STUDIES ON THE LIVER FLUKE FASCIOLA IN EGYPT: II. ULTRA-STRUCTURAL AND HISTOCHEMICAL OBSERVATIONS ON THE INTESTINAL CAECA

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ABSTRACT

The alimentary tract of adult Egyptian liver fluke Fasciola gigantica was studied by light and transmission electron microscopy, the intestinal caecum was found to be composed of a single layer of epithelial cells, which contain numerous organelles such as mitochondria, well-developed granular endoplasmic reticulum, few secretory gransules, and prominent nuclei, each containing a single nucleolus. The apical surface of the gut has numerous elongate lamellae extending into the lumen of the caecum. It was proposed that the intestinal epithelium has both secretory and absorptive functions. The localization of both carbohydrates and proteins within the intestinal cells of the fluke was also demonstrated. Results were discussed and compared with other studies on digenetic trematodes.

INTRODUCTION

In most digeneans the adult flukes possess a well developed gut, generally consisting of a mouth, pharynx, oesophagus and a single or more frequently two intestinal caeca. The trematode intestinal caecum is lined with a single layer of epithelial cells (mucosal layer) that vary in height.

The cells are lying on a thin basal lamina, and a subjacent muscular bilayer of circular and longitudinal muscular fibers. The morphology of the digenean caeca has been studied both at the light microscopical and ultrastructural levels by ***Gresson and*** Threadgold ***(1959);*** Dawes ***(1962) for Fasciola hepatica;*** Wotton et al. ***(1963) for Cleptodiscus kyphoris and C. reticulatus;*** Halton et al. ***(1986)*** for monogenetic trematode *Diclidophora merlangi;* Dike *(1969) for Paragonimus kellicotti;* Reader *(1971) for Bithynia tentaculata;* Halton *(1972) for Aspidogaster conchicola;* Hanna *(1975) for Fasciola hepatica;* Byram et al. *(1975) for Paragonimus kellicotti;* Dunn et al. *(1987) for Gigantocotyle explanatum, Gastrothylax crumenifer and Srivastavaia indica and* Mc Michael-Phillips et al. *(1994)* for *Sanguinicola inermis.* The ultrastructure of the intestinal caeca of *Fasciola* has been also studied by Thorsell and Bjorkman ***(1965)***; Gallagher and Threadgold ***(1975)*** Bennett ***(1975)*** and Fujino and Ishii ***(1979)***

Since it is not exactly known whether the Egyptian populatins of Fasciola belong to a single or several species. The present work was carried out in order to characterize the medically and veterinary important parasite; *Fasciola gigantica* isolated from Egypt and to clarify the descripancy in speciation within the genus Fasciola based on both ultrastructural and histochemical characteristics of the intestinal caecum of the flukes.

MATERIAL AND METHODS

Adult flukes were collected from Cairo abattoir, washed in saline, each fluke were cut transversely into three pieces, fixed in formaldehyde. For histochemical observations, 5 μ sections were prepared in paraplast wax and stained with Periodic Acid Schiff (PAS) for demonstration of glycogen (Hotchkiss, *1948*) or stained with Mercury Bromophenol Blue for demonstration of proteins (Maria et al., *1953*).

For transmission electron microscopy, the fluke were sliced transversly into two portions and fixed in 3% glutraldehyde in 0.12 M Millonig's phosphate buffer, pH 7.4, for 2-3 hours at room temperature, and post fixed for 3 hours in 1% Osmium tetroxide in 0.12 M Millonig's phosphate buffer, pH 7.4 at 4 C. These specimens were dehydrated in a graded series of ethanol, and then embedded in epoxy resin, semithin and ultrathin sections were cut on ultratome. The ultrathin sections were stained with both uranyl acetate and lead citrate, and examined in Philips 400T electron microscope at Ain Shams Specialized Hospital.

