Acta Medica Okayama

Volume 24, *Issue* 2 1970 *Article* 7

APRIL 1970

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The ultrastructure of helminth. 4. Cercaria of Schistosoma japonicum*

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Abstract

The body wall of the cercaria of Schistosoma japonicum is covered with a thin integument which is connected to epithel cells located under the uscle layer. On the outer and the basal surfaces of the integument are seen thin limiting membranes. In the matrix of the integument are distributed numerous dense granules, vacuoles and spines. The rootlet of the spine is attached to the basement membrane of the integument. The circular and longitudinal muscle layers, both underlying the integument, have smooth muscle fibers composed of thick and thin myofilaments. The cercaria possesses five pairs of secretion gland cells which are divided into two groups of three anterior and two posterior pairs. Both gland cells are filled with secretion balls. The tail of cercaria is likewise covered with a thin integumen t, whose structure is identically the same as the body integument. Beneath the integument are located thin circular and longitudinal muscle layers. The circular muscle cells have smooth muscle fibers, but the longitudinal muscle cells have striated muscle fibers. These muscle cells contain many large mitochondria. On observing the cross-sections of the tail at the flame cell level the arrangement of these muscle can be divided into four muscle groups and each muscle group reveals four or five muscle cells. The excretory system is well developed and has flame cells, excretory canal and bladder.

*PMID: 4247893 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **24**, 205—224 (1970)

THE ULTRASTRUCTURE OF HELMINTH
4) CERCARIA OF *SCHISTOSOMA JAPONICUM*

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Received for publication, December 25, 1969

In recent years a number of papers dealing with the ultrastructure of cercariae of Trematodes have been published. Especialy, the reports of KRUIDENIA & VAITER (1958), INATOMI (1960) and LUMSDEN & FOOR (1968) have respectively reported in some details the fine structure of the cercaria of Schistosoma. This study was undertaken to elucidate the fine structures of the cercaria of *Schistosoma japonicum*.

MATERIALS AND METHODS

The cercaria collected for the study were those that naturally escaped from infected snail host, *Oncomelania nosophora* (Kurume strain). The cercaria were immediately fixed in 1 % cold glutaraldehyde solution buffered with phosphate (pH 7.4) for 30 minutes, then washed with phosphate buffer. These were again subjected to the post-fixation with cold 2 % osmium tetroxide solution in phosphate buffer (pH 7.4) for two hours, dehydrated with ethanol series by routine methods immediately after the fixation and embedded in methacrylate or Epon 812. Specimens with these materials were cut with Porter-Blum ultramicrotome, and thin sections were stained with uranyl acetate and lead nitrate. For the observations the electron microscopes of Hitachi HS-6, HS-8, HU-11, and that of Nihondenshi JEM-7 type were used.

RESULTS

Integument: The body and tail of cercaria are covered with thin integuments from about 0.1 to 0.5 μ thick, and the integument is connected with epithelial cells located under the muscle layer, forming a large syncytium structure identical with that of the adult worm. On both outer and basal surfaces of the integument is seen a limiting membrane of about 80 Å in thickness. In integument matrix are distributed numerous discoidal or rod-shaped dense granules of varying sizes, vacuoles, and minute spines of about 0.1 μ or more in diameter and about 3 to 4 μ in

length. As to the structure of these spines, it presents a crystalloid structure congregated of high dense fine granules that measure about 80 Å in diameter and these granules run transversely and longitudinally to the long axis of the spine, forming a lattice structure. The rootlet of such spines reaches the basement membrane of the integument, and the part extending from the integument surface is covered with a thin plasma membrane which covers the surface of the integument itself.

The irregular projections of the integument are observed on the body surface, especially, many irregular finger-like projections are distributed at the head part of cercaria.

The basement membrane of the integument is often observed invaginating into the matrix. The mitochondria observable in the integument have relatively simple, small and clear cristae.

The fiber layer is of varying thickness, as connective tissue is situated between the integument and the muscle layer. This layer is constructed of the fine net-work with very fine fiber that is about 60 Å in diameter.

