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Scanning Electron Microscopic and Histochemical Observations on the Miracidium of *Gigantobilharzia sturniae* Tanabe, 1948

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Abstract

Miracidia of *Gigantobilharzia sturniae* were studied by light and scanning electron microscopy. Some morphological findings obtained were as follows: An apical gland connects with the apical papilla through a duct, and a pair of PAS-positive lateral glands opens on either side at the base of the apical papilla. Four pairs of single ciliated sensory projections situated in the four quarters, protrude on the surface of the apical papilla having cytoplasmic folds of a honeycomb-like appearance. Three secretory pores lie laterally on either side of the apical papilla. Two pairs of lateral papillae, a bulbous and a crescent in shape, and sixteen multiciliated nerve endings are located on the narrow transverse ridge between the first and second tiers of the plates. A pair of excretory pores surrounded by double circular brims of cytoplasmic evagination lie on the longitudinal ridges between two plates of the third tier of the epidermal plates. A pair of excretory bladders, thick-walled and elongated in shape, are located near the excretory pores. Two clusters of excretory ducts surrounded by numerous PAS-positive substances lie in two places, one located beneath the nerve mass and another at the posterior most portion of the body. Many nucleate cells are scattered among the germ cells.

Key words: *Gigantobilharzia sturniae*, miracidium, SEM, surface structure, histochemistry, secretory gland

Introduction

Under light microscopy (LM), the miracidia of schistosomes examined thus far were shown to be composed of a common inner structure, such as an apical gland, a pair of lateral glands, a nerve mass, two pairs of lateral papillae, and two pairs of flame cells, several germ cells, as in *Gigantobilharzia* (Iwasaki, 1960), *Schistosoma* (Faust, 1949), and *Trichobilharzia* (Ito, 1960). In the scanning electron microscopic (SEM) investigation, however, some differences in the surface structure of the "bald" miracidia from which cilia are artificially removed, have been recognized in those of *S. mansoni, S. intercalatum, S. japonicum* or *S. haematobium* (LoVerde, 1975; Køie and Frandsen, 1976; Eklu-Natey, *et al.*, 1985).

The miracidia of *G. sturniae,* an avian schistosome which is widely prevailing in Japan, have been studied by Tanabe (1948), Oda (1953) and Iwasaki (1960) at the LM level. Present authors compared the "bald" surface of *G. sturniae* miracidium with those of previously examined schistosomes and the other trematodes using SEM, and histochemically examined the details of the inner structure of the miracidium.

Materials and Methods

The miracidia of *G. sturniae* were obtained from the faeces of the naturally infected bird, gray starling, *Sturnus cineraceus.* The faeces collected at an endemic area of cercarial dermatitis in Yonago City, Tottori Prefecture, were washed several times in distilled water. To make

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the miracidia emerge, they were exposed to a light spot for 5 hr at about 28°C. The swimming miracidia were fixed in 2.5% glutaraldehyde for 4 hr at 4°C. After washing in 0.1M phosphate buffer (pH 7.2), the specimens were dehydrated in a graded series of ethanol, and then embedded in Acrytron E resin. After polymerization for 24 hr at 4°C, the embedded miracidia were sectioned into about 1-2 μm-thick sections by the use of a glass knife for LM observations. The sections were stained with toluidine blue (TB) and periodic acid-Schiff's method (PAS).

The cilia of some miracidia were removed by an ultrasonic cleaner in absolute ethanol for 2-3 min to study their surface structure. After washing in distilled water, the specimens were post-fixed in 1% osmium tetroxide for 1 hr at 4°C, dehydrated in a graded series of ethanol, substituted by amylacetate, critically dried, coated with gold, and examined in a Hitachi S-450 scanning electron microscope.

Results

Scanning electron microscopic observations

The free swimming miracidium has a gourd -shape in general (Fig. 1). The cilia cover the body surface except for apical papilla and intercellular ridge (Fig. 2).

