

HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE MALE ACCESSORY GLANDS OF CULICINE MOSQUITOES

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The paper deals with studies on the development, histology and histochemistry of the male accessory glands of *Aedes aegypti* and *Culex pipiens fatigans*. The paired accessory glands in both the species grow rapidly from pupal stage till they mature in two-day old adults. The glands have an outer layer of muscles and an inner layer of simple columnar secretory cells. In *Culex* the gland has a central lumen in pupal stage while it is partly filled with cells in *Aedes*. The secretory cells are similar morphologically but secretory activity is more in the posterior part of the glands than in the anterior part in both the species. The secretory granules in *Aedes* are small and are present in the lumen of the gland within polygonal cells whereas in *Culex* they are large and are not in bound form. Histochemical study suggests that the secretion is predominantly proteins, rich in SH-containing amino acids. Further histochemical evidences indicate the presence of glycogen in the secretions of *Aedes* and *Culex*, and mucopolysaccharide in *Culex* only. The relevance of these findings is discussed.

INTRODUCTION

Recent studies have shown that the passage of male accessory gland substance into female mosquito triggers many physiological changes in the female (LEAHY & CRAIG, 1965; CRAIG, 1967; DOWNE, 1975; ADLAKHA & PILLAI, 1975, 1976). The accessory glands in adult male mosquito enlarge prior to mating and diminish in size after mating (LUM, 1961; FOSTER & LEA, 1975). However studies on histology and histochemistry of accessory glands have not received much attention. Only ultrastructure of the male accessory glands of *Culex pipiens pallens* (TONGU *et al.*, 1972) and of the secretory cells in *Aedes aegypti* have been reported so far (DAPPLES *et al.*, 1974). Studies on the chemical nature of the accessory gland secretion are incomplete. In *Ae. aegypti*, the secretion of the gland is reported to be proteinaceous and two

proteins have been identified (FUCHS *et al.*, 1969). The present paper deals with a comparative study on the development, histology and histochemistry of the male accessory glands in two species of culicine mosquitoes *viz.* the yellow-fever mosquito, *Ae. aegypti* and the tropical house mosquito, *Culex pipiens fatigans*.

MATERIALS AND METHODS

Mosquitoes for the present study were taken from colonies of Delhi strains of *Ae. aegypti* and *C. p. fatigans* maintained at 28°C and 80% RH in an insectary (ADLAKHA & PILLAI, 1975). Accessory glands from male pupae and adults at different time intervals after emergence were dissected out in *Aedes*-Ringer solution. Size of the gland was measured by means of a calibrated ocular micrometer. For histological studies the glands were fixed in BOUIN'S fluid and processed for paraffin embedding and the sections were stained with either DELAFIELD or HARRIS haematoxylin and Alcoholic eosin. For histochemical study BOUIN'S fixative was used for carbohydrate, CARNOY'S fixative for DNA and RNA and 10% neutral formalin for proteins and lipids. The tissues were either embedded in

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paraffin and processed or they were embedded in gelatin and sectioned in a cryostat. The following histochemical methods have been performed according to the different methods cited in *Histochemistry* (PEARSE, 1968). Proteins were demonstrated by mercury bromophenol blue method, cysteine and cystine by paraldehyde fuchsin method (EWEN, 1962), arginine by SAKAGUCHI reaction, tyrosine by MILLON'S reaction, basic protein by fast green staining at pH 8, 1, 2-glycol by PAS method, acid mucopolysaccharides by alcian blue, lipids by sudan black B, neutral lipids by oil red O method, and nucleic acids by methyl green pyronin method and by FEULGEN-fast green method.

RESULTS

Morphology

In *Ae. aegypti* and *C. p. fatigans* the accessory glands develop as a pair of bud-like outgrowths from either side of the seminal vesicles in early pupal stage (Figs. 1 & 6). At this stage the glands are translucent and occupy two thirds of the last abdominal segment. In the adult, the glands increase in length and diameter and occupy almost the whole of the last abdominal segment. The glands in both *Culex* and *Aedes* exhibit a progressive increase in size from pupal stage till two days after adult emergence as evidenced by the data presented in Table 1. Two-day old males are mature enough to inseminate the females and normally the males mate only on the third day. The accessory glands in *Aedes* grow more in diameter, the increase being about 67% as compared to increase in length which was only about

30% from pupal to two-day old adults. On the other hand, in *Culex* the glands grow more lengthwise, the increase being about 55% while the diameter increased only about 30% from the pupal stage. The mature glands of *Aedes* are opaque white in colour while in *Culex* they are yellowish. At this stage the glands are fully filled with their secretions.