Body muscle: The muscle layer consisted of the outer circular and inner longitudinal muscle layers is located under the fiber layer. The fibers of these muscle cells are nonstriated but are not really smooth muscle. The myofibrils are constructed of both thick and thin myofilaments. And also, A and I bands, Z-discs are not observable on the myofibrils. However, the dense bodies are scattered occasionally on the myofibrils. The diameter of thick myofilaments is 250 Å and that of the thin ones 50 Å. The thick myofilament is surrounded hexagonally with about 12 thin myofilaments. The agranular endoplasmic reticula are distributed along the myofibil close to the sarcolemma. Almost all the mitochondria of muscle cells, numberless glycogen particles and the nucleus are located along the periphery of the myofibril.

Tail muscle: The muscle of the tail is constructed of an outer thin circular layer and an inner longitudinal muscle layer. The longitudinal muscle cell has striated myofilaments which are classified as thick and thin filaments. These myofilaments form different arrangements at A and I bands. The striations which are expresented by dense Z-discs observed in these cells reflect a regular transverse arrangement associated with the thin myofilaments. Such a striated muscle cell is very long, being as long as about 60 μ, and runs top to tip of the tail. The arrangement of muscle cells at the cross-section of the flame cell level of the tail can be divided into 4 muscle groups and each muscle group comprises 4 or 5 muscle cells. These muscle cells run diagonally, as they approach the end of the tail, the cell number gradually decreases. These muscle cells contain

many large mitochondria, sarcoplasmic reticula and glycogen particles between the myofilaments.

Epithel cell: The epithel cells are located in a deep portion of the body wall and connected to each other. These cells containing numerous annulated lamellar dense secretion granules of varying sizes, glycogen particles, endoplasmic reticula with ribosome granules, Golgi complex, mitochondria and nuclei, are connected to the integument by protoplasmic tubules. They are not so well developed and are not so numerous as in the case of adult worm.

Excretory system: The excretory system is well developed and has the flame cells, the excretory canal as its primitive nephron, and the excretory bladder. The flame cell measures about 14 to 15 μ in length, 3 to 5 μ in width, and at its tip it has a large nucleus with relatively small cytoplasm and several mitochondria around the nucleus. From this cytoplasm nearly 100 long cilia of about 0.1 μ in diameter are growing and running towards the excretory canal in a brush-like form. These cilia show typical cilium pattern. At the junction of the excretory canal and the cytoplasm there is observed a slit of filter part of funnel-like shape and the thickness of this part is about 0.2 μ.

Sense organ: A certain number of sensory hairs as terminal sense organ are observed on the integument. The sensory hair is constructed of two parts consisting of the cilia and bulb-like portion. The bulb and root of cilia are surrounded by the integument. The diameter of the bulb varies from 0.6 to 1 μ. The diameter of the cilia is 0.23 μ. The rootlet of the cilia reaches into the central part of the bulb. An open space of the inside of the bulb is filled with numerous small vesicles and mitochondria, and several microtubules are situated inside the bulb. The desmosomes are observed between the bulb and the integument surrounding the bulb itself. Two electron dense concentric rings associated with the microtubules are connected with the root of the cilia.

Secretion gland: In the posterior half of the body of cercaria, there are observed five pairs of penetration glands. They are classified into two groups by the morphology of secretion granules, and each penetration gland cell is filled with secretion granules. Three anterior pairs of these gland cells have rather dense secretion balls of varying sizes measuring about 0.05 μ in diameter. The two posterior pairs of gland cells have low dense secretion balls with numerous round vacuoles of about 0.1 μ in diameter. Many microtubules run along the inside of these gland cell membrane. The flattened nucleus which is compressed by producing the secretion granules themselves, is often found on the secretion gland cell

membrane.

Digestive organ : The digestive canal is of a very simple and not well-developed structure. The inner surface of the esophagus is covered with a thin integumental layer of about 0.1 μ in thickness. There can be seen several plait-like projections of the integumental layer at the inner surface of the esophageal and intestinal lumen, but not any real microvilli. The digestive canal is surrounded by small muscle groups.

Fig. 1 The body wall of the cercaria of *Schistozoma japonicum*. There are numerous rod-shaped or round dense secretion granules, and minute spines in the matrix of the integument covering the body surface. The muscle layer underlying the fibrous layer as connective tissue is consisted of two layers of circular and longitudinal muscles having smooth muscle fibers. The epithel cell contains numerous secretion granules.


(1)
Mi
PM
BM
Sp
SG

Fig. 2 A cross-section of the body wall. The outer covering of the body is the integument covered with a thin plasma membrane at the outer surface and bounded by a thin basement membrane at its lower margin. The muscle layer consisted of the outer circular and inner longitudinal muscle layers is located under the fiber layer.