The spherical apical papilla located in anterior tip of the miracidium is composed of a honeycomb-shaped cytoplasmic folds, eight sensory projections and six secretory pores. A pair of uniciliated sensory projections are situated at each corner of a square inscribed in a circular section of the spherical surface (Fig. 3). Three secretory pores are situated at each top of triangle section on both lateral surfaces of the apical papilla (Fig. 4). The globular secretions in a variety of sizes released from each honeycomb pore or the secretory pores are rarely observed.

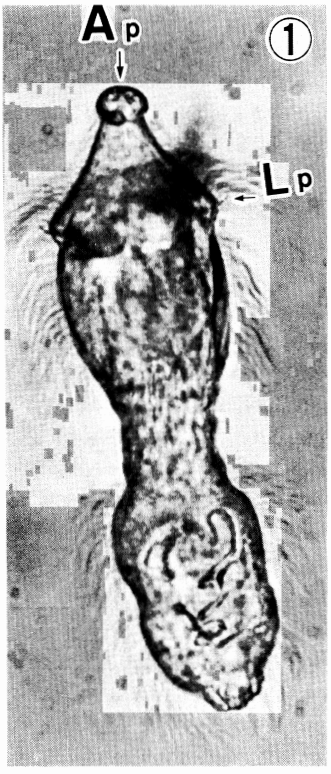
Four tiers of the epidermal plates are arranged in a pattern of 6, 9, 4 and 3 plates per tier antero-posteriorly. The plates on the first tier have a triangle shape with a somewhat rounded posterior margin, and those on the second tier are elongate ellipse in shape. The lateral papillae and the multiciliated nerve endings are seen on the intercellular ridge between the first and the second tiers of the plates (Fig. 5). The plates on the third and forth tiers are broad scale in shape (Fig. 6). The epidermal plates except for those on the first tier are covered with the cilia of uniform size about 10-15 μm by 0.2 μm (Fig. 7). The cilia on the first tier gradually increase in length from an anterior to posterior direction. These plates are separated from each other by non-ciliated, intercellular ridges, and bounded by a thin, wavy evagination of the cytoplasmic folds (Fig. 8). The narrow, transverse, body-encircled ridge between the first and second tiers form the anterior ring. The other ridges cover a larger area on the surface of the miracidial body, and do not form so -called median and posterior rings. The body surface of the "bald" miracidium is covered wholly with the fine microvilli, and numerous stubs of the cilia are observed on the epidermal plates (Figs. 7 and 8).

Three types of sensory organs are recognized exclusively on the anterior ring of the body surface (Figs. 9 and 10). Sixteen multiciliated nerve endings are situated on the anterior ring, although the cilia are broken by sonication. As shown in Fig. 10, two types of lateral papillae, a crescent and a bulbous-shaped, are observed close to each other.

Two excretory pores about 2-3 μm in diameter are located bilaterally on the longitudinal intercellular ridges between the plates of the third tier. They are surrounded by double circular brims of the cytoplasmic folds with fine microvilli (Fig. 11).

