ULTRASTRUCTURAL OBSERVATIONS ON CERCARIA OF *SCHISTOSOMA JAPONICUM*

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Abstract. The present paper describes the ultrastructure of the surface topography, head organ, tegument, musculature, glandular system, primary alimentary tract and flame cells of the Chinese mainland strain of *Schistosoma japonicum* cercaria, as visualized with both scanning and transmission electron microscopes. The results not only illustrate the morphological features of the cercarial surface and its internal structure reflecting an adaption to the aqueous habitat but also reveal the correlation between the morphological structure and physiological function.

INTRODUCTION

Schistosome cercaria is an infective stage which penetrates host skin. Studies on both morphological structure and ecological behavior of schistosome cercaria may provide valuable information about the mechanism of cercarial penetration. In the past two decades electron microscopy has become a useful tool for investigating in-depth characterization of schistosomal cercaria morphology. Several species of schistosome cercaria have been visualized by both scanning (SEM) and transmission (TEM) electron microscopy, more data being gathered on *S. mansoni* than on any other schistosome species (Lumsden and Foor, 1968; Ebrahimzadeh and Kraft 1969, 1971a, 1971b; Inatomi *et al,* 1970; Morris 1971; Race *et al,* 1971; Dorsey and Stirewalt 1971; Hockley 1973; Ebrahimzadeh 1974; Nuttman 1974; Dorsey 1976; Dorsey and Stirewalt 1977; Sakamoto and Ishii 1978; Davies 1983; He *et al,* 1985; Zhou *et al,* 1984, 1988; Lin 1989; Sobhon and Upatham 1990). However, detailed studies of the ultrastructure of surface characteristics and internal organs of *S. japonicum* cercariae are still lacking. Therefore, the present paper is devoted to the use of SEM and TEM for observation of the surface topography and the inner structures of cercaria from the Chinese Anhui-Hubei strain of *S. japonicum.*

MATERIALS AND METHODS

Source of cercariae

S. japonicum cercariae of Chinese Anhui-Hubei strain were collected from many F1 laboratory-bred and -infected *Oncomelania hupensis.*

SEM observation

Cercariae were fixed in cold 4% glutaraldehyde with phosphate buffer pH 7.4 at 4°C and then washed in the same buffer, dehydrated in graded ethanol series, followed by 100% acetone for 1-2 hours, and dried by critical point method in liquid CO_2. Specimens were observed with a JSM-15 scanning electron microscope, following coating with a layer of gold.

TEM observation

Cercariae were pre-fixed in cold 2.5% glutaraldehyde in 0.1 mol phosphate buffer pH 7.4 for 2 hours at 4°C, then washed twice in the same buffer. They were post-fixed in 1% aqueous osmium tetroxide for 1 hour, following further washing in buffer, dehydrated in graded ethanol series and 100% acetone, then transferred to 1:1 actone-Epon mixture. The specimens were embedded in Epon 812, after

which thin sections were cut with an LKB-V ultramicrotome and stained with uranyl acetate and lead citrate, then examined by JEM-200 CX transmission electron microscope.

RESULTS AND DISCUSSION

General features and surface topography

The cercaria of *S. japonicum* has an oval or elongated body and a long cylindrical tail which is divided into two furculae at the posterior extremity (Fig 1). The ventral sucker located in the post one third of the body is well developed, displaying numerous large sharp spines and four ciliated sensory bulbs on its rim (Fig 2). The anterior tip of the body is a specialized head organ which can slightly stretch out and retract (Fig 3). The posterior end of the body is tapered into a collar-like folding over the narrow connecting piece between the body and the tail (Fig 4).

With the exception of the apical region of the head organ of the cercaria, the entire surface is covered with backwardly-directed spines. The spines on the anterior part of the body are a little rounder and less spaced than those on the posterior of the body (Fig 3). While the spines on the tail are generally fewer but longer and thiner than those on the body. The furculae of the tail have less and relatively sharp spines but comparatively more ridges which are close and parallel (Fig 5). The apertures of the excretory ducts are usually seen at the tips of the furculae.

