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**ELECTRON MICROSCOPIC STUDY ON THE TEGUMENT
 OF FASCIOLA GIGANTICA**
 (With 9 Figures)

By

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دراسة التركيب الاليكتروني لجلد الدودة الكبدية
 (الجيجانتكا)

فاطمة هيكل

بتركيب جلد الدودة الكبدية من طبقة سطحية عديدة الأنوية متصلة بخلايا طلائية عميقة عن طريق امتداد أنبوبي . وقد تميزت الخلايا الجلدية الي نوعين نوع غامق ونوع فاتح وقد تميزت الخلايا الغامقة بوفرة الميتوكوندريا وحبيبات إفرازية كثيفة محاطة بغشاء . أما الخلايا الفاتحة فقد تميزت بوجود عدد قليل من الميتوكوندريا وأجسام مفرزة مفلطحة وقد أظهرت الطبقة السطحية هذين النوعين من الأجسام المفرزة .

SUMMARY

The tegument of *Fasciola gigantica* was composed of surface non-nucleated layer (syncytium) connected to deep tegument cells by tubular extensions. The tegumental cells were two types, dark cells and light cells. The dark cell was characterized by numerous mitochondria and dense membrane-bounded secretory granules, while the light cell was characterized by few mitochondria and flattened membrane-bounded secretory bodies. The surface syncytium showed both types of secretory bodies.

INTRODUCTION

The ultrastructure of the tegument of *Fasciola hepatica* was studied by BJORKMAN & THORSELL (1963); THREADGOLD (1963 & 1967), GALLAGHER & THREADGOLD (1967) and BENNETT & THREADGOLD (1973, 1975). It was composed of a syncytium, non-nucleated surface-layer joined by protoplasmic tubes to individual nucleated areas of cytoplasm (tegumental cells) which were embedded within the parenchyma.

THREADGOLD (1967) mentioned that the tegumental cells were of two types, type I characterized by presence of membrane-bounded dense secretory granules and type II cell characterized by presence of biconcave disk-like secretion bodies.

From the available literatures, no study was carried out on the tegument of *Fasciola gigantica* using transmission electron microscope. So the aim of this work

was to throw spot of light on the ultrastructure of the tegument of F.gigantica and to compare it with that of F.hepatica.

MATERIAL and METHODS

Fasciola gigantica adult worms were collected from the bile ducts of cattle which had been slaughtered only a short while. The flukes were placed in fixative and thin strips were cut from their initial, middle and caudal thirds. The fixative was 4% buffered glutaraldehyde at PH 7.4 for 2 hours followed by 2 h. postomission. The fixed samples were washed in 0.1 M sodium cacodylate containing 5% sucrose then processed through tannic acid, dehydrated in ascending grades of ethanol and embedded in epon. Ultrathin sections were cut with a diamond knife and stained with uranyl acetate for 20 minutes, followed by lead citrate for 5 minutes.

RESULTS

The tegument of Fasciola gigantica is composed of outer non-nucleated layer (Syncytium) connected to deep tegumental cells by cytoplasmic tubular extension. The syncytium is covered by trilaminar plasma membrane with many finger-like or irregular evaginations which contain electron dense granular material similar to that of the rest of the syncytium (Fig. 1). Invaginations were also observed containing membranous vacuoles and granular material. The syncytium has some mitochondria, few endoplasmic reticulum, vacuoles, dense secretory bodies and spines. It rests on a basement membrane which shows long invaginations into the parenchyma. The tubular extensions is made up of plasma membrane with a thin layer of cytoplasm. These tubes contain many dense secretory bodies and granules (Fig. 2). The tegumental cells are lying below the peripheral musculature and they are dark and light cells.

The dark cells are found in groups 2 to 3 in each group. Each cell is flask-shaped and connected to the surface syncytium through tubular cytoplasmic extension (Fig. 3). The cytoplasm is dense and full-of mitochondria, more than one well developed golgi complex, rough endoplasmic reticulum and free ribosomes (Fig. 4). This cytoplasm is characterized by the presence of membrane bounded very dense secretory granules of variable sizes distributed all over the cytoplasm and extend through the tubular extensions into the surface syncytium. These secretory granules originate from the rough endoplasmic reticulum golgi complex (Fig. 5). The dark cell has a large nucleus with darkly stained prominent nucleolus.

The light tegumental cells are also flask shaped being found singly among groups of dark tegumental cells (Fig. 6). They were clearly differentiated from the latter cells by their less electron dense cytoplasm. They have many free ribosomes and few mitochondria usually located at the supranuclear region. Flattened disc-like secretory bodies can be seen (Fig. 7). These bodies are scattered randomly throughout the cells and may occur in very few numbers in the tubular extension which connect the cell to the surface syncytium. The nucleus is large and contains large prominent nucleolus.