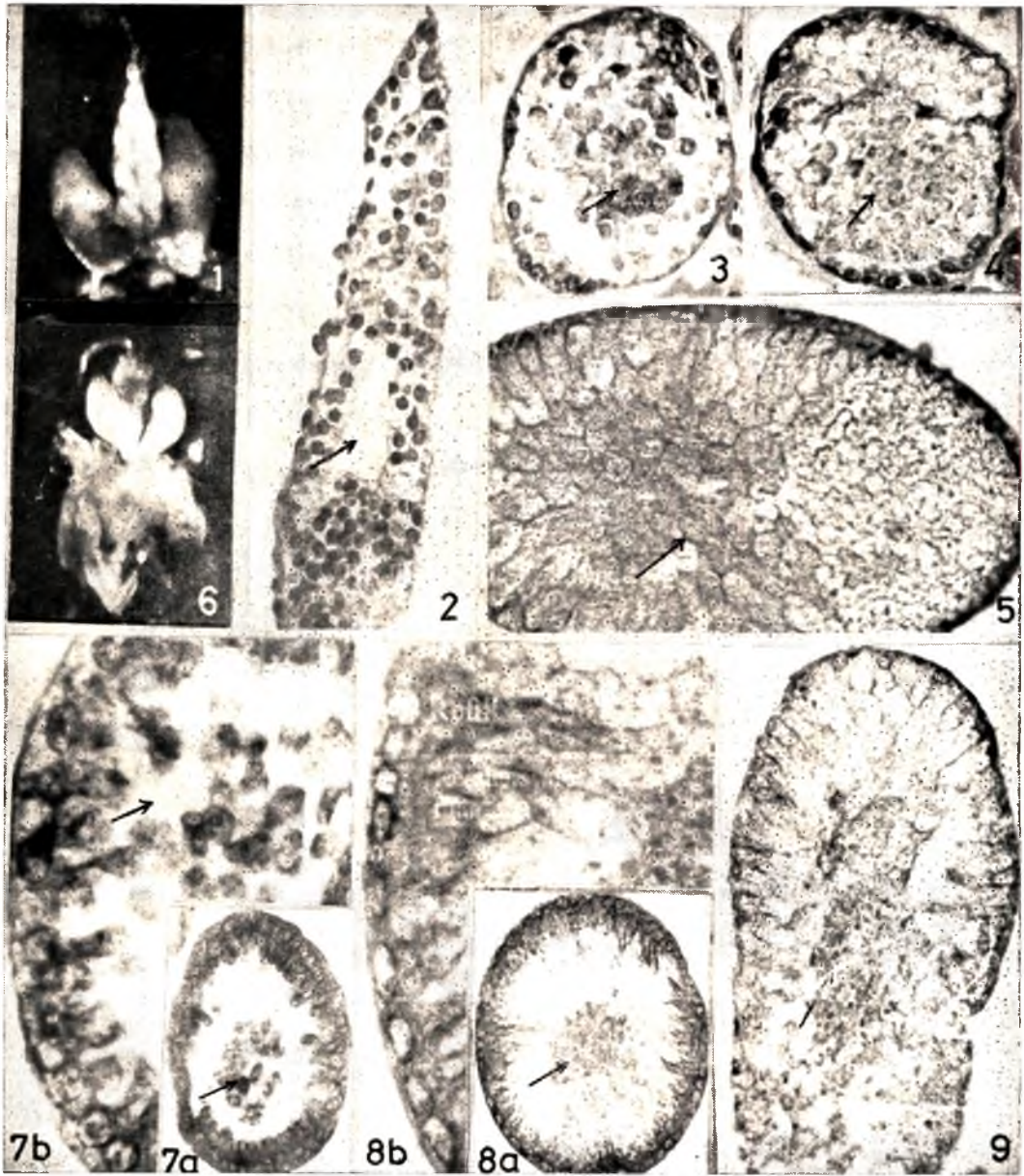
Histology

Accessory glands in both *Aedes* and *Culex* have an outer thin muscular layer and an inner layer of columnar cells. The centre of the gland has a lumen partly filled by secretory cells in the pupa which later on gets completely filled with secretions in the adults. Each secretory cell has a large nucleus with 12 to 14 nucleoli. The secretory cells are absent where the glands join the seminal vesicle. The glands in *Aedes* and *Culex* do not have sperms at any time in them.

