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ULTRASTRUCTURAL STUDIES ON THE DIGESTIVE GLAND OF *BIOMPHALARIA ALEXANDRINA* INFECTED WITH *ECHINOSTOMA LIEI*

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ABSTRACT

Ultrastructural examinations of the normal and parasitized digestive gland of Biomphalaria alexandrina suggests that parasitism by the biological control agent, Echinostoma liei, induces a series of pathological alterations changes in the snail digestive gland include reduce microvilli, increased cytoplasmic vaculation, reduction in mitochondrial number, increased density of the endoplasmic reticulum, and slight atrophy of Golgi apparatus.

INTRODUCTION

The use of biological agents in the control of human disease vector is gaining increasing prominence (Who, 1983). Yet, at the ultrastructural level, little is known about the specific cytoplasmic effects of such parasites on *Biomphalaria alexandrina*. As far as the available literature indicates, no reports have dealt with the consequences at the ultrastructural level of such infections in *B. alexandrina* with *E. liei*.

Nonetheless, a number of important general studies do exist on the ultrastructure of the digestive gland of mollusca, the most comprehensive of which are published by Southgate (1969); Abolins-Kirogis (1970); Walker (1970) and Pal (1971). In the same direction, reference should be made to the work of Reader (1976) who had extensively studied the digestive gland of the snail *Bithynia tentaculata* at the ultrastructural level in an attempt to relate the structure of the individual cells to their functions.

The pathological effects of larval digenetic trematodes on the digestive glands of their molluscan hosts have been reviewed by Pan (1963 & 1965) in *Biomphalaria glabrata*, infected with *Schistosoma mansoni* by Wright (1966) regarding helminth infections in their molluscan hosts.

On the electron microscopic level, Meuleman (1972)

elucidated the changes induced in the digestive gland of South Africans strains of *Biomphalaria pfeifferi* as a result of infection with *Schistosoma mansoni*.

Recently, the development of the trematode parasite *Echinostoma liei* in *B. alexandrina* was followed by Yousif and Haroun (1986).

The present study was designed to examine the ultrastructural changes which might take place in the digestive gland of the snail *Biomphalaria alexandrina* parasitized by *Echinostoma liei*.

MATERIALS AND METHODS

The specimens used in the present study, *Biomphalaria alexandrina*, were laboratory reared at Ain Shams University in Cairo as recommended by Laing *et al.* (1987).

Infection of snails with *Echinostoma liei* was achieved according to the procedure reported by Saad and Mohamed (1989).

For electron microscopical purposes, 1 mm of the digestive gland of both uninfected and infected snails were fixed in 3% gluteraldehyde in sodium cacodyolate buffer for 2-3 hours at 4 °C, then transferred to ice cold buffer solutoin for about 5 hours. This was followed by post fixation in 1% buffered osmium tetroxide for two hours at 4 °C, washed in the same buffer, cleared in toluene (Dawes, 1971) then embedded in Epon resin (812) in gelatin capsules which were left in an oven adjusted at 60 °C for polymerization (Luft, 1961). Ultrathin sections were cut and then mounted on copper grids and stained with uranyl acetate and lead nitrate for 20 min. The grids were examined with Philips 400 transmission electron microscope operating at 25 Kv and the electron micrographs were produced at the E.M. unit in Ain Shams Specialized Hospital in Cairo.

RESULTS

The digestive gland of *Biomphalaria alexandrina* consists of two main cell types, the digestive and secretory cells.

The electron microscope has revealed the presence of long, dense microvilli along the apical areas of the plasma membranes of the digestive cells (Fig. 1). The same figure also shows scattered cilia along the luminal surface of these cells, each containing an axial filament complex of the conventional 9 + 2 configuration. The microvilli, as the cilia, are bounded by apical extensions from the plasma membranes of the cells.

The ground cytoplasm of such cells contains a granular

endoplasmic reticulum, mitochondria and occasional Golgi complexes.

Also, the digestive cells poccess numerous digestive vacuoles of variable sizes; the larger ones are located in the basal regions of these cells (Fig. 2).

The nuclei of the digestive cells occupy the basal regions, each containing a conspicuous nucleolus.

In the secretory cells of the digestive gland of *B. alexandrina*, the microvilli are apparently fewer (Fig. 3) than those of the digestive cells, and further more, no cilia could be detected in those cells.