The number and distribution of the tegumentary sensory organelle on the surface are observed. They are arranged in an essentially symmetrical pattern. Two types of tegumentary sensory organelle are distinguishable: Uniciliated sensory bulbs, and sensory pits. The first type is the most common and appears as a rounded elevation with a single cilium (Fig 6). The second type occurs near the anterior end, appearing as a pit. The number of the sensory organelles on the dorsal body is 18 (9 pairs, D1-D9). D1-D4 are limited to the head organ, the distance between D1 and D2 being about equal to that between D2 and D3. D3 and D4 are at about the same level. D5 and D6 are slightly posterior to the head organ (Fig 7), while D7-D9 lie medial and posterior part of the body. There are usually 16 (8 pairs, V1-V8) sensory organelle on the ventral surface. V1-V4 are limited to the head organ. V3 lies anterior to the mouth and V4 lies posterior to the mouth (Fig 8). V5 and V6 locate anterior to the acetabulum, while V7 and V8 lie at the level of the posterior part of the body (Fig 2). The number of lateral sensory bulbs is 16, 8 are on each side. L1 is almost at the same level as V3. The tail sensory bulbs are not arranged in a symmetrical pattern being more than 12 in number. Both D1-D2 and V1-V2 are the second types in structure while the rests are of the first type.

Head organ

The apex of the head organ forms a slighly elevated, spine-free disc-like area in which are the openings of the acetabular gland ducts exist. They are linearly arranged in two separate lateral crescents. Each duct opening is encircled by a raised tegumentary fold and seven ciliated sensory papillae are situated on the convex edge of the outer tegumentary folds of each crescent (Fig 8). The possible function of the tegumentary folds may be related to the discharge of secretions from the acetabular glands during penetration to the final host, and these folds may thus prevent the secretions diffusing to the skin surface, so allowing a more efficient and directional discharge of secretions from acetabular glands.

There are seven ciliated sensory papillae on the convex edge of each crescent, and from dorsal view, 3 on each side (Fig 9). The remaining 4 pairs of papillae are located on ventrolateral side. These pear-shaped papillae consist of round base with a relatively long collar, and with a short cilium protruding from it. They differ morphologically from the uniciliated sensory bulbs with unsheathed setae on the body surface. The mouth is situated on mid ventral surface of the subterminal part and lay within a spineless depression of the tegument.

Body wall

Our results revealed that the typical body wall of *S. japonicum* cercaria includes the tegument, basal lamina, interstitial material and muscular layers (Fig 10). The entire organism is covered by a single, continuous cytoplasmic tegument. It consists of an outer plasmic membrane, matrix, basement membrane and is connected by cytoplasmic processes to nucleated regions lying in the parenchyma. The external surface of the tegument is bounded by a trilaminate plasma membrane about 7.5-8.8 nm in thickness which bears 0.34-1.21 μm thick glycocalyx consisting of very