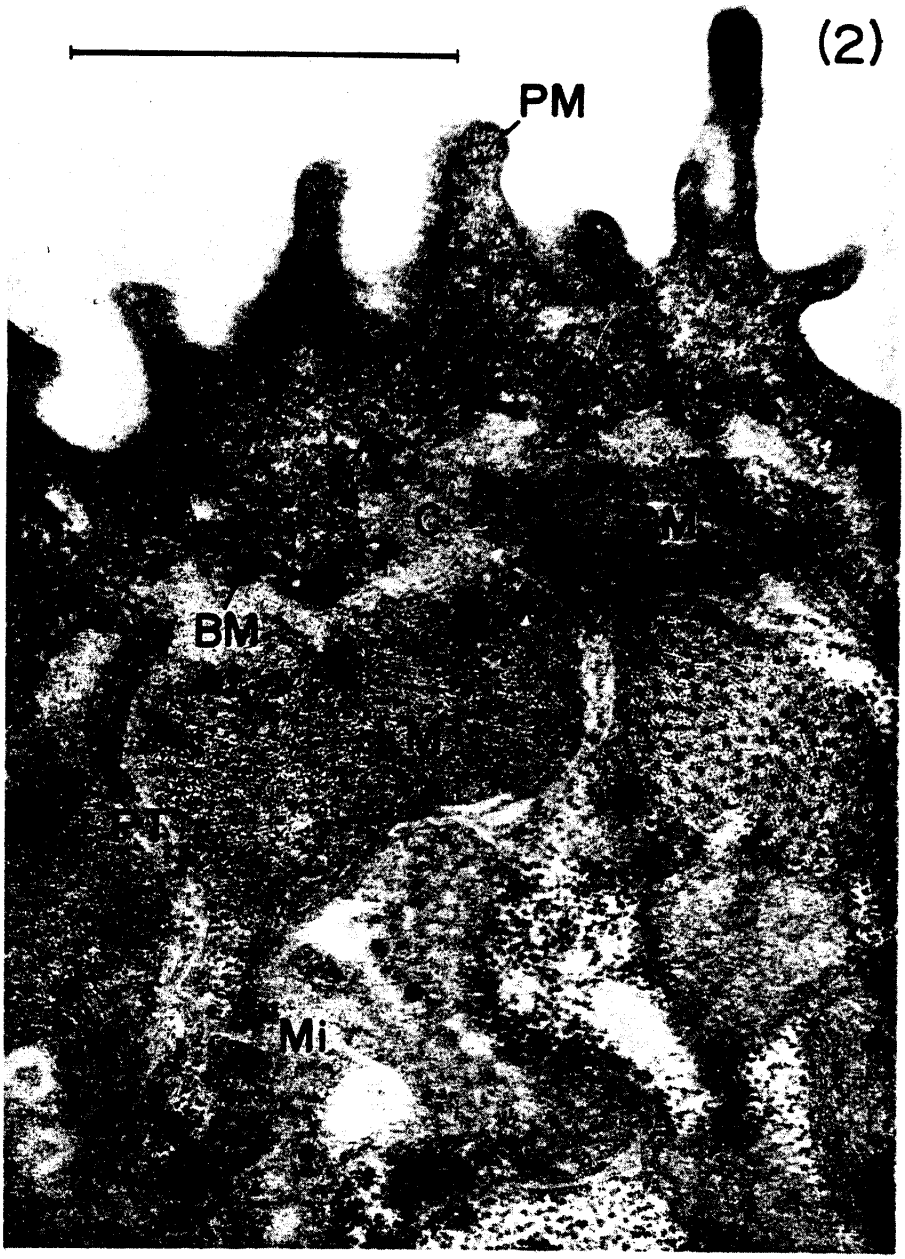
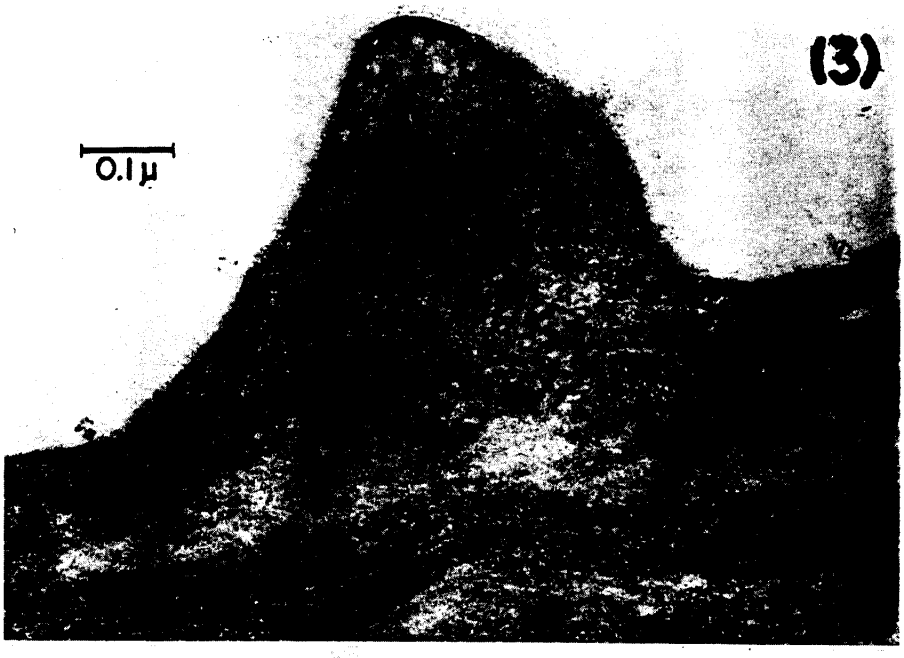

