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Light microscopic Identification Type II and Type III Acini Cells of Salivary Gland of Tropical Cattle Ticks *(Boophilus microplus)*

Thanmaporn Phichitrasilp[1], Worawut Rerkamnuychoke[1], Sirirak Chantakru[1], Daru Srangam[2], Somchai Nismeungpuk[2], Aswin Gingkaew[2] and Sathaporn Jittapalapong[3]

ABSTRACT

Light microscopic study the cell types in the acinus of salivary gland of tropical cattle tick (*Boophilus microplus*). The result showed that six different types of cells can be identified in thick-sections stained with Methylene blue Azure II - Basic fuchsin. The identification of the cells was based on the difference in staining of their granules as well as their morphology of the cells. In type II acinus 'a' cells containing granules which were spherical, packed tight together and stained in deep blue whereas 'b' cells had a less granules than 'c' cells, their granules appeared in loosely pattern and most of granules were stained in blue and some in pink. The 'c' cells were classified into two types, granular and agranular cells. The granular 'c' cell, contained pink and blue granules. In type III acinus 'd' cell had granules which were similar to those of 'a' cells. Granules of 'e' cells were larger than those of 'd' cell and stained in blue to light blue and 'f 'cell were agranular cells.

Key words: *Boophilus microplus*, salivary gland, ticks

INTRODUCTION

Study the salivary glands of ticks is consisted of paraffin - embedded sections with light microscope (LM) and electron microscope technique (EM). However, LM technique has a limitation due to size and shape of tick salivary gland acinus cells. Cells of tick salivary gland are morphologically identified. EM even though has more advantages in identifying cell types than LM but it is required more time and skill. The paired grape-like salivary glands of tropical cattle ticks lie in the anteriolateral region of the body and posteriorly to the fouth coxa. The gland is composed of main ducts which enter the salivarium and posteriorly, branch into secondary ducts that terminate in efferent ducts which are attached to the acinus (Megaw and Beadle,1979). The salivary gland of ixodidae ticks have three types in females and four types in males (Binnington,1978; Binnington and Stone,1977; Chinery,1965; Coon and Roshdy,1973; Krolak *et al*.,1982; Meredith and Kaufman,1973; Till,1961). The acini of salivary glands contain three types. Type I acinus are directly attached to the anterior region of the main salivary duct. The type I acinar cells appear as the cells with large nuclei. The numbers of cell, shapes of the cells as well as granularity do not change during the period of feeding. (Chinery,1965; Megaw and Beadle,1979). Type II acinus connects

1 Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
2 Rajamongala Institute of Technology Chanthaburi Campus Chanthaburi, Thailand.
3 Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.

to lobular ducts. This type is consisted of three cell types; 'a', 'b' and 'c' cell (Coons and Roshdy,1973; Megaw and Beadle,1979). Type III acinus are occupied posterior part of the lobulated mass of the gland. They connect to the main salivary duct or to its branches by compound lobular ducts. Type III contains 'd', 'e' and 'f' cell (Coons and Roshdy,1973; Fawcett *et al*.,1981). Type II and III acini are focused in many studies due to morphological changes of these acini during different stages of life cycle.

In several studies of tick salivary glands, several dyes have been used in paraffin - embedded sections including Periodic acid - Schiff (PAS) (Binninton,1978). Haematoxyline and Eosin (H and E), Mallory's triple stain (Chinery,1965) and in thick-section 1% azure II, Analine black blue (ABB), PAS (Coons and Roshdy,1973) and Giemsa's stain (Walker *et al*., 1985; Gill and walker,1987). Although special staining helped identify the cell types in tick salivary gland as well as the granular components within each cells type (Binington,1978), simple methods to identify the cell may require to reduce the cost and time. Our study showed that the standard staining with Methylene blue Azure II - Basic fuchsin for epoxy-embedded sections successfully differentiated the cells of all types in type II and III acini.

