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Abstract

Electron microscopy of the body wall of Opisthorchis viverrini shows the integument which is connected to the epidermal cell with fine protoplasmic tubules, to form a syncytium, as in Clonorchis sinensis and other trematodes. Vacuole-like secretory granules are distributed in the matrix of the integument, and mitochondria are arranged at the proximal outer surface of the integument. The crystalline inclusions are observed in the perinucleus of some epidermal cells.

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THE ULTRASTRUCTURE OF HELMINTH VI) THE BODY WALL OF *OPISTHORCHIS VIVERRINI* (POIRIER, 1886)

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Recently many workers have investigated the ultrastructure of trematode integument and have concluded that it consists of a large syncytium.

The ultrastructure of the integument of *Clonorchis sinensis*, belonging to the same family of *Opisthorchis viverrini*, was reported by INATOMI *et al.* (1968). The integument, which is composed of anucleated integument layer and nucleated epidermal cells lying beneath the muscle layer under the anucleated integument layer, consists of a sponge-like syncytium. Both, the integument layer and nucleated epidermal cells contain numerous vesicles, secretory granules and mitochondria. However, no spine has been distributed on the integument as in some other trematodes.

The present paper deals with the ultrastructure of integument of *Opisthorchis viverrini* to compare with *Clonorchis sinensis*.

MATERIALS AND METHODS

The adult *Opisthorchis viverrini* was obtained from the liver of a cat purchased from a farmer at Udon in Thailand, March 7th, 1969.

The cat was sacrificed, the liver was quickly removed in the 0.85% saline solution, and the worms were collected. Flukes were washed with 0.85% saline solution and immediately fixed with phosphate buffered cold 1% glutaraldehyde solution at pH 7.4 for 30 minutes and were cut into four pieces. These materials were washed well with phosphate buffer. Then they were postfixed for an hour in phosphate buffered cold 2% osmium tetroxide solution at pH 7.4 and embedded in Epon after dehydration with ethanol series.

The sections were cut on a Porter-Blum microtome, stained with uranyl acetate and lead nitrate, and observed with a Hitachi HS-8 electron microscope.

OBSERVATIONS

The integument, anucleated integument layer, is well developed to about 3 microns in thickness and has an irregular surface. The outer surface of integument is covered with a thin plasma membrane about 80 Å and the basal surface with a thin basal plasma membrane about 80 Å in thickness (Figs. 1, 2, 3). The fine fibril network layer which is constructed from collagen-like fibers, is located under the integument as the connective tissue (Fig. 2). The thickness of the layer is about 1 micron. The integument, without any nuclei, is composed of a syncytial structure. No spine can be seen on the integument. In the matrix of the integument are seen numerous vacuole-like secretory granules and mitochondria of varying sizes. These secretory granules coming from epidermal cell, are surrounded by a thin double membrane 70 Å thick and some of these contain dense secretory products (Fig. 3). The mitochondria are well developed and have numerous cristae. The vesicles or tubular-like infoldings of the basal plasma membrane protrude into the matrix of integument (Figs. 2, 3). The protoplasmic tubules originating from the basal plasma membrane of integument pierce through the basement membrane at various points, and are connecting to the integument and deep interior epidermal cells located in the parenchymal layer under the muscle layers, as well as the cells themselves (Figs. 1, 2). Then the integument is a large syncytium which is constructed of the anucleated integument layer and nucleated epidermal cells. The epidermal cells have nuclei, and numerous mitochondria, endoplasmic reticula with ribosome granules, Golgi complexes, and electron dense secretory granules which are surrounded by a thin membrane as can be seen inside of the matrix of integument (Figs. 4. 5). Some of epidermal cells have the crystalline inclusion bodies which are arranged regularly with fine round particles, about 260 Å in diameter (Fig. 5). But the substances and functions of these particles are unknown. The muscle layer which consists of somatic muscle cells, is located between the fiber layer as connective tissue under the integument, and parenchyma (Fig. 7). These somatic muscle cells are constructed of both the thick and thin filaments, as in some other trematodes.

The collagen-like fiber, having seven different-dense bands at regular

Fig. 1 Cross-section of the body wall. The integument which is about 2 to 3 microns in thickness, is located on the body surface and bounded by thin plasma membrane at both the outer and basal surface of integument. There can be seen the protoplasmic tubules which connect the outer covering anucleated integument layer with epidermal cell (nucleated integument cell).