Light microscopic observations

In the horizontal section, the apical gland stained with TB occupies most of the anterior region above the nerve mass. The gland cell communicates with the apical papilla through an elongated duct (Figs. 12a and 12b). The germ cells stained intensely with TB occupy the major portion of the posterior half of the body. Many nucleate cells stained slightly with both TB and PAS are scattered between the germ cells. A pair of elongated, thick-walled excretory bladders stained with TB are located at posterior about one-third of the body length (Figs. 12a). The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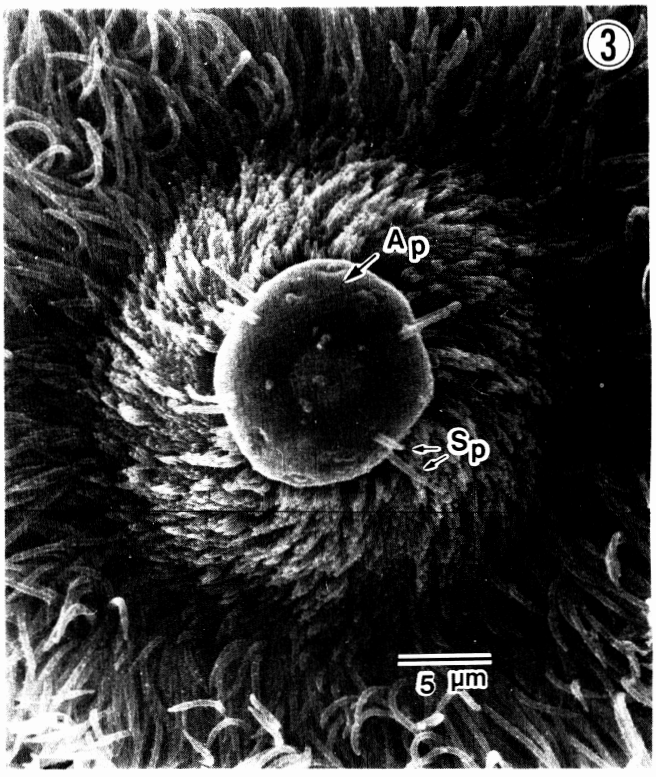
Lp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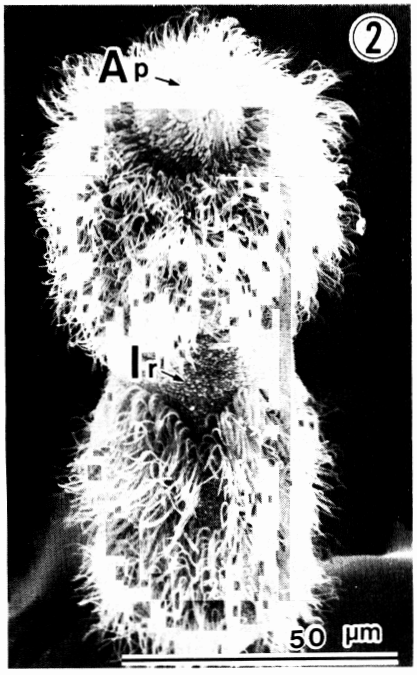
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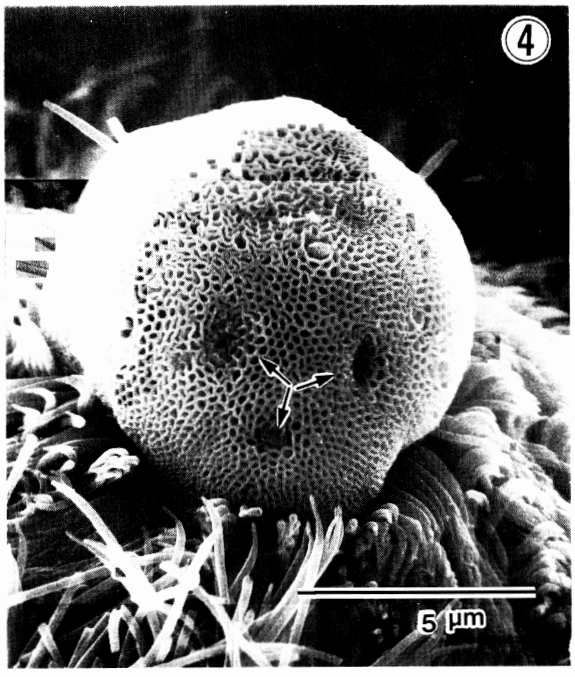

④
5 μm

clusters of the excretory ducts surrounded with numerous PAS-positive deposits are observed in two places, beneath the nerve mass and posterior portion of the body (Fig. 13). A pair of ducts from the lateral glands containing numerous PAS-positive granules open on both sides at the base of the apical papilla (Fig. 14).

A pair of small lateral glands and several multiciliated nerve endings are observed at the level of the anterior ring in the transverse section (Fig. 15). The transverse section of the miracidium shows a hexagonal shape at the level of the anterior ring (Fig. 15) or of the ridge lacking the cilia between the second and third tiers of the epidermal plates (Fig. 16). At the level of the third tiers, a pair of the elongated excretory bladders and four groups of the cilia are observed (Fig. 17). In the transverse section of the first tiers of the plates with the short cilia, a pair of ducts stained with PAS and a duct stained with TB are observed (Fig. 18). At the level the second tiers, nine groups of the cilia, a nerve mass and flame cell are observed (Fig. 19).