In *Aedes* the accessory glands in the pupal stage show loosely packed secretory cells with round nuclei and large intercellular spaces (Figs. 2 & 3). In newly emerged adults the secretory cells enlarge and also increase in number thereby reducing the intercellular spaces (Fig. 4). In the pupal stage the lumen is partly packed with secretory cells and later these cells filled with more secretion and become polygonal in shape. In 1-day males the number of polygonal cells become more and in 2-day

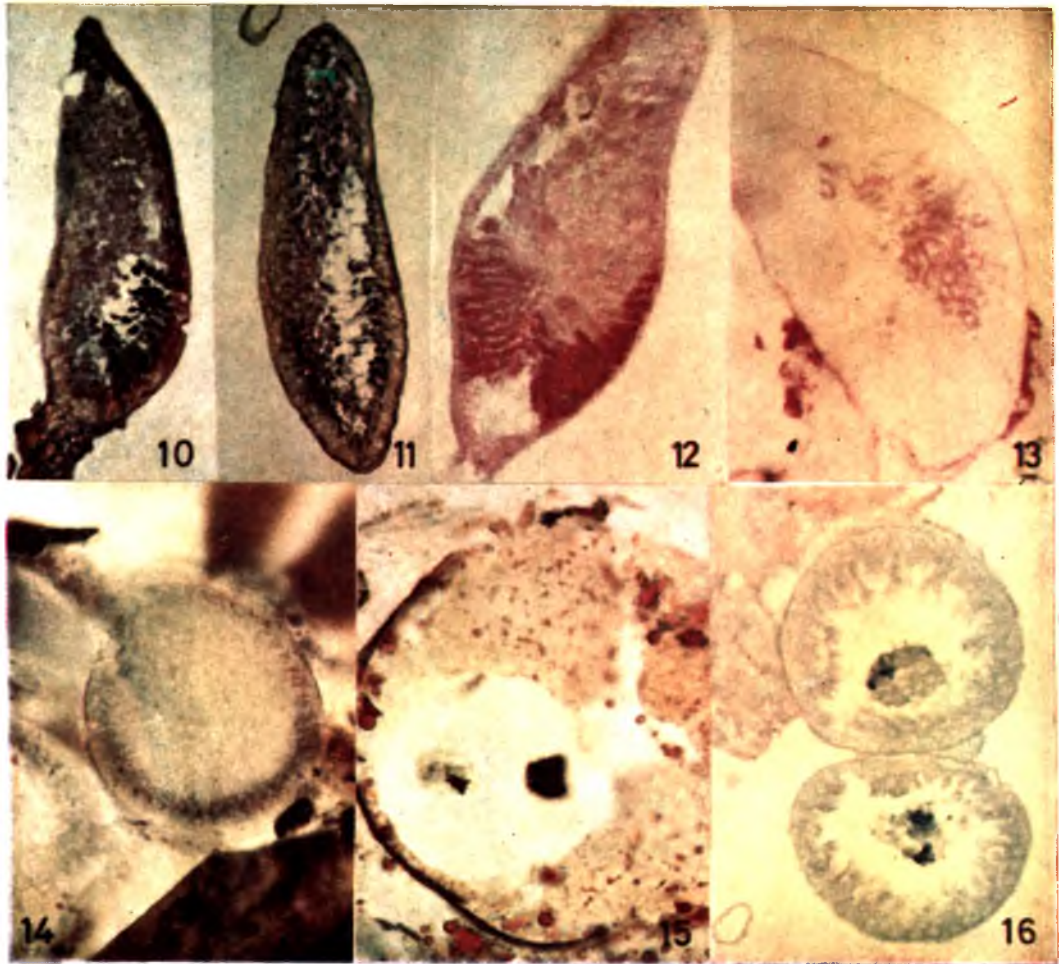
TABLE 1. Measurements of the male accessory glands of *Ae. aegypti* and *C. p. fatigans*

Stage	Length in micron		Diameter in micron	
	<i>Aedes</i>	<i>Culex</i>	<i>Aedes</i>	<i>Culex</i>
Pupa	293.3 ± 16.7	272 ± 12.6	108.3 ± 2.5	132.2 ± 5.8
Newly emerged adult	330.3 ± 10.3	319 ± 6.3	119.2 ± 5.9	137.5 ± 3.3
1 day old adult	374.7 ± 7.4	334 ± 2.7	163.9 ± 6.1	151.8 ± 5.3
2 day old adult	391.7 ± 12.8	492 ± 11.5	177.1 ± 5.0	170.5 ± 9.3



Figs. 1-5. Male accessory glands of *A. aegypti*. Fig. 1. accessory glands of 2-day old adults; Fig. 2. l. s. of the gland of early pupa showing the secretory cells and central lumen; Fig. 3. t. s. of the gland of late pupa showing the central lumen filled with secretory cells; Fig. 4. t. s. of the gland of newly emerged adult and 5. t. s. of the gland of 2-day old adult showing secretory granules in polygonal cells.