RESULTS

The alimentary canal of the adult Fasciola generally consists of a most anterior mouth opening, a short pharynx, an extremely short, thin oesophagus, which is less than half of the pharynx and the intestine. It divides into two, right and left branches called intestinal caeca. Each branch extends to the posterior part of the body giving rise to some short branches called diverticula, that end blindly. Histological examination showed that, the walls of the intestinal caeca of Fasciola are composed of a single layer of epithelial cells resting upon a basal lamina. These cells have a more or less similar structure. The only obvious variation in structure is in the height of the cells.

Several cytoplasmic projections of the plasma membrane of the caecal epithelium extend into the caecal lumen forming lamellae (Fig. 1). These lamellae appear numerous, long and some of them are short, which extend into the lumen. Below the basement membrane a thin external layer of muscle fibers and a thick layer of interstitial materials are found. It was noticed that shallow cells commonly line caeca that contain much food and taller cells occur in other parts of the intestine in which there may be little or no food. The short cells are most commonly seen in the larger primary and secondary branches of the intestine, while tall cells are seen in the smaller caeca.

The intestinal wall is supported with systems of transverse and longitudinal muscles which may modify the epithelium by their various contractions and relaxations.

Transmission electron micrographs of transverse sections passing through the intestinal caeca revealed that, the intestine of *Fasciola gigantica* is formed of a single layer of epithelial cells, which has pyramid-shaped and distinct lateral membranes separating the cells. Its cytoplasm includes numerous mitochondria which are rounded or elongate with short cristae, and dense matrix, well developed granular endoplasmic reticulum (GER), few secretory granules, and septate desmosomes are clearly seen at the apex of adjacent "cells".

The nucleus has an irregular boundary and is located in the middle or near the apical surface of the cytoplasm, it has distinct nucleoli, the nucleolus contains a considerable amount of dense material. Granules and irregularly shaped bodies are distributed throughout the nucleoplasm (Fig. 2). The apical border of the cells has numerous long thin microvilli or lamellae, which extend into the lumen.

The lamellae are relatively long and straight being well separated at their origin, some of the lamellae branch. In cross-section, the lamellae appear as round or oval bodies according to the angle at which they are cut (Fig. 3). Each lamella is tubular and bounded with a typical trilaminar membrane or (unit membrane). The outer surface of the lamellae was found covered entirely with a thin dense glycocalyx-like coat, from which fine filaments extend and join with the adjacent lamellae forming ladder-like connections (Fig. 4). A distinct core was also seen running medially along each lamella. This was clearly seen in both longitudinal and transverse sections of the lamellae. Desmosomes joining caecal cells were also seen (Fig. 3).

Membrane vesicles were seen situated between the caecal lamellae, which may enclose other vesicles or laminar bodies. The epithelial cells rest on a thin, dense basement membrane, below, which is a thick layer of intestinal material enclosing the circular muscle layer of the intestine. These muscles are presumably responsible for the vigorous peristaltic movement of both caecal and parenchymal tissues. A finger like projections of surrounding parenchymal cells pass between the muscle layers and penetrate the interstitial to form close junctions with the base of caecal cells.

The surface of the lamellae and the apical margin of the cell assumed a deep pink coloration when sections were treated with the PAS technique demonstrating high concentrations of carbohydrates (Fig. 5).

The intestinal caeca displayed a fairly high concentration of protein in the apical cytoplasm (Fig. 6).

DISCUSSION

Transmission electron microscope (TEM) studies on the gut epithelium of adult Egyptian Fasciola were found basically resembling the ultrastructure of the intestinal caeca of most other digenetic trematodes.

The intestinal caecum in most digeneans is formed of a single layer of epithelial cells that rest on a relatively thin basal lamina. The present result is in agreement with that presented by Halton ***(1972)*** on ***Aspidogaster conchicola***, Fujino and Ishii ***(1979)*** on ***Fasciola hepatica***, and Dunn et al. ***(1987)*** on *Gigantocotyle explanatum; Gastrothylax crumenifer* and *Srivastavaia indica*.