The cytoplasmic inclusions also embody a granular endoplasmic reticulum and certain secretory granules presumably produced by the Golgi bodies. These granules are located in the basal regions of the cells. Also mitochondria are hapnazardly distributed in the secretory cells.

It is also apparent that the secretory cells differ from the digestive ones in the fact that they poccess fewer vacuoles with different sizes than those observed in the latter cells.

The nuclei have a basal location in the secretory cells each having a prominent nucleolus and patches of chromatin (Fig. 4).

In the *Echinostoma liei* infected *Biophalaria alexandrina*, the digestive cells display various signs of degeneration. Microvilli seemed almost to disappear or at least obviously degenerated along large areas of the apical surfaces of those cells. However, the remaining ones appeared as simple finger-like projections being fewer in number than those in the normal condition (Fig. 5).

The endoplasmic reticulum became distinctly swollen, and the mitochondria were reduced in number and much thinned out.

The cytoplasmic vacuoles became abundant without any apparent inclusions.

No apparent alterations were obtained in the secretory cells of the digestive glands of infected snails (Fig. 6).

DISCUSSION

The digestive gland of the investigated molluscan specimen (*Biomphalaria alexandrina*) is composed of two cell types, digestive and secretory ones as had also been elucidated by Lutfy et al., 1973 and Meuleman, 1972 in

their studies on *Bulinus truncatus* and *Biomphalaria pfeifferi* respectively.

The digestive cells were previously recognized as liver cells (Hurst, 1927), vacuolated cells (Yonge, 1926), absorptive cells (Mc Lean, 1970), absorptive secretory cells (Weiss, 1968) and digestive cells (Schnebel and Wechsler, 1968 a,b; Walker, 1970 and Owen, 1970).

At the same time, the secretory cells were designated as calcium cells (Pan, 1958), basophil cells (Summer, 1966 a,b) and secretory cells (Meuleman, 1972 and Ibrahim, 1977).

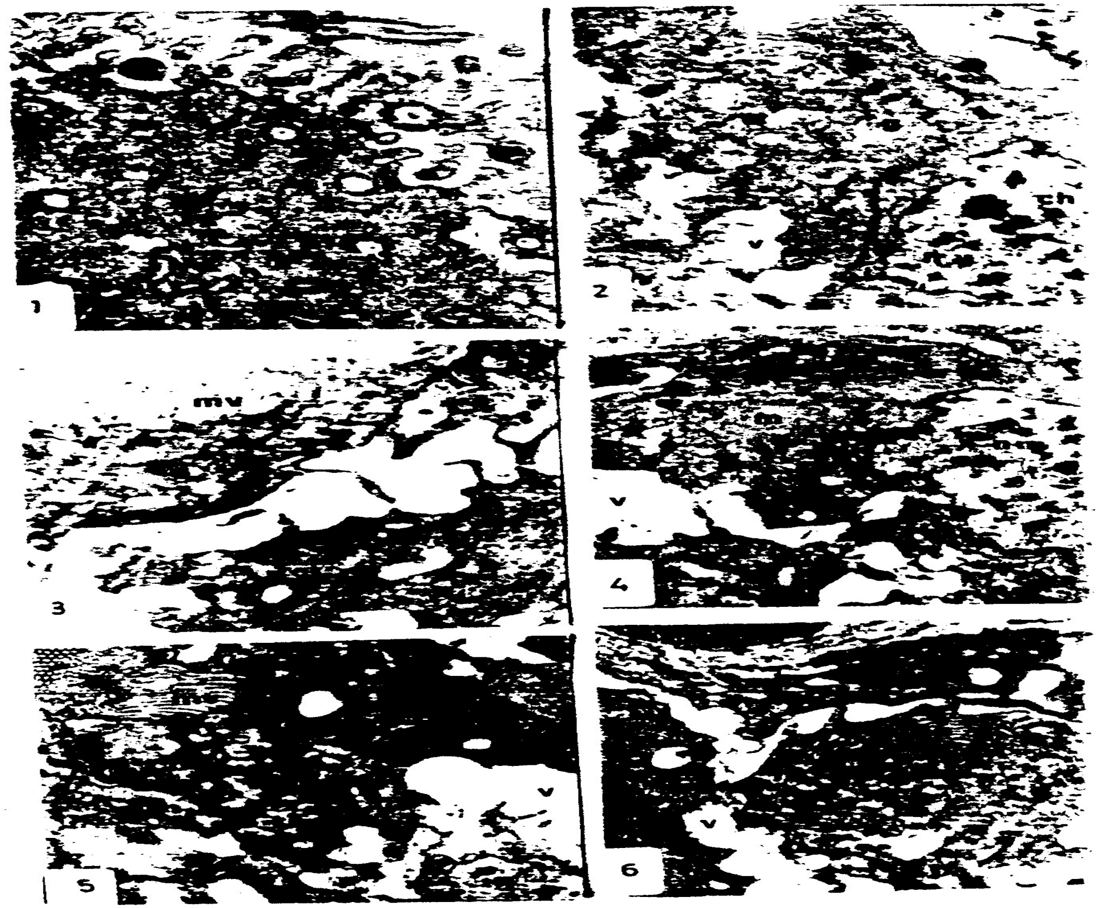
The apical portions of the plasma membranes in the digestive cells of the uninfected snail extended into numerous microvilli, an observation confirming the results recorded in the digestive glands of *Biomphalaria pfeifferi* by Meuleman (1972) and *Bithynia tentaculata* by Reader (1976). However, the microvilli had almost disappeared from digestive cells of the infected snails.