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intervals, each band is 150 Å in length, is observed between the somatic muscle fibers (Fig. 7). Although the substances of this fiber are unknown, such fibers can be seen inside the muscle cells.

The outer surface of the entire body, oral sucker, ventral sucker, pharynx and esophagus is covered by integument layer (Fig. 9). The integument of these portions is bounded by a plasma membrane at the outer surface and the basal surface of the integument, and the basal plasma membrane is acutely infolded into the integumentary matrix in many places. These infoldings of basal plasma membrane reach apex portion of the integumentary matrix. Some mitochondria and numerous round vacuole-like secretory granules are likewise located in the matrix. The somatic muscle layer lies internal to the connective tissue at this portion.

Fig. 2 Cross-section of the integument. Most mitochondria are distributed near outer surface of the matrix of integument. Many of the membrane-limited secretory granules and vesicles scatter in the matrix of integument, and there are infoldings of the basal plasma membrane (arrow).

Fig. 3 The epidermal cell which is located beneath the muscle layer, has nucleus, Golgi complex, endoplasmic reticula with dense granules and secretory granules which is bounded by thin limiting membrane.

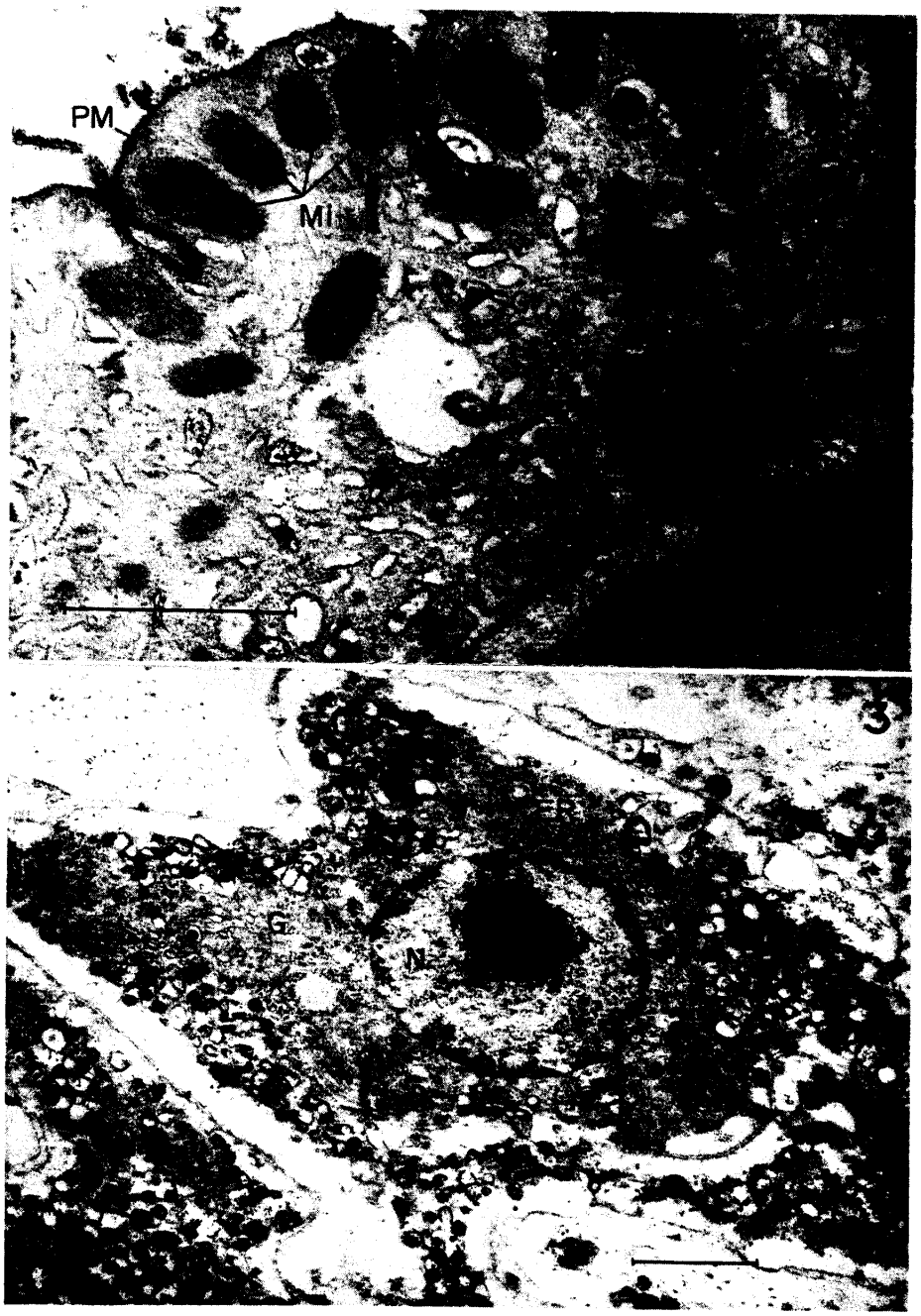
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Fig. 4 High magnification of the epidermal cell. There can be seen a nucleus with nucleolus and Golgi complexes.