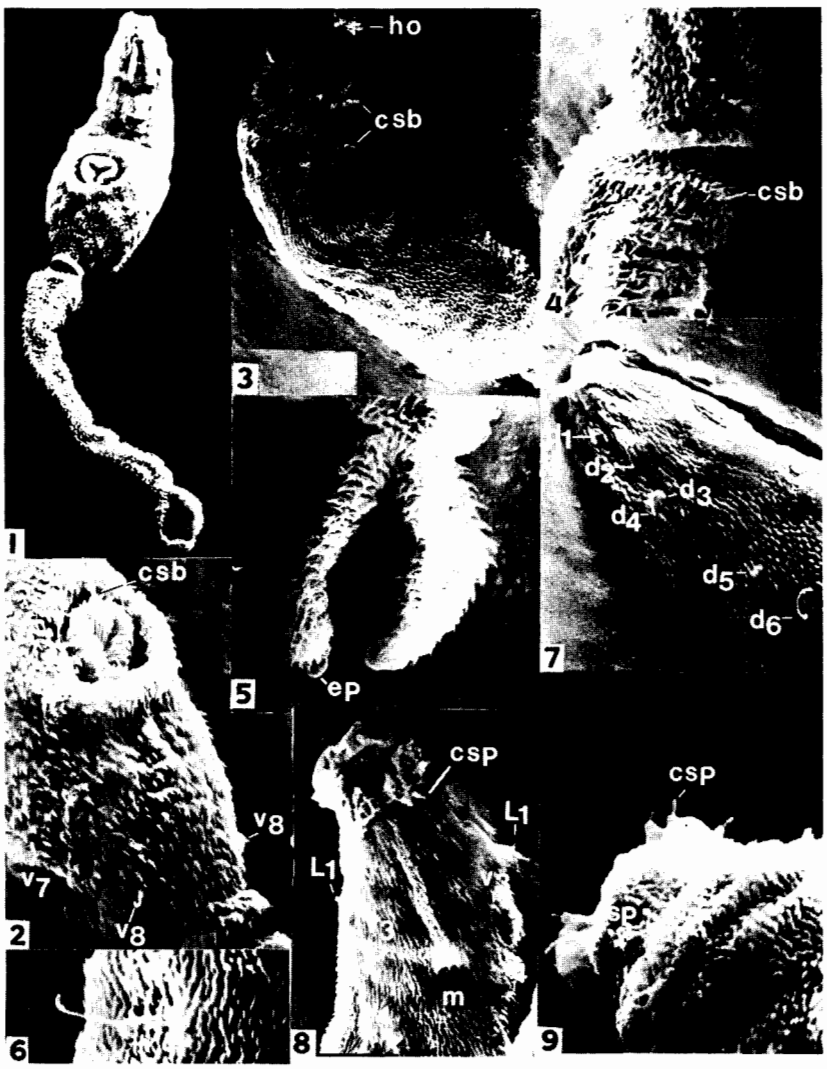

Fig 1–SEM of the ventral surface of *S. japonicum* cercaria showing the body, the tail and the junction between body and tail. × 1,125.

Fig 2–An acetabulum showing many spines and 4 ciliated sensory bulbs (csb) on it rim, and the 7-8th pair of ciliated sensory bulb (V7-8 csb) on the ventral surface of the posterior body. × 3,000

Fig 3–Whole cercarial body showing the head organ (ho) on the anterior tip, the surface of the body is covered with two types of spines and several siliated sensory bulbs (csb). × 1,500

Fig 4–Body-tail junction of cercaria showing the body and tail are separated by a structure of collar × 3,000

Fig 5–Caudal furculae showing sharp spines and the excretory pore (ep) at the tips × 3,000

Fig 6–Scanning electron micrograph showing one uniciliated sensory bulb on the body surface. × 5,000

Fig 7–arrangement of tegumentary sensory organelle on each side of the dorsal part of anterior body, showing the 1st and 2nd pairs of sensory pit (dl, d2) and 3th to 6th pairs of ciliated sensory bulbs (d3-d6). × 3,000

Fig 8–Head organ showing acetabular gland duct openings and ciliated sensory papillae (csp), mouth (m) is situated on the subterminal part, around it the 3th and 4th pair of ventral ciliated sensory bulbs (V3, V4) and 1st pair of lateral ciliated sensory bulbs (L1) can be observed. × 3,000

Fig 9–Dorsal view of head organ, showing 3 ciliated sensory papillae (csp) on each side and one sensory pit (sp).

finely branched anastomosing filaments (Fig 11). The internal boundary of the tegument is also delimited by a trilaminate basal membrane. Inclusion bodies indentified within the matrix of the cercarial surface syncytium include spine, mitochondria, spherical bodies and membraneous bodies. The spherical bodies are oval in shape with 0.10-0.29 × 0.13-0.48 μm in size, and are limited by a plasma membrane; they contain a peripheral electron-lucent region and somedense granules. The membrenous bodies are some what oval in shape with 0.13-0.18 × 0.23-0.36 μm in size and are constituted by the parallel multilamellated membranes.

Just beneath the basement membrane of the tegument there is a layer of basal lamina which is homogeneously electron-dense about 37.5-43.8 nm in thickness. A medial electron-lucid layer, interstitial material, about 0.14-0.19 μm thick lies immediately beneath the basal lamina, and the fibers of the layer are branched and interconnected, forming a diffuse network.