MATERIALS AND METHODS

Salivary glands were collected from 72 hrs fed 10 female cattle tick (*Boophilus microplus*). They were fixed in 2.5% glutaradehyde in cacodylate buffer for 2 hrs. They were rinsed in cacodylate buffer and postfixed in 1% osmium tetroxide for 2 hrs. The specimens were then dehydrated in a gradual series of ethanol and embedded in the mixtures of Epon 812. The blocks were cut into 2 mm thick section, were stained with Methylene blue Azure II - Basic fuchsin (Hayat,1989) and studied by light microscope.

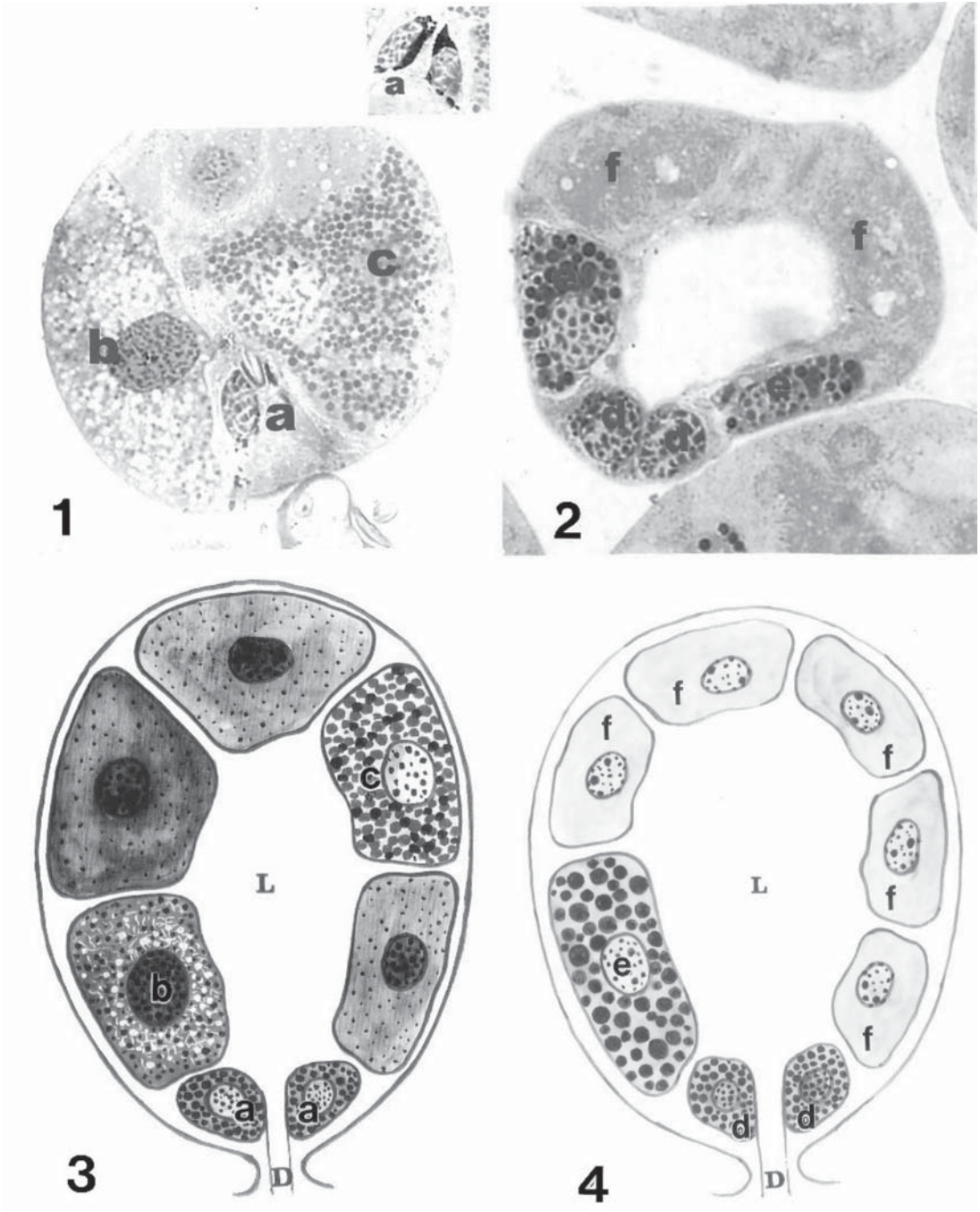
RESULTS

Serial thick-section of salivary glands stained with Methylene blue Azure II - Basic fuchsin were studied for histological structure. The demarcation of cells and granules were clearly shown structural details.