Fig. 5 The cell inclusion granules in epidermal cell. The inclusion granules are arranged regularly.


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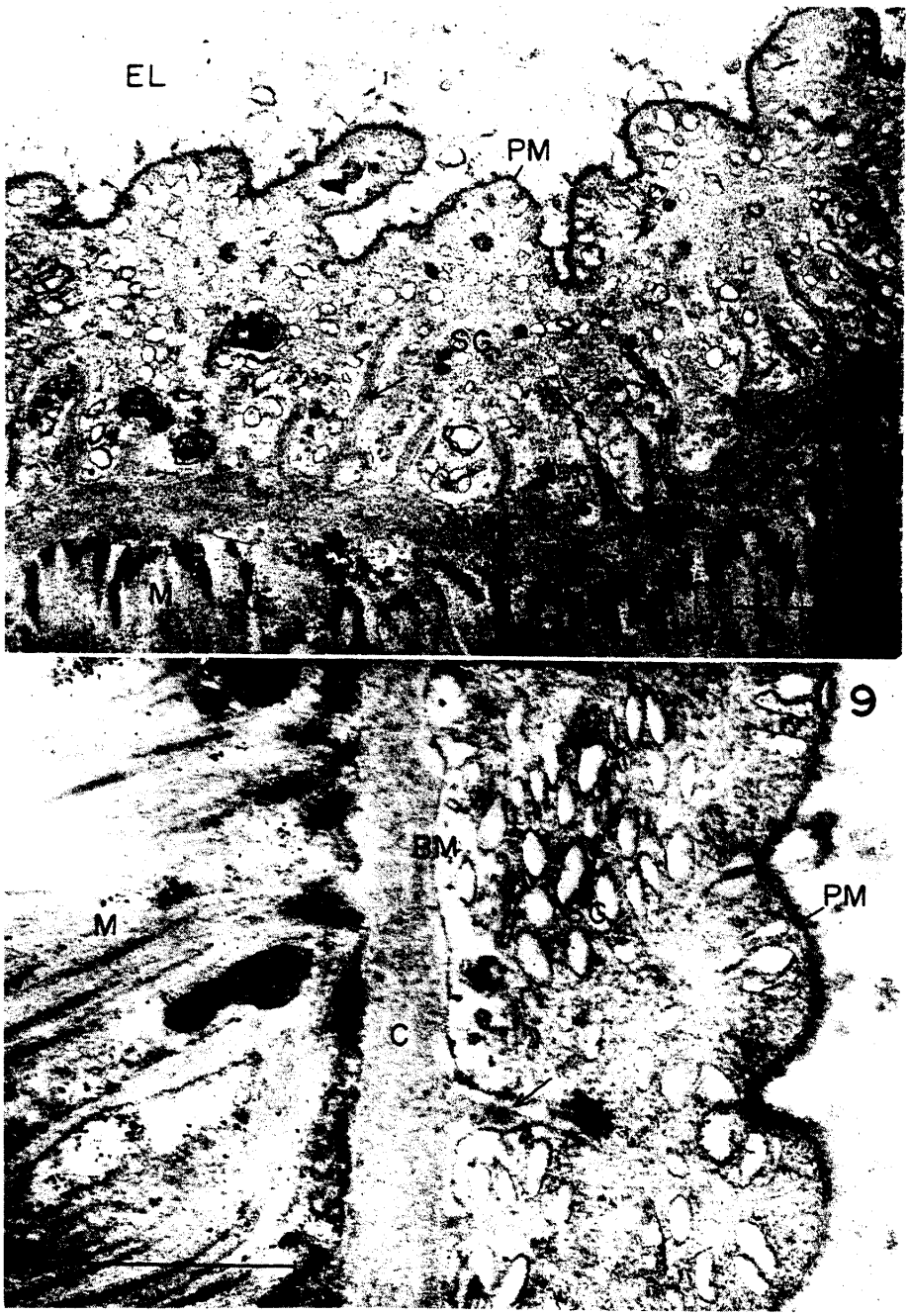
Fig. 6 Muscle layer. Battom right is a cross section of the circular muscle and left is longitudinal muscle. Both muscles are constructed of somatic muscle which are containing the thick and thin myofilaments.