Protoplasmic projections in the form of lamellae, occur at the free surface of intestinal caeca of the present Fasciola and although similar structures appear to be of general occurrence in the gut of digenetic trematodes (Thorsell and Bjrokman, ***1965***; Halton ***1966***; Dike ***1967***; Morris, ***1968*** and Davis and Bogitsh, ***1971***). These lamellae have a surrounding unit membrane and an inner core, and rootlets extending into the apical region of the cell. The inner central core of the lamellae consists of an ill defined narrow strand. Fujino and Ishii ***(1979)*** similarly reported the presence of a central core in the species of the genus *Paragonimus*. Threadgold ***(1978)*** concluded, after a close examination of the lamellae in *Fasciola hepatica* with TEM that the central cores were fine fibers running from side to side of the lamellae in parallel with each other. He mentioned that, "the fibers" are also attached laterally to the plasma membrane and perhaps to adjacent "fibers by amorphous material". It may be inferred that the cores of the lamellae are involved in the movement of the lamellae themselves. Dike ***(1967)*** observed lamellae engulfing luminal substances in Haematolechus medioplexus.

The function of the lamellae is probably related to the increase in surface area of the intestinal epithelium, or act as an absorbing surface (Sheffield, ***1964***; Thorsell and Bjorkaman, ***1965***; Halton, ***1972*** and Robinson and Threadgold, ***1975***). The presence of microvilli or lamellae projecting from the luminal surface is similar to the structure of those lining the alimentary tract of vertebrates.

The presence of abundant ribosomes, granular endoplasmic reticulum (GER), and mitochondria in the intestinal epithelium are related to the active formation of secretory granules or bodies which according to Dike ***(1969)*** and Davis and Bojitsh ***(1971)***, are supposed to be digestive enzymes.

Gresson and Threadgold ***(1959)***; Thorsell and Bjorkman ***(1965)*** and Robinson and Threadgold ***(1975)***, pointed out that in *F. hepatica* epithelial cells showed a cyclical transformation between secretory and absorptive forms.

The intestinal material may have a role in bringing precursors within the range of the transport membranes since it occurs as a thick layer immadiately beneath the basal lamina of the cells. On the other hand the inpushings of the parenchymal cells into the base of the gut epithelial cells may be a more important region of transfer and the junctional complexes between parenchymal and gut cells may be the sites of precursor transport and are known to contain acid and alkaline phosphatases (Gallagher and Threadgold, ***1967*** and Threadgold, ***1968***). The presence of mitochondria and lysosomes in the parenchyma beneath the gut may have a role in the processing of nutrients in transit between the gut and deeper tissues (Dunn et al., ***1987***).

Secretory granules are commonly found in the intestinal cells of digeneans including *Gorgodera amplicava* (***Dike, 1967***), *Haematolechus medioplexus* (Dike, ***1967*** and Davis et al., ***1968***) *F. hepatica* (Thorsell and Bjorkman, ***1965***; Robinson and Threadgold, ***1975***), *Gorgoderina attenuate* (Davis and Bogitsh, ***1971***) and *Sanguinicola inermis* (Mc Michael-Philis et al., ***1994***).

There is little precise information on the nature of secretory material in the intestinal caeca of digeneans, although assumption was made that secretory bodies contain hydrolytic enzymes. Degitsh ***(1972)*** and Hanna ***(1975)*** suggested that the secretory bodies contain both carbohydrates and proteins possibly indicating the presence of a glycoprotein. The proteolytic and enzymatic nature of the secretory granules in *Fasciola hepatica* was provided by Gresson and Threadgold ***(1959)***; Thorsell and Bjorkman ***(1965)*** and Halton ***(1967)***.

Threadgold ***(1968)*** has linked the frequent occurrence of phosphatases in digenean tegument, intestinal caeca and excretory systems with sites of active transport, in the junctional complexes where intercellular exchange takes place.

Gallagher and Threadgold ***(1967)*** and Threadgold and Gallagher ***(1966)*** reported the presence of close contact between the base of the caecal cell and parenchyma cells, which project into the caecal cells.

In the absence of a circulatory system this intimate intercellular contact might provide a route by which nutrients pass from the gastrodermis into the surrounding tissues.