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Sometimes free ribosomes appear in the form of many polyribosomes and the cytoplasm shows large membrane bounded spherical secretory granules of variable sizes and in different stages of synthesis (Fig. 8). These granules contained densely packed granular material and many of them were observed in the tubular extensions and the intercellular spaces. These granules have a structure similar to that of the spines.

Each of the tegumental cells is in contact with at least one of the parenchymal cells, which is found individually between the groups of the former cells (Fig. 9). The parenchymal cell has relatively small darkly stained nucleus with clear nucleolus and long cytoplasmic processes (pseudopodia) of irregular shapes. The cytoplasm had fine granules and fibrils interspersed with clear areas or compact granular mass.

DISCUSSION

The present study showed that the cuticle of *F. gigantica* is a cellular tegument. This result coincides with the findings of BJORKMAN and THORSELL (1963); THREADGOLD (1963); THREADGOLD and GALLAGHER (1966); THREADGOLD (1967); GALLAGHER & THREADGOLD (1967) and BENNETT & THREADGOLD (1975) who were working on the tegument of *F. hepatica*.

The tegument was composed of non-nucleated surface layer (Syncytium) connected by cytoplasmic tubular extensions to deep tegumental cells located in the parenchyma. These cells were of two types, dark cells and light cells. THREADGOLD (1967) described similar findings in the tegument of *F. hepatica* and named these cells type I and type II cells respectively. The dark cell was characterized by its dense cytoplasm which was full of mitochondria and contained many membrane-bounded very dense secretory granules. These granules were clearly observed in the surface syncytium in addition to few flattened disk-like secretory bodies of the light tegumental cells. THREADGOLD (1967) mentioned that neither type of secretion has been seen in process of release onto the apical surface, and were not acid mucopolysaccharides. He added that neither do these bodies appear to contribute to the formation of the spines. So, the present data confirm his opinion that these bodies may have a protective function through their combination with other substances absorbed either selectively or because the fluke cannot prevent their entry.

From the above mentioned observations it appears that the tegument of *F. gigantica* serve as both secretory and a protective covering.

In the present work, the large spherical secretory granules which appeared in the light cells and have closely packed granular material similar in its structure to that of the spines let me suggest that these granules are the forerunner of the spines thus the light cells are the site of synthesis of spine material. BENNETT and THREADGOLD (1975) mentioned that no study of adult trematodes has yet yielded any explanation of the origin or development of the spines. They added that the tegumental cells must be the origin after excluding the syncytial layer since it does

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not contain either Golgi complex or ribosomes. They also suggested that spine material may be synthesized on polyribosomes but it did not appear in the cytoplasm because it is in a monomer form.

In the present work, the synthesis of the spine material was observed on polyribosomes, then in the cytoplasm and the tubular cell extensions as membrane bounded secretory granules of variable size. Deep in the syncytium these secretory granules unite together to form the spine and their growth comes as a result of adding new secretory granules to the base of the spine.

ACKNOWLEDGEMENTS

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KEY OF FIGURES

Fig. 1: Electron micrograph of syncytium layer of *F. gigantica* tegument showing finger-like evaginations (E) covered by trilaminar plasma membrane. Very dense secretory granules (G). X 20000.

Fig. 2: Electron micrograph of tegumental tube (T) penetrating the peripheral musculature (M) and contains large secretory granules of variable size (G). Plasma membrane of the tube (P). X 12000.

Fig. 3: Electron micrograph of tegumental dark cell having flask shape. Cytoplasm (C) is darkly stained. Nucleus (N) with clear nucleolus. X 8000.