Fig. 7 The collagen-like striated fiber can be seen between the somatic muscle filaments. One periodic which is composed of seven different dense bands, is 150 Å in length.


6

Fig. 8 The luminal surface of esophagus is covered with integument which has the same structure of body integument. A few mitochondria can be seen in this layer. There are many infoldings of basal plasma membrane at the area of integument.

Fig. 9 High magnification of the esophageal integument. There are numerous vesicles, membrane-limited secretory granules, and muscle layer (left) beneath the connective tissue layer.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DISCUSSION

The basic form of the integument resembles that reported by THREADGOLD (1963 a, b) for *Fasciola hepatica*, by BURTON (1964, 1966) for *Haematoloechus* and *Gorgoderina*, by INATOMI *et al.* (1968 a, b, 1969) for *Clonorchis sinensis*, *Metagonimus takahashii*, and *Schistosoma japonicum*. There are two points at issues on the integument. The first is secretory granules and the second is the distribution of the mitochondria.

The secretory granules are surrounded by thin double membranes and the form is of an irregular small vesicle-like shape of about 0.2 micron in diameter. The form, structure and size of these secretory granules are very similar to those of *Clonorchis sinensis*. THREADGOLD (1968) reported that *Fasciola hepatica* have two different kinds of epidermal cells which are classified by secretory granules. But a single type epidermal cell was found in *Opisthorchis viverrini*, and also *Clonorchis sinensis* just as with *Haplometra cylindracea* which was reported by THREADGOLD (1968 b).

Numerous mitochondria of the integument of both *Opisthorchis* and *Clonorchis* are distributed in the proximal outer surface of the integument. The fact that numerous mitochondria of the integument of trematodes belonging to Opisthorchidae are distributed in the proximal outer surface of the integument, is necessary for securing nutrients and protecting themselves in the environment of their parasitic locality. This point differs from that of *Schistosomatidae* as stated by BURTON (1966), THREADGOLD (1968), and INATOMI *et al.* (1969).

THREADGOLD (1968) reported that *Fasciola hepatica* has two different kinds of epidermal cells which are classified by secretory granules. However, epidermal cells of a single type were found in *Opisthorchis viverrini*, and also in *Clonorchis sinensis* as in *Haplometra cylindracea* reported by THREADGOLD (1968).

The crystalline inclusions were found at the perinuclear portion of the epidermal cell. These crystalline inclusions were reported by THREADGOLD (1968) for *Haplometra cylindracea*, by INATOMI *et al.* (1968) for *Clonorchis sinensis*. These are similar in shape, size, and arrangement. But the nature, substance and function of these inclusions are still unknown.

The muscle of *Opisthorchis viverrini* belongs to the somatic muscle as in some other trematodes. But some of the muscle cells have the collagen-like fibers which are constructed of numerous periodics where seven bands of different kinds of density gather between the muscle fibers. Although the nature, substances, and functions are unknown, it can be presumed, that these fibers are useful in association with the contraction of the

somatic fibers.

SUMMARY

Electron microscopy of the body wall of *Opisthorchis viverrini* shows the integument which is connected to the epidermal cell with fine protoplasmic tubules, to form a syncytium, as in *Clonorchis sinensis* and other trematodes. Vacuole-like secretory granules are distributed in the matrix of the integument, and mitochondria are arranged at the proximal outer surface of the integument. The crystalline inclusions are observed in the perinucleus of some epidermal cells.

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ABBREVIATION OF FIGURES

BM·····basal plasma membrane
Ci ·····cilia
CI ·····crystalline inclusion
EC ·····epidermal cell
ER ·····endoplasmic reticulum
I ·····integument
M ·····muscle
N ·····nucleus
PM·····plasma membrane
SG ·····secretory granule
C·····connective tissue
CM·····circular muscle
HL·····esophagus lumen
G·····Golgi complex
LM·····longitudinal muscle
Mi ·····mitochondria
No ·····nucleolus
PT ·····protoplasmic tubule
SR ·····sarcoplasmic reticulum

(Scale is one micron in each figure.)