

Histochemical Studies on the Male Accessory Glands of the Tenebrionid Beetle *Blaps polychresta* (Coleoptera, Tenebrionidae)

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ABSTRACT

The aim of the present study is to describe the male accessory glands of the tenebrionid beetle *Blaps polychresta* using histochemical methods and to detect the nature of their secretions. In the adult male tenebrionid beetle *Blaps polychresta*, there is one pair of bean – shaped and other pair of long tubular accessory reproductive glands. Each bean – shaped gland consists of a single layer of secretory columnar epithelium, surrounded by a muscular coat which is highly tracheated and innervated. Each tubular gland consists of one type of secretory columnar epithelium, surrounded by thin muscular coat which is innervated and highly tracheated. The nuclei of secretory cells in both glands are situated in the basal zone. Histochemical tests of the bean – shaped glands indicate that the secretion of these glands is mainly composed of neutral mucopolysaccharide, glycogen, proteins and lipids. Histochemical tests of the tubular glands indicate the presence of neutral mucopolysaccharide and scanty acidic mucopolysaccharide secretion inside the lumen of these glands, the positive reaction for glycogen and proteins in the cells and secretion inside the lumen of these glands indicate the glycoprotein nature of the secretion.

Keywords: *Blaps*, Bean – shaped, tubular male accessory glands, Histochemistry.

INTRODUCTION

Male accessory glands are commonly found in insects except for some Apterygota and Diptera (Grimes & Happ, 1980) and they play several key roles in their reproduction. One of the main functions of these glands is to secrete the substances involved in the production of spermatophores structures that protect the sperms and facilitates their transfer from the male to the female (Leopold, 1976; Muzzi, 2020). Nevertheless, male accessory gland secretions are often involved in many other processes such as the structural organization of spermatozoan bundles (Viscuso *et al.*, 2001) or the promotion of their activation and motility ensuring the successful storage inside the female genital tract (Lung *et al.*, 2001; Mueller *et al.*; 2007). In some other cases, the products of the male accessory glands are directly involved in spermatozoan competition by either inactivating / reducing the effective number of germ cells from the previous mating (Muzzi, 2020). Other subsidiary effects of accessory gland secretions include the ability to directly affect and modulate the post mating behavior of females, causing a vast array of responses that include increasing in egg production and maturation, enhancement of oviposition and decrease in re-mating receptivity (Avila *et al.*; 2011; Hayashi and Takami, 2014; Yu *et al.*; 2014; Yamane *et al.*, 2015; Carmel *et al.*, 2016).

The aim of the present study is to describe the male accessory glands of the tenebrionid beetle *Blaps polychresta* using histochemical methods and to detect the nature of their secretions.

MATERIALS AND METHODS

The male adults of *Blaps polychresta* were collected from gardens during the breeding season (September – November) and reared in the laboratory, Faculty of Science, Alexandria University, under normal conditions.

The bean – shaped and tubular accessory glands were dissected out using a dissecting stereo binocular microscope and fixed in alcoholic Bouin for light microscopy. Paraffin sections (5-7 μ m thick) were stained using Ehrlich's haematoxylin and eosin. Unfixed cryostat sections (10 μ m thick) were stained using Sudan III for the demonstration of lipids. For the detection of carbohydrates, they were stained using the PAS-Alcian blue method (Mowry, 1963). For the detection of proteins and glycogen, paraffin sections fixed in 10% neutral buffered formalin, stained with mercuric bromophenol blue for proteins (Mazia *et al.*, 1953) and Best's Carmine for glycogen (Graumann and Neumann, 1971).

RESULTS AND DISCUSSION

In an earlier work, El- Zoheiry, (1999 a,b) referred to that the reproductive system of adult male beetle *Blaps polychresta* consists of two testes, two vasa deferentia, two seminal vesicles, an ejaculatory duct and two pairs of accessory glands, one pair is long and tubular and the other is bean – shaped (Fig. 1). The two seminal vesicles and the two pairs of accessory glands open into the proximal end of the ejaculatory duct. The author studied the ultrastructure of accessory glands in *Blaps polychresta*. Paraffin sections of bean – shaped gland showed secretory columnar epithelium

surrounded by a muscular coat which is highly tracheated (Fig. 2), and the tubular gland showed also secretory columnar epithelium - with apocrine secretion in the apical part of cytoplasm - and surrounded by a thin muscular coat (Fig. 3).