(2)
PM
BM
Mi

Fig. 3 A longitudinal section of minute spine. The spine showing a lattice-like crystaloid structure, has a rootlet which reaches the basement membrane of the integument.

Fig. 4 A cross-section of a minute spine of the integument reveals a lattice-like crystaloid structure.


(3)
0.1μ

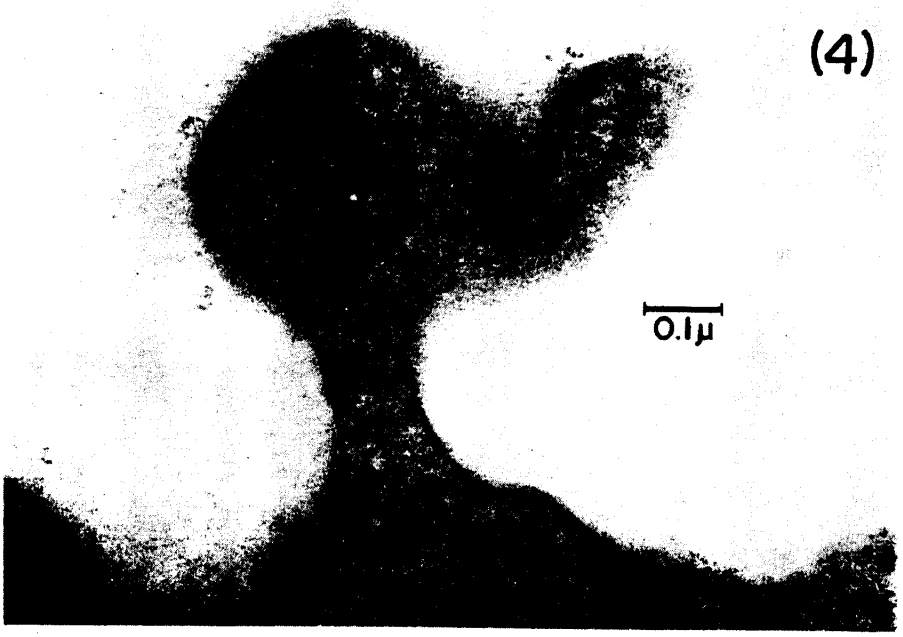

(4)
0.1μ

Fig. 5 The upper part of the picture shows A type penetration gland cells and the lower the B type. A-type gland cell reveals secretion balls containing numerous fine highly dense granules. B type gland cell contains rather dense secretion balls having many small vacuoles. Many microtubules, which measure about 260 Å in diameter, are arranged along the inner surface of the cell membrane.