Figs. 6-9. Male accessory glands of *C. p. fatigans*. Fig. 6. accessory glands of late pupa; Fig. 7a. t. s. of the gland of late pupa showing central lumen; Fig. 7b. the same enlarged; Fig. 8a. t. s. of the gland of 1 - day old adult showing secretion in the lumen; Fig. 8b. the same enlarged and Fig. 9. t.s. of the gland of 2- day old adult showing the lumen completely filled with secretory granules.



Figs. 10-11 L. S. of the gland of *Aedes* and *Culex* respectively showing glycogen (PAS method).

Figs. 12-13. L. S. of the gland of *Aedes* and *Culex* respectively showing PAF response (cysteine and cystine bound proteins)

Fig. 14 T. S. of the gland of *Aedes* showing lipids (Sudan black B method).

Fig. 15 T. S. of the gland of *Aedes* showing neutral lipids (Oil red O method).

Fig. 16. T. S. of the gland of *Culex* showing acid mucopolysaccharide (Alcian blue method).

old glands the lumen shows the secretion partly free and partly within the cells (Fig. 5). The secretory granules are small in size.

In the pupal stage of *Culex* the secretory columnar cells are closely packed leaving no intercellular spaces (Figs. 7a, b) and the central lumen of the gland has less secretory cells than in *Aedes*. In 1-day old males the lumen shows presence of free secretory granules which are bigger in size (Figs. 8a, b). The nuclei in the secretory cells are oval in shape. In 2-day old glands the entire lumen is filled with secretory

granules (Fig. 9). In mature glands the secretory cells recede to the lining epithelium thereby increasing the space of the lumen.

Histochemistry

The results of the histochemical tests are summarized in Table 2. In the adult *Aedes* posterior region of the gland shows more intense PAS positive response as compared to the anterior region indicating difference in the secretory activity of the cells (Fig. 10). In the adult *Culex* the PAS positive granules were confined to the central lumen only (Fig. 11). Acetylation

TABLE 2. Data on the histochemical tests performed on the male accessory glands of *Ae. aegypti* and *C. p. fatigans*

Test performed	Adult							
	Pupa		Newly emerged		1 day old		2 day old	
	<i>Aedes</i>	<i>Culex</i>	<i>Aedes</i>	<i>Culex</i>	<i>Aedes</i>	<i>Culex</i>	<i>Aedes</i>	<i>Culex</i>
A. Protein and amino acids								
i) General (bromophenol blue)	*	*	**	*	***	***	****	****
ii) Arginine (SAKAGUCHI oxine reaction)	—	—	—	—	—	—	—	—
iii) Tyrosine (MILLON'S reaction)	—	—	—	—	—	—	—	—
iv) Cysteine and Cystine (Paraldehyde fuchsin method)	*	*	**	*	***	***	****	****
B. Carbohydrates								
i) General (Periodic Acid Schiff reaction)	*	*	**	*	***	*	****	**
ii) Acid mucopolysaccharide (alcian blue)	—	—	—	—	—	*	—	*
C. Lipids								
i) General (Sudan-black B)	Not observed						*	*
ii) Neutral lipids (Oil red O)	Not observed						*	*
D. Nucleic acids								
i) Methyl green pylonin	***	***	***	**	**	**	*	*
ii) Feulgen fast green	***	***	*	*	*	*	*	*

— No response to tests
 * Faint reaction
 ** Moderate reaction
 *** High intensity

with acetic anhydride and pyridine blocks the PAS response. This confirms the presence of neutral polysaccharides in the secretion. Treatment with 1% diastase before PAS treatment showed almost no staining with PAS further confirming that the polysaccharide is glycogen. Presence of acid mucopolysaccharide is tested by alcian blue method. The secretion of mature glands of *Culex* has showed positive response (Fig. 15) but not so of *Aedes*.

Mercury bromophenol blue method shows positive response in pupal and adult accessory glands of both the species. With increase in age the intensity of staining is more in the secretion than in the secretory cells and thus it indicates it is mainly proteinaceous. SAKAGUCHI reaction for arginine and MILLON'S reaction for tyrosine are negative in all developing stages of both the species. However, the secretory granules are found to be positive to PAF reaction for cysteine and cystine. In the adult glands the staining is more intense in the posterior region than in the anterior region both in *Aedes* and *Culex* (Figs. 12 & 13). Glands stained with fast green at pH 8.0 show positive response indicating the presence of more of basic amino acids in proteins.