Histochemical tests of bean - shaped gland showed that both the secretion inside the lumen of the gland and the cells reveal strong reaction (blue colour) with mercuric bromophenol blue indicating the presence of proteins (Fig. 4). Paraffin sections of the same gland stained with PAS - Alcian blue showed pink secretion inside the lumen of the gland and in the apical parts of their secretory cells indicating the presence of neutral mucopolysaccharides (Fig. 5). Moderate positive reaction for glycogen is shown in the basal parts of the cells of bean - shaped gland, positive reaction for glycogen is also shown inside the lumen of the gland (Fig. 6). Unfixed cryostat sections of bean - shaped gland showed positive reaction when stained with Sudan III indicating the presence of lipids (Fig. 7). Histochemical tests of tubular gland of *Blaps polychresta* give negative reaction for lipids which are strongly detected in the paragonadal fat body surrounding the gland with faint blue secretion inside the lumen of this gland (Fig. 8). Protein is detected in both the secretions and the cells of this gland (Fig. 9). Neutral mucopolysaccharide is detected in the secretory cells and scanty acidic mucopolysaccharide is detected inside the lumen of the tubular gland (Fig. 10). Strong reaction with Best's carmine in both the secretion inside the lumen of gland and the cells is shown indicating the presence of glycogen (Fig. 11). In the mucoid glands, the staining affinity for Best's carmine is very strong (Graumann & Neumann, 1971). This fact agrees with the obtained results in *Blaps*

polychresta. The present observations in *Blaps polychresta* coincide with the conclusion of Mowry (1963) who stated that the Alcian blue - PAS method stains the neutral mucopolysaccharides red and the acidic mucopolysaccharides blue.

It is suggested that some precursor material, or even prefabricated molecules from the paragonadal fat body - which is considered as an important center of intermediary metabolism - may enter the accessory glands for further elaboration into the characteristic secretions of the accessory glands. This seems rather unlikely, however, since fat body cells are dominated by glycogen and neutral lipid deposits in contrast to the non glycogen polysaccharide and acidic lipid normally found in accessory glands (Odhiambo, 1967; c.f., Odhiambo, 1969). Leopold (1976) reported that proteins and carbohydrates appear to be ubiquitous components of accessory reproductive glands of Orthoptera, Dictyoptera, Hemiptera, Coleoptera, Lepidoptera and Diptera. Gadzama *et al.* (1977) showed that proteins and glycoproteins are detected in secretion of the tubular gland of *Tenebrio*. Chen (1978) reported that proteins are more concerned with the transfer and utilization of the sperm rather than the stimulation of fecundity. In *Drosophila*, the sperm may have the capacity to utilize amino acids for energy production.

It is concluded that these studies have confirmed and agreed with most histological, ultrastructural and histochemical observations in similar glands in other species. This knowledge of male accessory gland ultrastructure and nature of their secretory product should facilitate the elucidation and gives the interpretation for the function of these important organs in the reproductive behaviour of these insects.

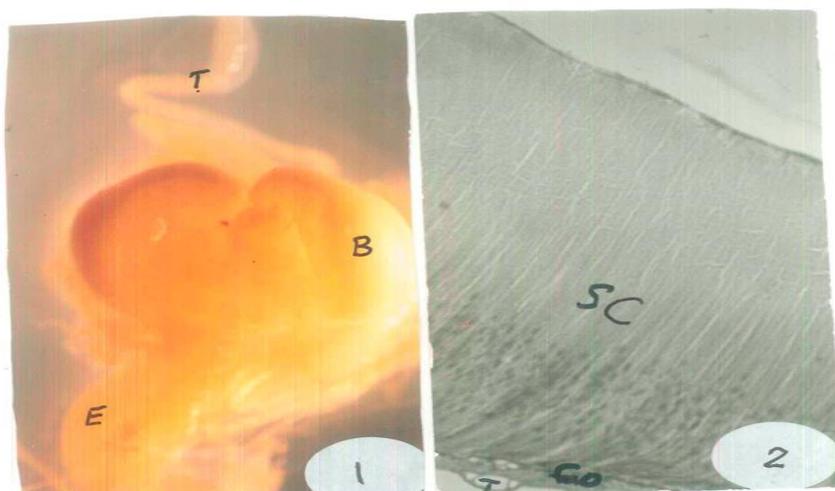


Fig. 1: Showing the bean - shaped accessory gland (B), tubular gland (T) and ejaculatory duct (E) of male *Blaps polychresta*. X10