Discussion

In most species of trematodes, the miracidia possess a pair of lateral glands and a large apical gland (Wright, 1971). The miracidium of *G. sturniae* possesses a large apical gland and a pair of small lateral glands similar to those of other schistosome species. Previous studies with SEM have shown that it is difficult to reveal the gland openings. On the apical papilla in *F. hepatica,* two pairs of lateral gland openings and their ducts have been described under LM or TEM by Wilson (1971) and Buzzell (1983). In SEM investigation, a pair of sucker-like lateral gland openings have been shown in *S. japonicum, S. haematobium, S. intercalatum* and *Gigantocotyle explanatum,* but no gland openings have been recognized in *S. mansoni* and *F. hepatica* (LoVerde, 1975; Scheerboom *et al.*, 1975; Køie and Frandsen, 1976; Eklu-Natey *et al.*, 1985; Dunn *et al.*, 1987). The apical gland opening of *G. sturniae* located on both lateral surface of the apical papilla is composed of numerous honeycomb pores, and are presumed to correspond to the openings on the lateral glands.

Several workers have suggested that the folds of the apical papilla serve to attach the miracidium to the body surface of snail host (Kinoti, 1971; Willson, 1971; LoVerde, 1975). Conversely, Køie and Frandsen (1976) suggest that the apical papilla is not fit for this use. In SEM investigation, the surface of the apical papilla of the schistosome miracidia is shown to be composed of elongated, branched or membraneous folds, and to forms a rosette pattern in *S. haematobium* and *S. intercalatum*, or of a honeycomb pattern at part of the apex in *S. japonicum* and *S. mansoni* (Eklu-Natey, *et al.*, 1985). The apical papilla of *G. sturniae* forms wholly a honeycomb pattern, and many pores (0.1-0.3 μm in diameter) formed by the tegumental folds are extremely smaller than those of other schistosome species. It may be unreasonable that such a structure functions as the tool for attachment to the snail, but the contents of the gland cells may be able to serve as a lubricant during the penetration process.

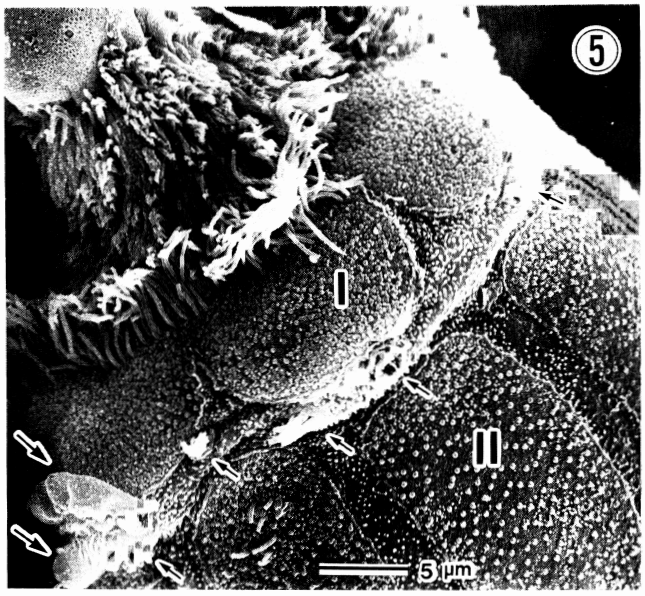
Various types of sensory organs have been observed on the intercellular ridges of some trematode species: e.g. *S. mansoni, S. haematobium* and *S. intercalatum* (Eklu-Natey, *et al.*, 1985), *S. margrebowiei* (Southgate and Knowles, 1977), *Fascioloides magna* (Coil, 1977) and *F. hepatica* (Køie, *et al.*, 1976). In many

Fig. 1. A gourd-shaped swimming miracidium of *Gigantobilharzia sturniae* with a spherical apical papilla (Ap) and the lateral papillae (Lp).

Fig. 2. A scanning electron micrograph (SEM) of *G. sturniae* miracidium, showing apical papilla (Ap) and intercellular ridge (Ir).