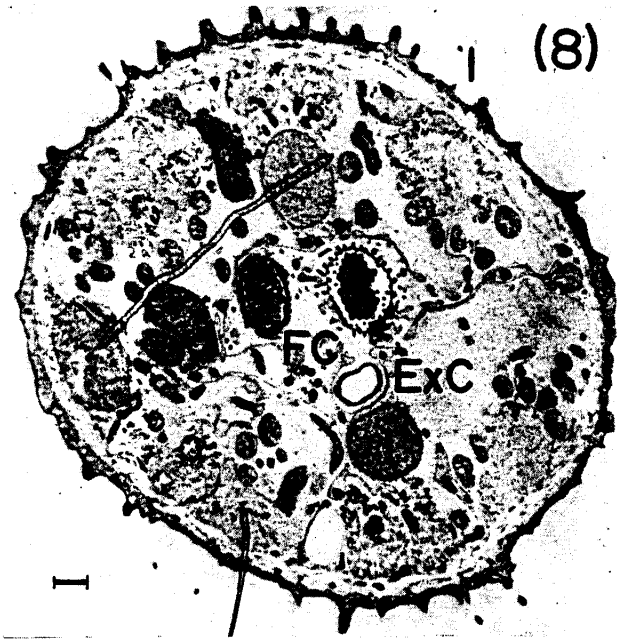
Fig. 6 A longitudinal section of the esophagus. The inner surface of the esophagus is covered with thin integumental layer. Several plait-like projections can be seen on the inner surface of the esophagus. The esophagus is surrounded by a thin muscle layer.

Fig. 7 A longitudinal section of the junction part of the body and tail. There is a small excretory bladder at the center of narrow junction. The flame cell is located near the anterior part of the tail.


M
ES
I
FC
(Tail)
(Body)

Fig. 8 A cross-section of the tail at the flame cell level. There are two flame cells with numerous cilia and an excretory canal at the central part of the tail. The cross-section reveals longitudinal muscle groups each having four or five striated muscle cells, under the integument thinner than that of the body wall.

Fig. 9 A semi-longitudinal section of the striated muscle of the tail. The muscle cell contains striated myofilaments of which I and A bands are not distinct, but Z band is usually clear-cut.


(8)
FG
ExC

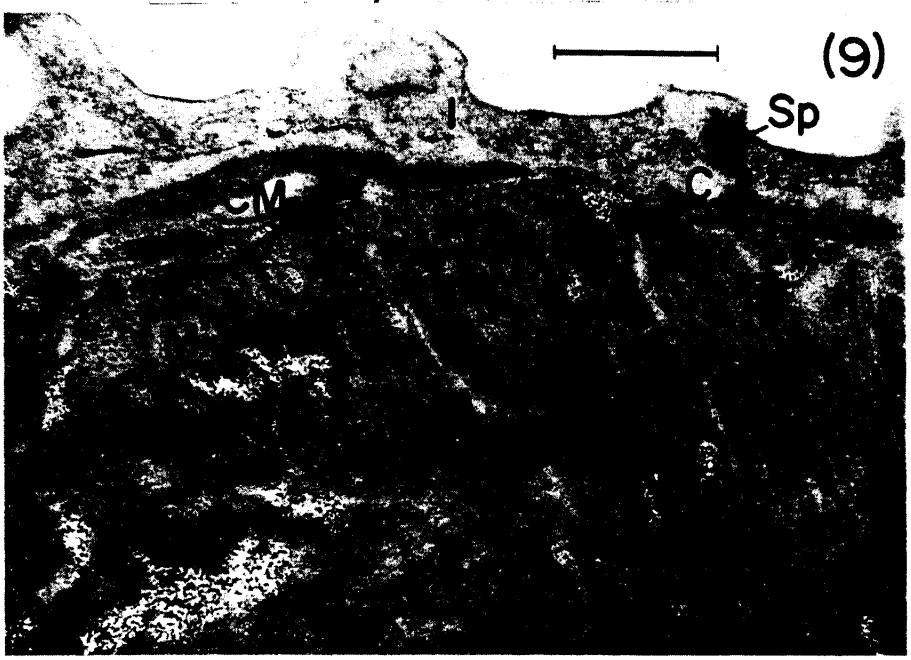

(9)
Sp
C

Fig. 10 A longitudinal section of the tail showing the integument and striated muscle cell. This picture seems to show Z, I and A bands on the striated muscle fibers. Numerous mitochondria with many fine cristae can be observed under the muscle fiber.

Fig. 11 A cross-section of the flame cell. There are 34 cilia with typical cilium pattern, with a pair of microtubules at the center and nine pairs in the periphery.

