan 1981). The presence of identical proteins in the haemolymph as well as anterior and posterior lobes presumably indicate the transportation of haemolymph protein into the salivary system. This observation coincides with the results obtained in phytophagous heteropteran by Miles (1967), who stated that haemolymph transports the essential amino acids, glycerol and glucose to the saliva secretion.

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References

Baptist B A 1941 The morphology and physiology of the salivary glands of Hemiptera; *Q. J. Microsc. Sci.* **83** 91–139

Bronskill J F, Salkeld E H and Friend W G 1958 Anatomy, histology and secretion of the salivary glands of the large milk weed bug, *Oncopeltus fasciatus* (Dallas) Hemiptera—Lygaeidae; *Can. J. Zool.* **30** 961–968

Edwards J S 1961 The action and composition of the saliva of an assassin bug, *Platymeris rhadamanthus* Gaerst (Hemiptera: Reduviidae); *J. Exp. Biol.* **38** 61–67

Davis B J 1964 Disc electrophoresis II. Method and application to human serum proteins; *Ann. N. Y. Acad. Sci.* **121** 404–427

Haridass E T and Ananthakrishnan T N 1981 Functional morphology of the salivary system in some Reduviidae (Insecta—Heteroptera); *Proc. Indian Acad. Sci. (Anim. Sci.)* **90** 145–160

Hori K 1969 Some properties of salivary amylases of *Adelphocoris suturalis* (Miridae) *Dolicoris baccarum* (Pentatomidae) and several other heteropteran species; *Entomol. Exp. Appl.* **12** 454–466

Louis D and Kumar R 1973 Morphology of the alimentary and reproductive organs in Reduviidae (Hemiptera—Heteroptera) with comments on interrelationships within the family; *Ann. Entomol. Soc. Am.* **66** 635–639

Miles P W 1959 The salivary secretion of the plant bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera—Lygaeidae) I. The type of secretion and their roles during feeding; *J. Insect Physiol.* **3** 243–245

Miles P W 1964a Studies on the salivary physiology of plant bugs: Oxidase activity in the salivary apparatus and saliva; *J. Insect Physiol.* **10** 121–129

Miles P W 1964b Studies on the salivary physiology of plant bugs: The chemistry of formation of sheath material; *J. Insect Physiol.* **10** 147–160

Miles P W 1967 Studies on the salivary physiology of plant bugs: Transport from haemolymph to saliva; *J. Insect Physiol.* **13** 1787–1801

Miles P W 1968 Studies on the salivary physiology of plant bugs: experimental induction of galls; *J. Insect Physiol.* **14** 97–106

Miles P W 1972 The saliva of Hemiptera; *Adv. Insect Physiol.* **9** 183–255

Miles P W and Slowiak D 1976 The accessory salivary gland as the source of water in the saliva of Hemiptera—Heteroptera; *Experientia* **38** 1011–1012

Miyamoto S 1961 Comparative morphology of the alimentary organs of Heteroptera, with phylogenetic consideration; *Sieboldia* **2** 197–259

Salkeld E H 1960 A histochemical study of the secretions in the principal salivary glands of the large milk weed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae); *Can. J. Zool.* **38** 449–454

Saxena K N 1963 Mode of ingestion in a heteropterous insect, *Dysdercus koenigii* Fabr. (Pyrrhocoridae); *J. Insect Physiol.* **9** 47–71

Wigglesworth V B 1943 Haemoglobin studies in the Reduviid Rhodnius; *Proc. Roy. Soc.* **B131** 313–339