Tegumental spines are found over the entire surface of the cercaria, much of the spines is in direct contact with the tegumental matrix and in this region the sides of the spine are often parallel (Fig 12). The spines protrude from the matrix to outer membrane and the glycocalyx covering the spines is thiner. In the case of uniciliated sensory bulbs founding in the tegument of both body and tail, they are embeded in the tegument and lie at the end of a dendron. The bulb contains numerous vesicles of various sizes, a fine granular material and microtubules (Fig 13). The sensory pits have a depth of 1 to 2 μm and are open to the exterior.

Musculature

Body muscularity is best exemplified by the peripheral body muscles and muscles associated with both ventral sucker and head organ. The body peripheral muscle appears as circumferential and longitudinal layers of muscle fibers (Fig 14) the latter layer is conspicuously thicker than the former. Usually these fibers are segregated from each other according to orientation, suggesting bundles of myofibrils proceeding in a paticular direction. Between these muscular bundles are accumulations of mitochondria with numerous cristae. Muscle cross section shows that the myofibril consists of both thick (25 nm in diameter) and thin (5 nm) filaments. Dense bodies in body muscle are scattered among the filaments, making them difficult to observe at lower magnifiication. However, these dense bodies are more evident in tail muscle, because in the longitudinal muscle they are aligned, giving it a striated appearance (Fig 15).

The structure of body and tail muscle in cercaria has been compared. The more obvious differences between body and tail muscle are as follows: 1) There are four distinct groups of longitudinal muscle in an arrangement of dorsal, ventral and two lateral in the tail stem, lying immediately beneath the circum-ferential muscle layer and each muscle group contains 4-5 muscular fibers; 2) The crossbands appear in the caudal myofibrils; 3) Tail muscle contains large and numerous mitochondria and many glycogen granules; 4) Large amounts of tubular arrays of smooth sarcoplasmic reticulum occurs in the tail muscle (Fig 16). These variations reflect the differences between the slow-working body muscle and rapidly contracting muscle in the tail. The smooth sarcoplasmic reticulum, mitochondria and glycogen granules associated with tail muscle are typical characteristics of fast-contracting muscle.

Glands

The glandular system of *S. japonicum* cercaria consists of 3 different types of large unicellular glands, namely, one head gland located in the center of head organ and 10 glands positioned around the acetabulum. The ten glands are symmetrically in two sets, each set consisting of two pre-acetabular glands and three post-acetabular glands. They occupy a large volume of the body.

In the pre-acetabular glands there are to morphologically distinct types of secretory globules. Type A is electron-dense, oval in shape, and 0.46 × 0.32 μm in size. Type B secretory globules are more abundant than type A, measuring about 1.02 × 0.82 μm in size (Fig 17). The type B globules have a matrix of medium electron density, and within the matrix are numerous small round electron-lucid areas of about 0.12 μm in diameter. A thin network of anastomosing components can be seen within the lucid areas. changes in the appearance of the type B globules occur as they move toward the distal part of the duct, and the electron-lucid areas become fewer. As they move toward the duct opening, the globules are mostly of a homogeneous nature.

The most abundant cytoplasmic inclusions of the post-acetabular glands are the secretory globules. Each globule is elongate oval or irregular in shape, and