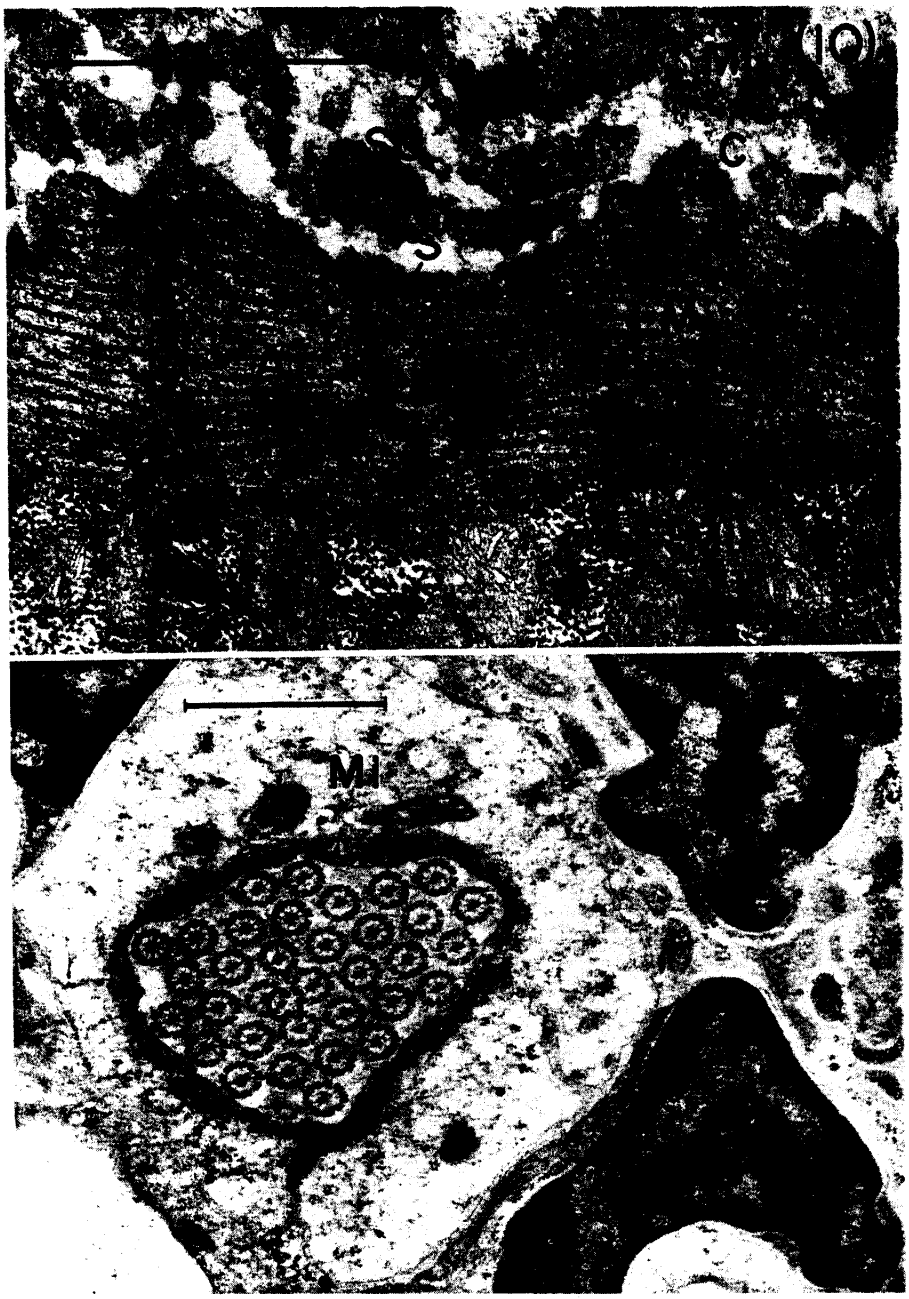

(10)
C
C
M

Fig. 12 A longitudinal section of the flame cell. It reveals several small mitochondria and little cytoplasm. Long cilia are growing and intruding towards the excretory canal in a long brush-like shape from the cytoplasm. There is observed a slit of filter part of funnel-like shape at the junction of the excretory canal and cytoplasm.
The scale in each photograph is in 1 μ.