Tests for lipids are performed only in the adult glands. Sudan black B for general lipids is positive in the proximal part of the secretory cells in both the species, though the staining was faint as compared to fat cells. Oil red O staining shows a small amount of neutral lipids detected as small droplets uniformly distributed in the secretory cells of the glands. The staining reaction is not intense as normally seen in the fat cells (Figs. 14 & 15).

Methyl green pyronin staining is positive in the gland. In the pupal stage the cells show mainly bluish green colour of the methyl green but in the adult stage the

cells become pinkish due to pyronin staining. These indicate that there is abundant DNA in the secretory cells of the pupal stage and RNA in the secretory cells of the adult stage of both *Aedes* and *Culex*. Control slides hydrolysed by 1 N HCl at 90°C did not take up the stain. These results are further confirmed by FEULGEN fast green method.

DISCUSSION

The present study has revealed that the accessory glands of *Ae. aegypti* and *C. p. fatigans* have an outer circular muscle sheath and an inner layer of simple cells. The development and subsequent increase in size of the glands in mature males are almost identical. The increase in size during post emergence maturation period is due to the accumulation of secretory materials in the gland cells (DAPPLES *et al.*, 1974). Accessory glands of *Aedes* differ from those of *C. p. fatigans* in having small central lumen. In *C. p. pallens* (TONGU *et al.*, 1972) the glands have narrow canals in the centre in place of lumen. The cells of the glands are all morphologically identical and are found to be secretory. Ultrastructural studies have shown that the cells of the accessory glands in *Ae. aegypti* are morphologically similar (DAPPLES *et al.*, 1974) unlike in *C. p. pallens* wherein there are four types of morphologically different secretory cells in the accessory glands (TONGU *et al.*, 1972).

In *Ae. aegypti* the secretion of the mature glands is bound in polygonal cells. This is supported by the findings of DAPPLES *et al.* (1974) as they have suggested that the secretion is apocrine in *Ae. aegypti* and that the secretion-laden cells are pinched off forming membrane bound packets of secretory granules and other cytoplasmic components and that the loose cytoplasmic material is also released during the apocrine process and before the cell membranes are

reformed. However, in *C. p. fatigans* the secretion is not in bound form. In *C. p. pallens* three types of secretory granules have been observed; of these only one type is found to be free and the other two types are bound in membranes (TONGU *et al.*, 1972). Whether the release of secretion in *C. p. fatigans* is holocrine or apocrine is not clear from the present study. The glands in both the species are devoid of secretory granules in their pupal stage. The secretory granules of *Aedes* seem to be finely granular and in bound form while in *Culex* they are bigger and not in bound form.

In both the species the secretory cells of the posterior region are shown to be more active with regard to PAS staining and PAF staining suggesting the existence of two types of secretory cells. Electron microscopic studies of the glands of *Ae. aegypti* have revealed only two types of secretory cells, the anterior cells being less active than the posterior ones (DAPPLES *et al.*, 1974). Histochemical studies on nucleic acids reveal that the DNA content was more in early stages of the development of gland and later more RNA could be seen in cells near the centre. This evidently coincides with the biosynthesis of secretory substances in the cells of the glands. The present study indicates that the secretions in both the glands are predominantly proteaceous. The accessory gland substance of *Ae. aegypti* known as matrone (CRAIG, 1967) consists of two protein fractions. Of these only one fraction stimulates oviposition, but both together ensure monogamy in females (FUCHS *et al.*, 1969; FUCHS & HISS, 1970, HISS & FUCHS, 1972). The accessory gland secretion of *Aedes* and *Culex* is found to be rich in SH-containing amino acids. Similarly in *C. p. pallens* the secretion of the gland is stainable with aldehyde fuchsin and the authors have suggested a probable hormonal function for the accessory glands (TONGU *et al.*, 1972).

The presence of the polysaccharide glycogen in adult gland suggests that the secretion may be a glycoprotein. Earlier biochemical evidence in *Ae. aegypti* indicates that the main constituents of matrone may be glycoproteins or a protein and a polysaccharide (FUCHS *et al.*, 1969). The secretion of the accessory glands of *Culex* shows the presence of acid mucopolysaccharides though its significance is not clear. Recent studies have shown that the accessory gland substance is involved in blood intake and blood digestion of the female mosquitoes (ADLAKHA & PILLAI, 1976; DOWNE, 1975) and it is also essential for the fertility of the eggs (ADLAKHA & PILLAI 1975). The multifarious functions of glands do suggest the possibility of many chemical components in their secretion.

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