The cells of the trematode intestinal epithelium have been described as subject to transformation between absorptive and secretory stages (Gresson and Threadgold, ***1959***; Dawes, ***1962***; Thorsell and Bjorkman, ***1965***; Halton, ***1967b*** and Robinson and Threadgold, ***1975***).

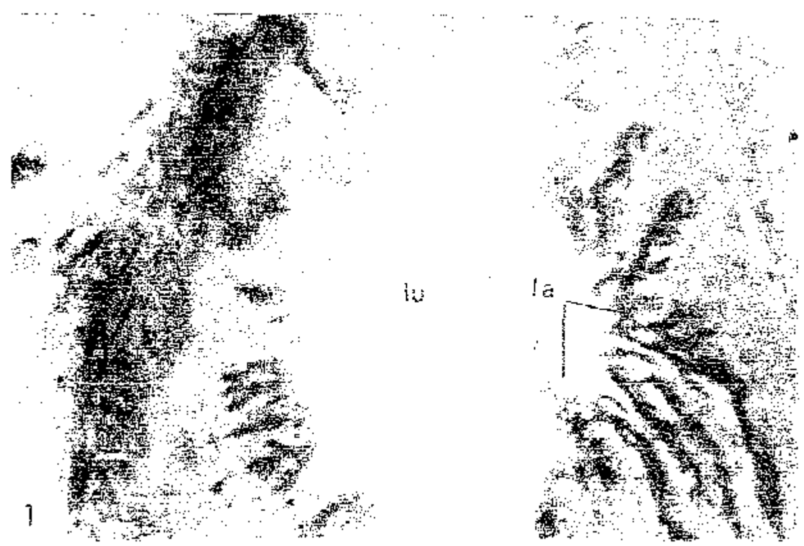

Fig. (1): Section of intestinal caeca of adult fluke showing the apical plasma membrane of the caecal epithelium, forming lamellae (la) and projecting into the lumen (lu). (x580)

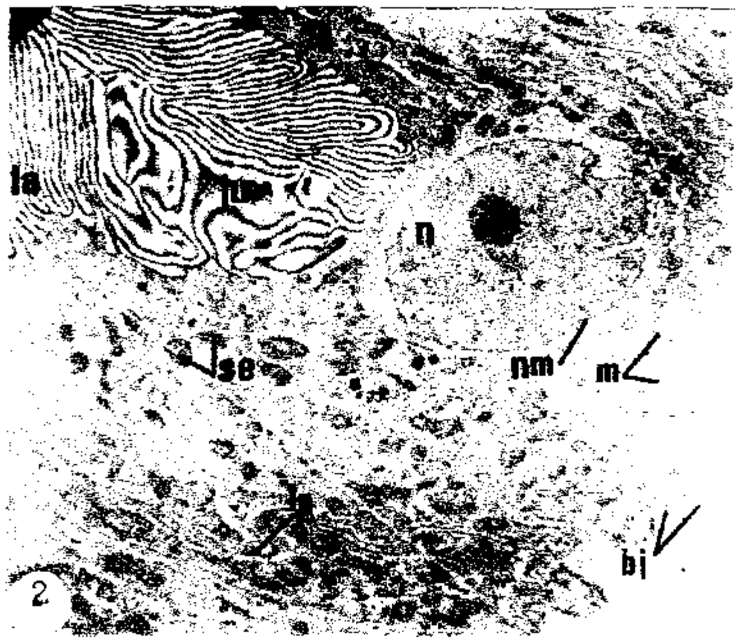

Fig. (2): TEM of the gut epithelium of Egyptian Fasciola at view of apical part of epithelium in intestinal caeca showing lamellae (la), lumen (lu), a flattened apithelial cell containing a nucleus (n), nucleolus (nu), and invaginated nuclear membrane (nm), mitoohondria (m), secretory granules (se), lateral plasma membrane separating the caeca (lp). The basal cytoplasm of the intestinal caeca forms a pseudopodium-like process which projects into the underlying parenchymal tissue, basal invagination, (bi). (X7600)