The vacuolation observed in the digestive gland cells of the snail *Biomphalaria alexandrina* are believed to be closely related to the digestive and absorptive processes going on in these cells, which supports the views postulated by Summer (1965 & 1968) in their studies on *Helix* and Saad and Mohamed, 1989 concerning *Bulinus truncatus*. However, such vacuoles were presumably produced as a result of the coalesence of the pinocytotic vesicles which develop along the apical portions of the plasma membranes adjusted to the tubular lumen.

In the present investigation, the digestive cells of uninfected *Biomphalaria alexandrina* poccess numerous and relatively small vacuoles. However, snails infected with *Echinostoma liei* showed a marked increase in the cytoplasmic vacuolation to the extent that they almost occupied the whole cells. This observation agrees with the findings of Reader (1976) presented in such cases.

The ultrastructural changes observed in the secretory cells of *Biomphalaria alexandrina* shedding cercariae included reduction in number of mitochondria, appearance of dense material in parts of the endoplasmic reticulum and a slight atrophy of Golgi apparatus. Many of the cell apices had become irregular in outline as compared with those of the normal ones. These observations were similar to those described by Southgate (1969) and Meuleman (1972).

The present observations indicate the breakdown of the digestive gland of *B. alexandrina* due to the presence of the parasite *Echinostoma liei*. Such modifications may provide the foundation for the observed mortality-associated effects of this parasite on the snail.



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ABBREVIATIONS

C. Cilia.
er. endoplasmic reticulum.
g. Golgi apparatus.
mv microvilli.
n. nucleus.
V. Vacuole.

EXPLANATION OF FIGURES

An electron micrograph of part of a section of the digestive gland of uninfected *Biomphalaria alexandrina* showing:-

Fig. 1: Apical region of the digestive cells with microvilli, cilia and mitochondria. X 6000.

Fig. 2: Basal portion of the digestive cells with Golgi apparatus, endoplasmic reticulum, nucleus and chromatin particles. X 6000.

Fig. 3: Apical region of secretory cells showing the microvilli. X 6000.

Fig. 4: Basal region of the secretory cells revealing the endoplasmic reticulum, nucleus and chromatin bodies. X 6000.

An electron micrograph of part of a section of the digestive gland of uninfected *Biomphalaria alexandrina* infected with *Echinostoma liei* showing:-

Fig. 5: The digestive cells with microvilli, vacuoles and nucleus. X 6000.

Fig. 6: Secretory cells with vacuoles and endoplasmic reticulum. X 6000.

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دراسات بالمجهر الاليكترونى على تأثير طفيل ايكينوستوما لياى على الغدة الهضمية فى قوقع بيومفلاريا الكسندرينا

أوضحت الدراسة بالمجهر الاليكترونى على الغدة الهضمية المصابة بطفيل ايكينوستوما لياى فى قوقع بيومفلاريا الكسندرينا على حدوث تغيرات بالغة الأهمية مثل نقص واضح فى اعداد الخلايا الدقيقة و الميتوكوندريا، هذا بجانب ازدياد الفجوات الستوبلازمية و ارتفاع معدل كثافة وحدات الشبكة الاندوبلازمية و ضمور فى أجسام جولجى