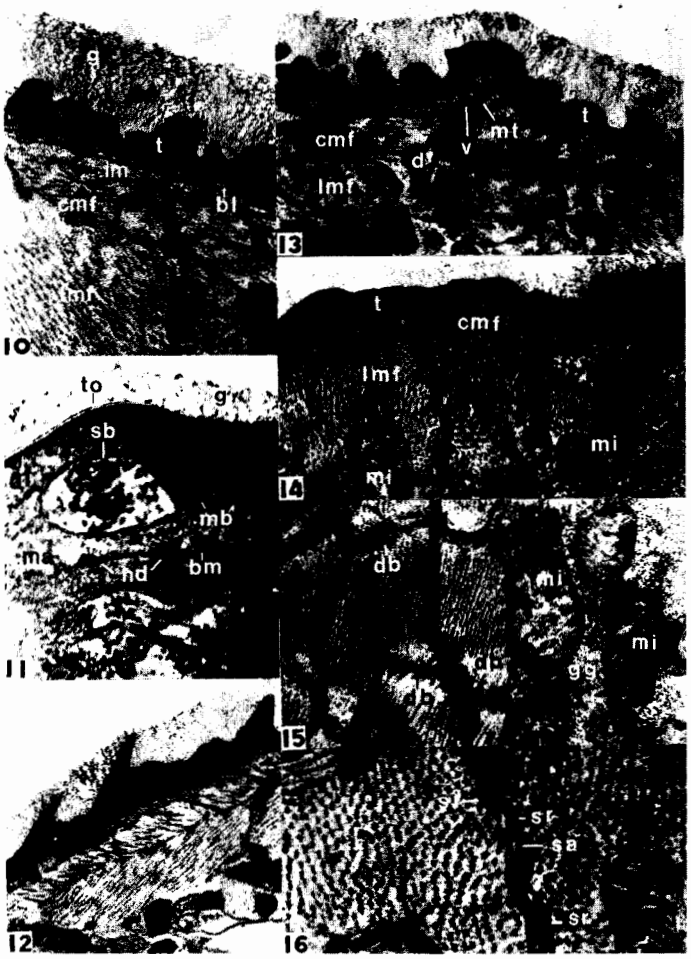

Fig 10–A typical ultrastructure of the body wall of *S.japonicum* cercaria, showing the glycocalyx (g), tegument (t), basal lamina (bl), interstitial material (im) and muscular layers consisting of circularly arranged muscle fibers (cmf) and longitudinally arranged muscle fibers (lmf) × 20,000

Fig 11–Ultrastructure of the tegument, showing trilaminate outer membrane (tom) and dense layer (dl) under it, matrix (ma), spherical body (sb), membraneous body (mb), hemidemosome (hd) and basement membrane (bm) × 60,000

Fig 12–Spines protrude from matrix to outer membrane, the glycocalyx covering the spines is thiner × 14,000

Fig 13–Tegument (t) containing a ciliated sensosry bulb (csb) and associated dendron (d), the bulb contains many vesicles (v), microtubules (mt) and granules × 14,000

Fig 14–Transverse section of tail, showing circumferential muscle fibers (cmf), longtudinal muscle fibers (lmf) and many large mitochondria (mi) with numerous cristae in the caudal muscle × 20,000

Fig 15–Longitudinal section of tail muscle, showing cross-bands arranging by dense bodies (db), large mitochondria (mi) numerous glycogen granules (gg) and packed smooth sarcoplasmic reticulum (sr) × 20,000

Fig 16–Section of the sarcolemmae (sa) of two adjacent caudal axial fibers which are closely approximated, showing an alingent of the sarcoplasmic reticulum (sr) in each to form the paired dyads illustrated × 40,000