Type II acini were identified from the presence of 'a', 'b' and 'c' cells and type III acini by the presence of 'd', 'e', and 'f' cells. The 'a' cell was located adjacent to lobular duct and it had small size. Its cytoplasm contained a few granules which were tightly packed together. The granules were stained in deep blue. The 'b' cell was located either adjacent to 'a' cell or adjacent to lobular duct. Cytoplasm of 'b' cell showed mesh - like structure with scattered granules within the mesh. Most granules were stained in blue while a few of them were stained in pink. The 'c' cells were located at intermediate and fundus of acinus and were divided into two type, granular and agranular cells. Granular cells contained tightly packed granules which were mostly stained in pink. Some granules were stained in blue. The acinus contained one or two 'd' cells located adjacent to lobular duct. Granules of the 'd' cells were packed and stained in deep blue. The 'e' cell was found between 'd' and 'f' cells and was immediately situated to lobular duct. Granules of 'e' cell were blue to light blue. Agranular 'f' cells were found close to intermediate region or fundus of acinus.

DISCUSSION

The cells of tick salivary gland are only identified by their granularity and sizes of granules which are shown only when using the TEM technique. This is the main reason that hampers studies of tick salivary gland using the LM. In this study we used thick-sections which were produced by epoxy-embedded blocks. Thick-section which were 2 mm in thickness allowed the cells to appear more clearly. The thick-section also enhance the



Figures 1-2 Photomicrographs of feed female *Boophilus microplus* salivary gland 1. Type II acini, showing 'a', 'b' and 'c' cell ¥ 600 (inset) a cell of Type II acinus ¥ 700. 2. Type III acini, showing 'd', 'e' and 'f' cell ¥ 850.

Figures 3-4 Diagrams shows Type II and III acinus. Type II acini, showing 'a', 'b' and 'c' cells. 2. Type III acini, showing 'd', 'e' and 'f' cells. Lumen (L), Duct (D).

Table 1 Sammary of staining of cells in type II and III acini and positions of cells in each acinus.

Cell	Granule staining	Position of cells in acinus
Type II acinus		
a	Deep blue	Lobular duct
b	Most were stained in blue and a few Granules are stained in pink	Between 'a' and 'c'cell Or adjacent to lobular duct
c	Most is stained in pink and a few Granules are stained in blue	Intermediate to fundus of acinus
Type III acinus		
d	Deep blue	Lobular duct
e	Blue to light blue	Between 'd' and 'f' cell
f	No granules present	Intermediate to fundus of acinus

dye to penetrate and thereby stain the granules better than sections prepared from paraffin-embedded sections which have more thickness i.e. 3-5 mm in thickness. This is the first report of the success in cell identification of tick salivary gland using the LM.

Methylene blue Azure II – Basic fuchsin staining technique used in studies of tick salivary gland was shown in many reports. This technique gives an advantage over the others because their ability to stain granules of all cell types in both type II and III acini. In this studies the morphology of the cells was clearly shown and the sizes as well as the shape of the granules were well demarcated. The difference of granules of the cells in taking up the different dyes suggests the different chemical components in secretion in each types of cells.

CONCLUSION

Six different types of cells in type II and type III acinus of tropical cattle tick can be differential identified in thick-sections stained with Methylene blue Azure II - Basic fuchsin. In type II acinus 'a' cells containing granules which were spherical, packed tight together and stained in deep blue whereas 'b' cells granules appeared in loosely pattern and most of granules were stained in blue and some in pink. The 'c' cells were classified into two types, granular and agranular cells. The granular 'c' cell, contained pink and blue granules. In type III acinus 'd' cell had granules which were similar to those of 'a' cells. Granules of 'e' cells were larger than those of 'd' cell and stained in blue to light blue and 'f 'cell were agranular cells.

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