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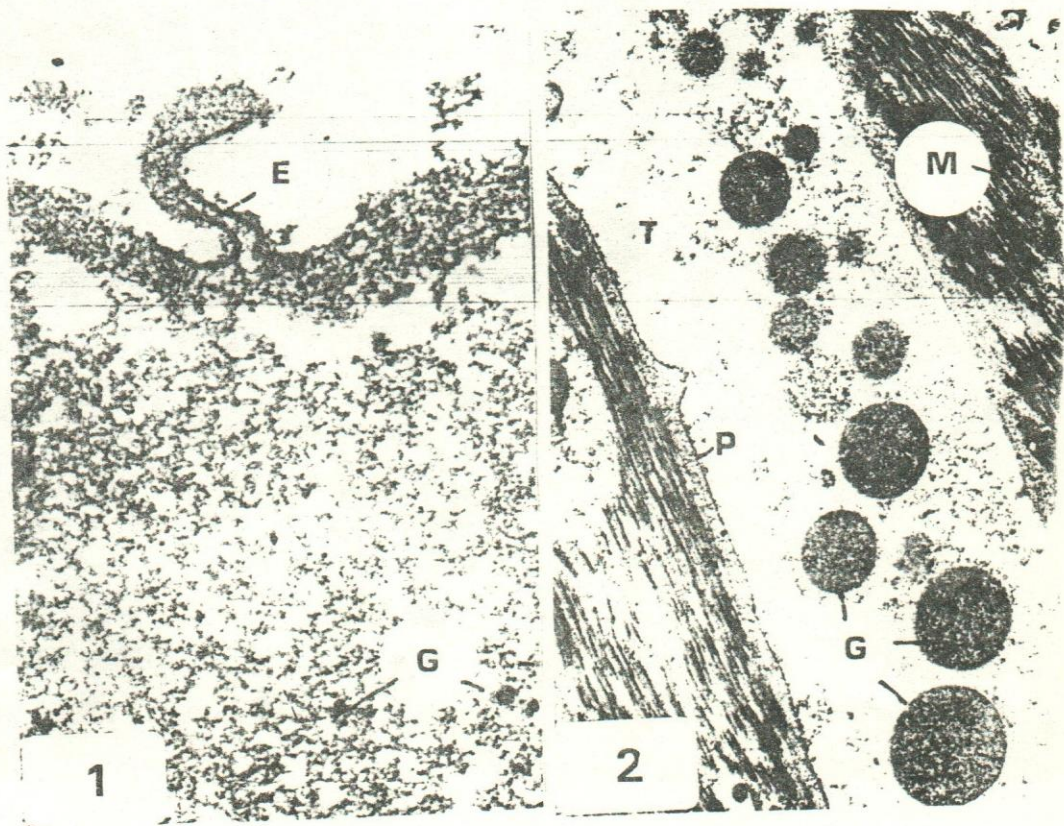
Fig. 4: Cytoplasm of dark tegumental cell showing numerous mitochondria (M), rough endoplasmic reticulum and free ribosomes (R) and very dense secretory granules (S). Nucleus (N), X 22000.

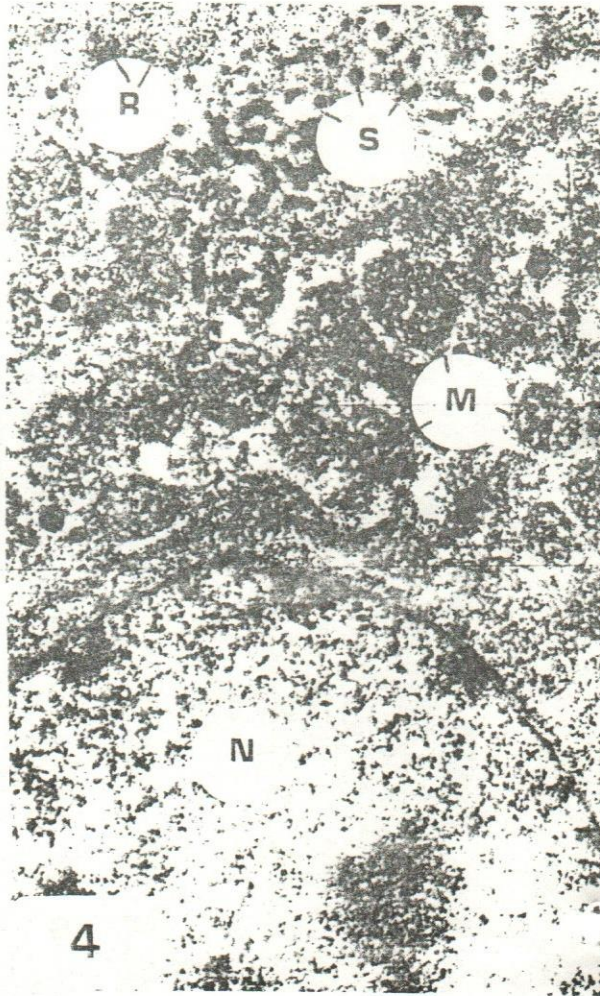
Fig. 5: At high magnification, cytoplasm of dark cell show well-developed Golgi complex (Gc), very dense secretory granules (S), rough endoplasmic reticulum and free ribosomes (R). X 46000.

Fig. 6: Electron micrograph of light tegumental cell (L) found between dark cells (D). X 12000.

Fig. 8: Light tegumental cell showing many polyribosomes (Pr) and spherical secretory granules (Ss) at different stages of synthesis. Dark tegumental cells (D). X 7000.

Fig. 9: Electron micrograph showing parenchymal cell (Pc) having cytoplasmic processes (Cp) in contact with dark tegumental cell (D). X 7000.





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