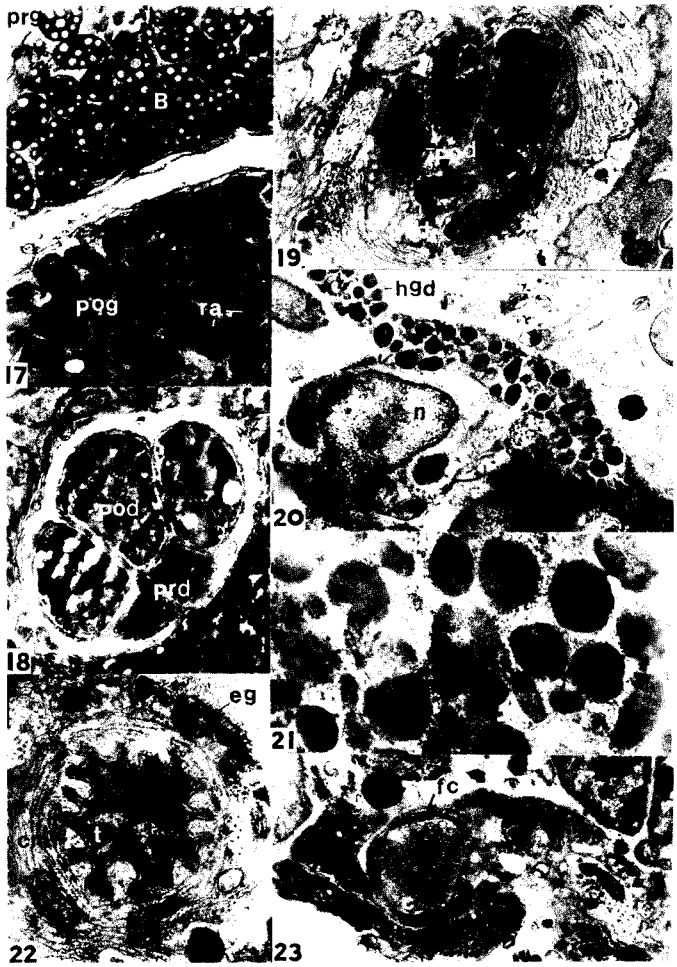

Fig 17–Acetabular glands of cercaria, showing type B secretory globules (B) packed within the pre-acetabular gland (prg) and secretory globules in post-acetabular gland (pog) consist of homogeneous ground substance in which is embeded round electron-dense aggregates (ra) × 12,000

Fig 18–Transverse section of one duct buncle, showing each bundle, includes three large post-acetabular ducts (pod) and two small pre-acetabular ducts (prd) × 12,000

Fig 19–Anterior part of one duct bundle, showing the five ducts are encompassed by two layers of muscle (m) forming as a sheath × 14,000

Fig 20–A section through one duct of head gland (hgd), showing the secretory globules and the space between the duct and the surrounding tissue is filled with nucleus (n) of parenchymal cell, extracellular fibrous and mitochondria × 14,000

Fig 21–Secretory globules within head gland, showing each secretory globule is concentric with multilamellated membranes × 43,500

Fig 22–Transverse section of esophagus, showing esophageal glands (eg) closely invest the esophagus with thick circular muscle fibers (cmf) and constricted tegument (t) × 20,000

Fig 23–Transverse section of a flame cell (fc) in body, showing many cilia (c) × 10,000

averages about 0.97 × 0.62 μm in size (Fig 17). The globule matrix is homogeneous, in which are embeded round, electron-dense aggregates measuring about 0.2 μm in diameter. As the secretory globules move from the fundus through the duct, they become more homogeneous and the electron-dense aggregates decrease progressively. Apart from the secretory globules, small mitochondria, electron-dense granules (probably granules of glycogen) occur in the cytoplasm of the post-acetabular glands.

The ducts of these glands are joined into two duct bundles which run anteriorly and become narrow. Each bundle of five is encompassed by two layers of muscle (Fig 18, 19). The extracellular space between the gland ducts is filled with an extracellular fibrous network and microtubules lining the inner-side of the duct membrane.

The head gland consists of a fundus and multiple ducts which open to the surface of the anterior end of the head organ (Fig 20). The fundus contains most of the secretory globules with concenric multilamellated membranes (Fig 21). Each globle is round or oval in shape and averages about 0.29 × 0.23 μm.

Alimentary tract

The primary alimentary tract of the cercaria composes of esophagus, esophageal glands and bifid short ceca. The upper part of the alimentary tract is lined by tegumental which resembles that of the outer body wall. However, at the region of the esophagus the tegumental surface attains 12.5 nm in thickness. The esophagus measures about 2.5 μm in width and there are two layers of muscle fiber bundles below the interstitial material. The circular muscle layer with 0.35-0.58 μm is markedly thicker than the layer of longitudinal muscle. In the middle part of alimentary tract some esophageal glands closely invest the esophagus (Fig 22). The inclusion materials in the esophageal glands are spherical or oval electron-dense secretory granules with 0.14-0.25 μm in diameter. A marked alteration in structure occurs at the junction of the oesophagus with the cecum. On the luminal surface of epithelial layer of the cecum, there are many long branching microvilli projecting into the dilated lumen.

Flame cells

The excretory system is well developed with flame cells and excretroy canal as a primitive nephron, which are prominent on either side of the cercarial body as well as an excretory bladder at its posterior tip. A flame cell measures about 3-5 μm in width as shown in transverse section (Fig 23). The flame cells have nearly 100 long cilia delimited by slender tubular cytoplasmic strands joined side-to-side. These cilia have a typical cilia pattern, with 2 filaments in the center and 9 filaments at the periphery.

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