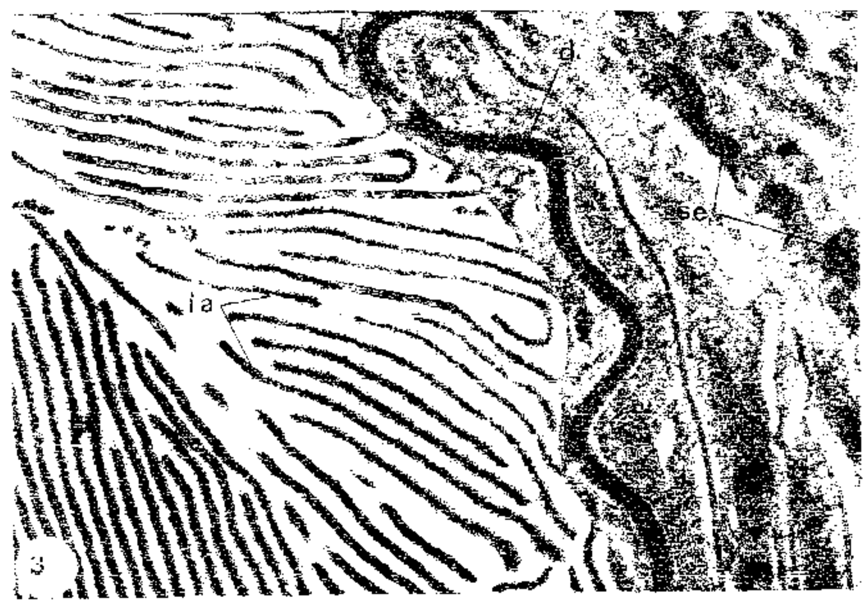

Fig. (3): T.E. micrograph of the gut epithelium of adult Egyptian *F. gigantica*, showing the apical plasma membrane which is extended into numerous lamellae (la), secretory granules (se), desmosome (d) are present. (X: 16000)

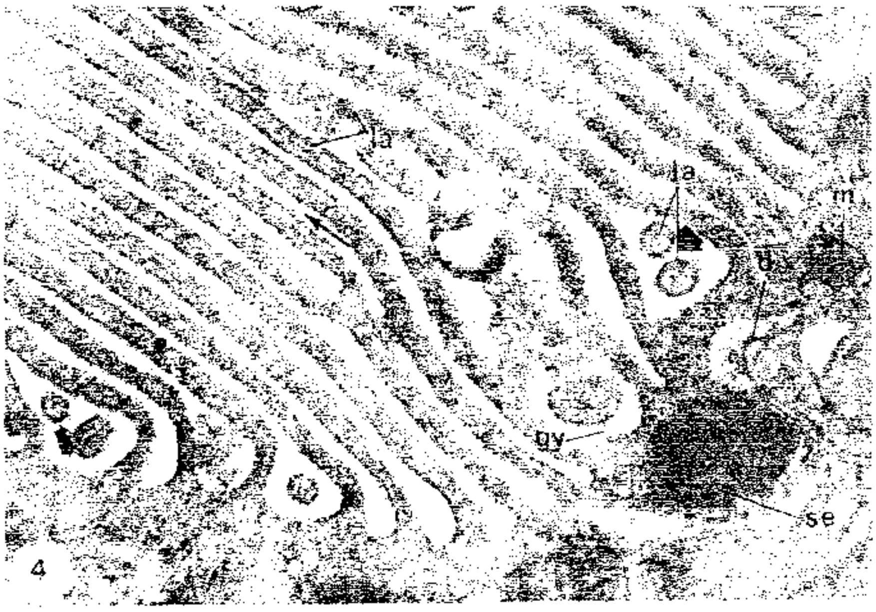

Fig. (4): T.E. micrograph of intestinal caeca of *Fasciola gigantica* showing high magnification view of the lamellae (la) which are numerous, long and well spaced at their origin, and some of the lamellae branch. Note the ladder-like connection of glycocalyx-like filaments (solid arrow), leaf arrows indicate cross section of lamellar extensions, secretory granules (se) and desmosomes (d) are present close to the apical plasma membrane, mitochondria (m) are present. (X: 44000)