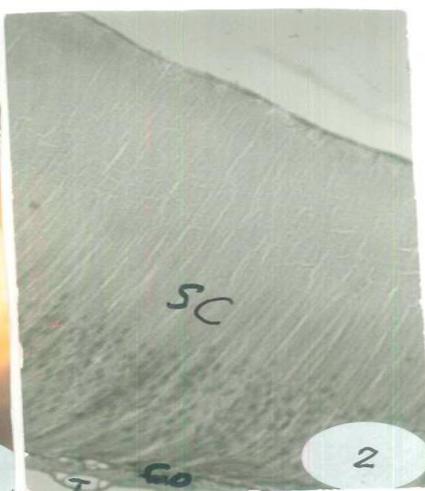


Fig. 2: Paraffin section of the bean- shaped gland showing secretory columnar epithelium (SC), muscular coat (CO) and trachea (T). X500

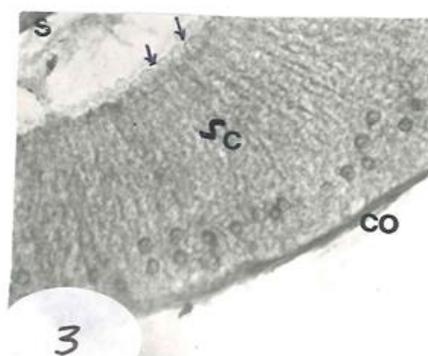


Fig. 3: Paraffin section of the tubular gland showing secretory columnar epithelium (SC) with apocrine secretion (arrows) in the apical part of cytoplasm, muscular coat (Co) and secretory product inside the lumen of the gland (S). X 500

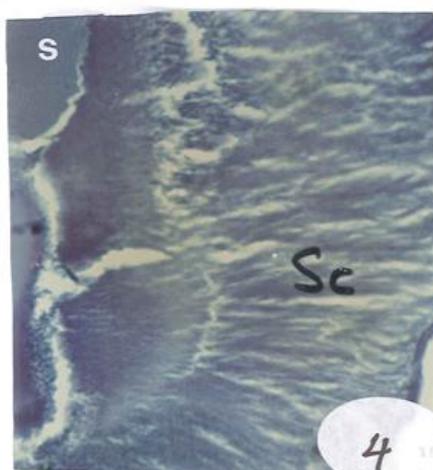


Fig. 4: Paraffin section of bean – shaped gland showing that both the secretion inside the lumen of the gland (s) and the secretory cells (SC) reveal strong reaction (blue colour) with mercuric bromophenol blue indicating the presence of proteins. X 190

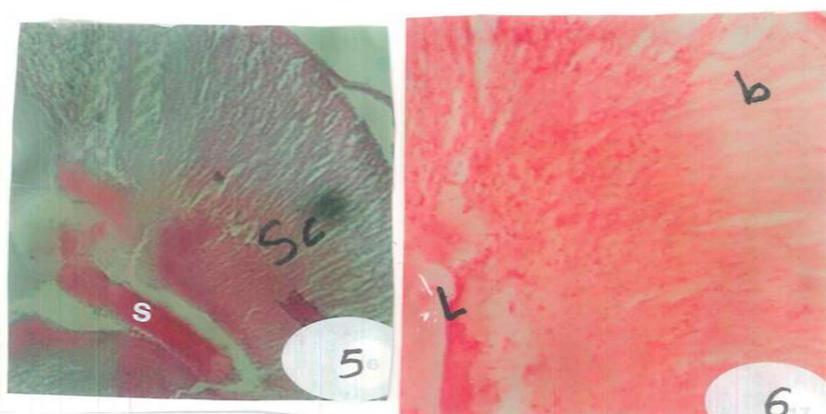


Fig. 5: Paraffin section of bean – shaped gland stained with PAS- Alcian blue showing pink secretion (s) inside the lumen of the gland and in the apical parts of the secretory cells(SC) indicating the presence of neutral mucopolysaccharides. X 190

Fig. 6: Paraffin section of bean – shaped gland stained with Best's carmine showing moderate positive reaction for glycogen in basal parts of the cells (b) which are faintly stained and positive reaction for glycogen is also shown inside the lumen (L) of the gland (red colour). X 190



Fig. 7: Unfixed cryostat section of bean – shaped gland showed positive reaction when stained with Sudan III indicating the presence of lipids. X 30