DISCUSSION

Concerning the cercaria of *Schistosoma*, there are reports by KRUIDENIER *et al.* (1958), LUMSDEN *et al.* (1968) and INATOMI (1960). KRUIDENIER *et al.* described about the muscle of *Schistosoma mansoni* and LUMSDEN *et al.* about the muscle of *Heterobilharzia americana*. Prior to these reports TAKAHASHI (1928) made detailed observations on the structure of the cercaria of *Schistosoma japonicum* at the light microscope level. In addition, INATOMI reported the ultrastructures of the tail of *Schistosoma japonicum* and *S. mansoni*, particularly of its muscle and flame cells. Apart from these, CARDELL *et al.* (1960) reported about the muscle of cercaria tail of *Himasthla* and INATOMI *et al.* (1964) about the cercaria of *Clonorchis sinensis*. In any case, it has become clear that the muscles observable in the cercaria of *Trematoda* are composed of smooth muscle cells with somatic fibers in the body while those in the tail are composed of striated muscle cells. This fact amply indicates durable and more rapid movements of the tail of cercaria. In addition, the observations of the cross-section of the tail reveal that these striated muscle in any case can be divided into four muscle groups, and there is a limit in the number of muscle cells belonging to each group; namely, at the flame cell level there are five muscle cells in each muscle group in the case of *S. japonicum* and *S. mansoni*. These are less than ten muscle cells in each muscle group of *Clonorchis sinensis*. However, in the tail near the tip the number grows less. These striated muscle cells measure approximately 60 μ in length.

As regards flame cells KÜMMEL (1958) observed of *Fasciola hepatica* and demonstrated its structure, and INATOMI (1960) also made observations on the flame cells of funnel-like shape in the cercaria of *S. japonicum*, that had cytoplasm with a large nucleus, having about 100 cilia and slit-like filters, as described by KÜMMEL.

MORRIS and THREADGOLD (1967) observed the sensory hair, in the integument of adult *Schistosoma mansoni* and demonstrated its fine structure in their brief report.

Descriptions on the gland cells of cercariae are found in the reports of REES (1967) on the cytogeneous gland cells of *Parorchis acanthus* and of INATOMI *et al.* (1968) on the gland cells of *Clonorchis sinensis* adults. However, the cytogeneous gland and secretion gland cells have different structures and functions, and also, the form, density and structure of secretion granules produced by themselves differ considerably.

The secretion gland cell was already described in detail by TAKAHASHI (1957) in his light microscope study, and we demonstrated these ultra-

structures more precisely.

The present description on the digestive organ of the cercaria is the first of its kind. Both the esophagus and primitive intestine have been proven to be a canal without any characteristic digestive epithelium.

SUMMARY

The body wall of the cercaria of *Schistosoma japonicum* is covered with a thin integument which is connected to epithel cells located under the muscle layer. On the outer and the basal surfaces of the integument are seen thin limiting membranes. In the matrix of the integument are distributed numerous dense granules, vacuoles and spines. The rootlet of the spine is attached to the basement membrane of the integument. The circular and longitudinal muscle layers, both underlying the integument, have smooth muscle fibers composed of thick and thin myofilaments. The cercaria possesses five pairs of secretion gland cells which are divided into two groups of three anterior and two posterior pairs. Both gland cells are filled with secretion balls.

The tail of cercaria is likewise covered with a thin integument, whose structure is identically the same as the body integument. Beneath the integument are located thin circular and longitudinal muscle layers. The circular muscle cells have smooth muscle fibers, but the longitudinal muscle cells have striated muscle fibers. These muscle cells contain many large mitochondria. On observing the cross-sections of the tail at the flame cell level the arrangement of these muscle can be divided into four muscle groups and each muscle group reveals four or five muscle cells.

The excretory system is well developed and has flame cells, excretory canal and bladder.

ACKNOWLEDGEMENT

The authors wish to thank Dr. K. Okabe, (Professor of Parasitology, Kurumé University, School of Medicine) for providing us with *Schistosoma japonicum* infected snail host, *Oncomelania nosophola*.

This study was supported by a research grant from Japan-U. S. Cooperative Medical Sciences.

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ABBREVIATIONS OF FIGURES

BM ……basement membrane
Ci……cilia
D ……desmosome
Ex C …excretory canal
G ……glycogen particle
I……integument
LM ……longitudinal muscle
Mi ……mitochondria
PM ……plasma membrane
SG ……secretion granule
Sp……spine
C……connective tissue
CM……circular muscle
Es ……esophagus
FC ……flame cell
HG……head gland
IS ……intercellular space
M ……muscle
N ……nucleus
PT……protoplasmic tubule
SHS …sensory hair sheath
SR……sarcoplasmic reticulum