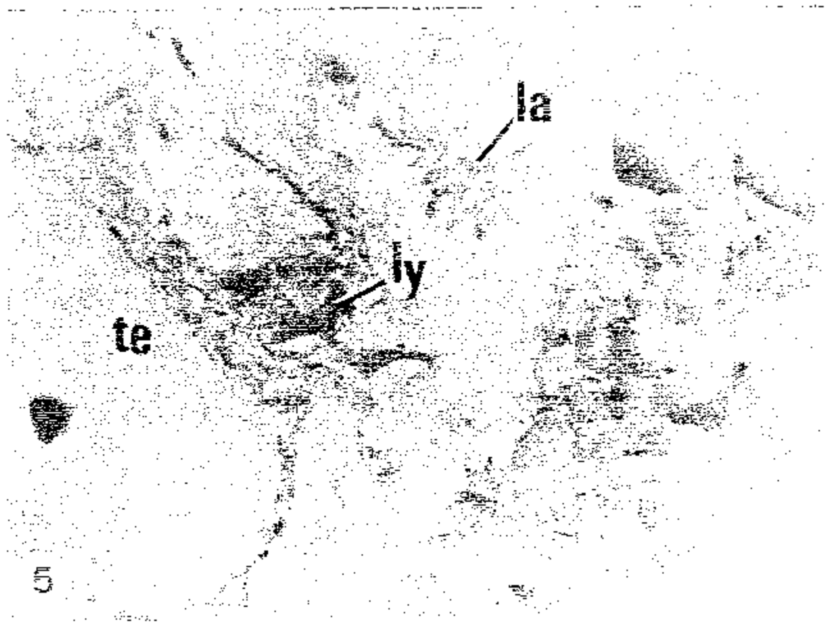

Fig. (5): Thin section in *F. gigantica* from sheep stained with PAS demonstrating the presence of high concentrations of carbohydrates in lymphatic duct (ly), and gut lamellae (al), while it is scarce in the testis (te). (X: 200)

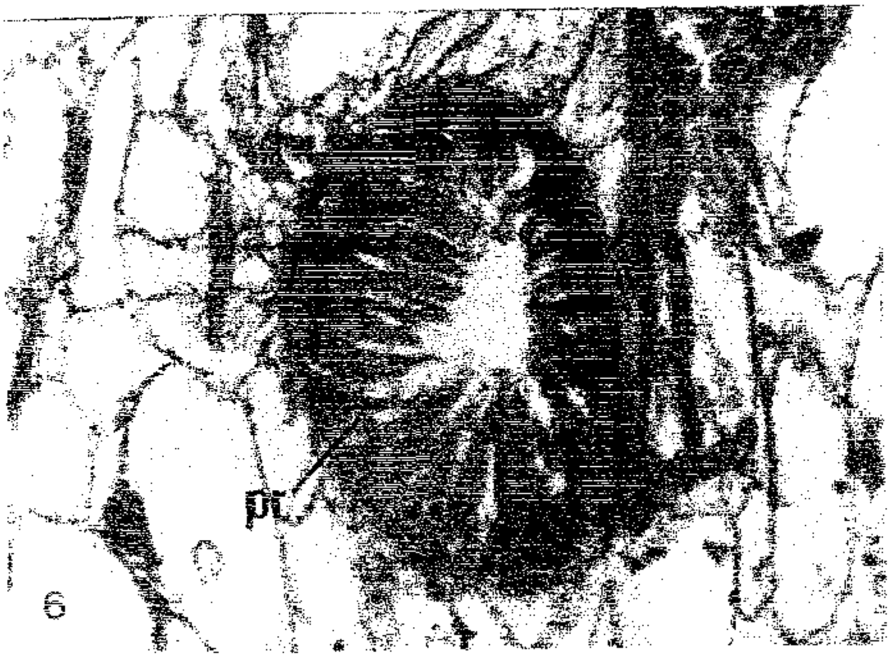

Fig. (6): Transverse section in *F. gigantica* from sheep stained with Bromophenol blue to demonstrate the presence of proteins (pr) in the intestinal epithelium. (X: 450)

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