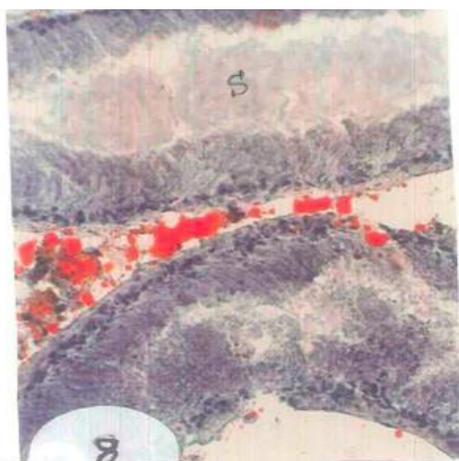


Fig. 8: Unfixed cryostat section of the tubular gland stained with Sudan III showing negative reaction for lipids which are strongly detected in the paragonal fat body surrounding the gland (orange colour) with faint blue secretion (S) inside the lumen of the gland. X 120

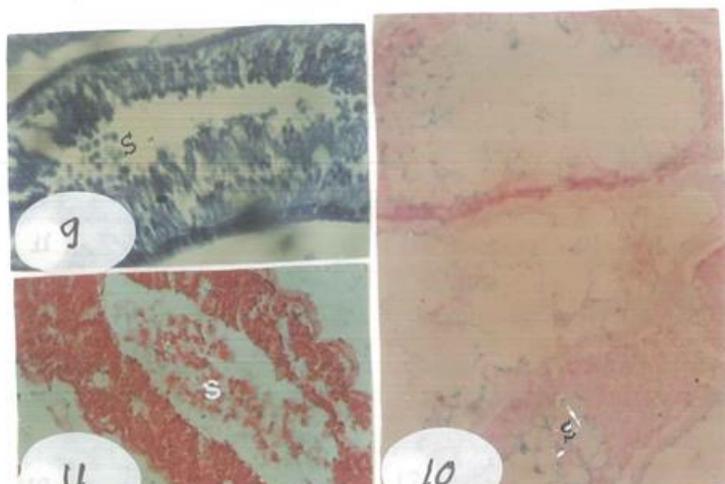


Fig. 9: Paraffin section of tubular gland showing that both the secretion inside the lumen of the gland (s) and the cells reveal strong reaction (blue colour) with mercuric bromophenol blue indicating the presence of proteins. X 190

Fig. 10: Paraffin section of tubular gland stained with PAS- Alcian blue showing neutral mucopolysaccharide (pink colour) in secretory cells and scanty acidic mucopolysaccharide secretion inside the lumen of the gland (blue colour). X 120

Fig. 11: Paraffin section of tubular gland showing strong reaction (red colour) with Best's carmine in both the secretion inside the lumen of the gland (S) and the cells indicating the presence of glycogen. X 190

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الملخص العربي

دراسات هستوكيميائية على الغدد الزائدة في ذكر خنفساء البلبس بولي كريستا (رتبة غمدية الاجنحة - عائلة تنبريونيدي)

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الغرض من هذه الدراسات هو وصف الغدد التناسلية الزائدة في ذكر خنفساء البلبس بولي كريستا باستخدام الطرق الهستوكيميائية بالإضافة الي معرفة طبيعة هذه الإفرازات في هذه الغدد. يوجد زوج من الغدد الكلوية الزائدة التناسلية وزوج آخر من الغدد الأنبوبية الزائدة التناسلية في الذكر البالغ لخنفساء البلبس بولي كريستا. تتكون كل غدة كلوية من طبقة وحيدة طلائية عمودية إفرازية محاطة بغلاف عضلى مزود بقصيبيات هوائية وأعصاب. وتتكون كل غدة أنبوبية من طبقة واحدة من نوع واحد من الخلايا الطلائية العمودية الإفرازية وتحاط هذه الخلايا بطبقة رقيقة من غلاف عضلى مزود بأعصاب وقصيبيات هوائية. وأنوية الخلايا الإفرازية في كل من هذه الغدد تقع فى المنطقة القاعدية.

ولقد أوضحت الإختبارات الهستوكيميائية للغدد الكلوية أن إفرازات هذه الغدد تتكون أساساً من سكريات عديدة مخاطية متعادلة وجليكوجين وبروتينات ودهون.

أما الإختبارات الهستوكيميائية للغدد الأنبوبية فلقد أوضحت وجود سكريات عديدة مخاطية متعادلة وقليل من السكريات العديدة المخاطية الحامضية فى الإفرازات التى توجد فى تجويف هذه الغدد، كما وجد الجليوكوجين والبروتينات داخل الخلايا وفى الإفرازات التى توجد فى تجويف هذه الغدد مما يوضح الطبيعة البروتينية السكرية لهذه الإفرازات.