Fig. 3. A pair of uniciliated sensory projection (Sp) are situated at each corner of a square inscribed in a circular section of the spherical surface of the apical papilla (Ap).

Fig. 4. Secretory pores arranged in an inversed-triangle (arrows) on the cytoplasmic folds of the apical papilla having the appearance of a honeycomb pattern.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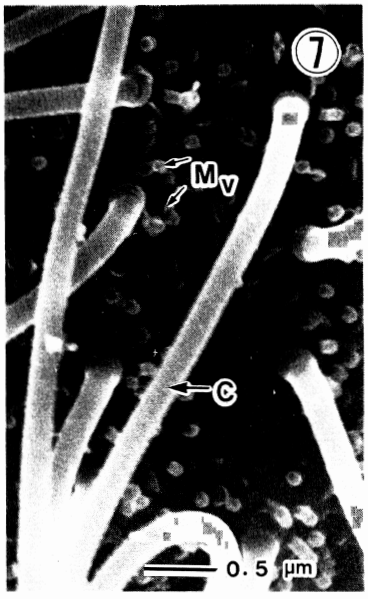
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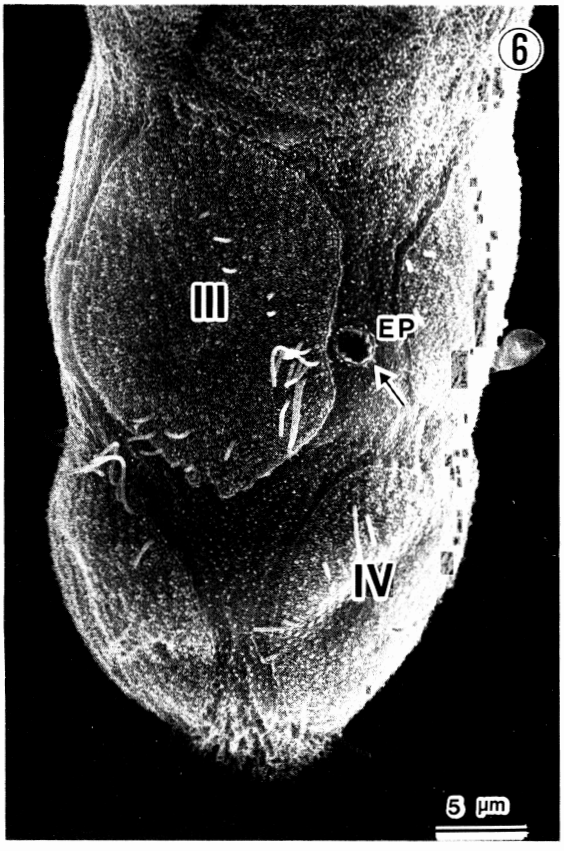
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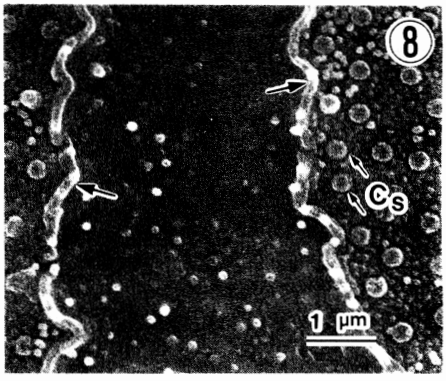
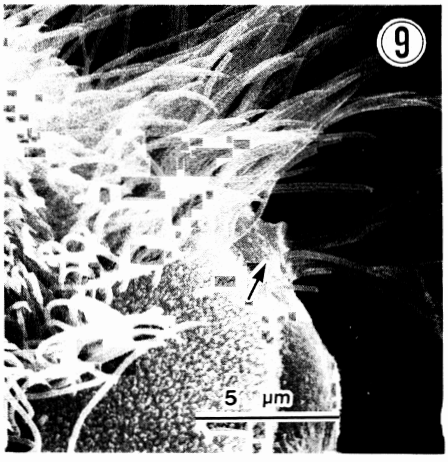

8
Cs
1 μm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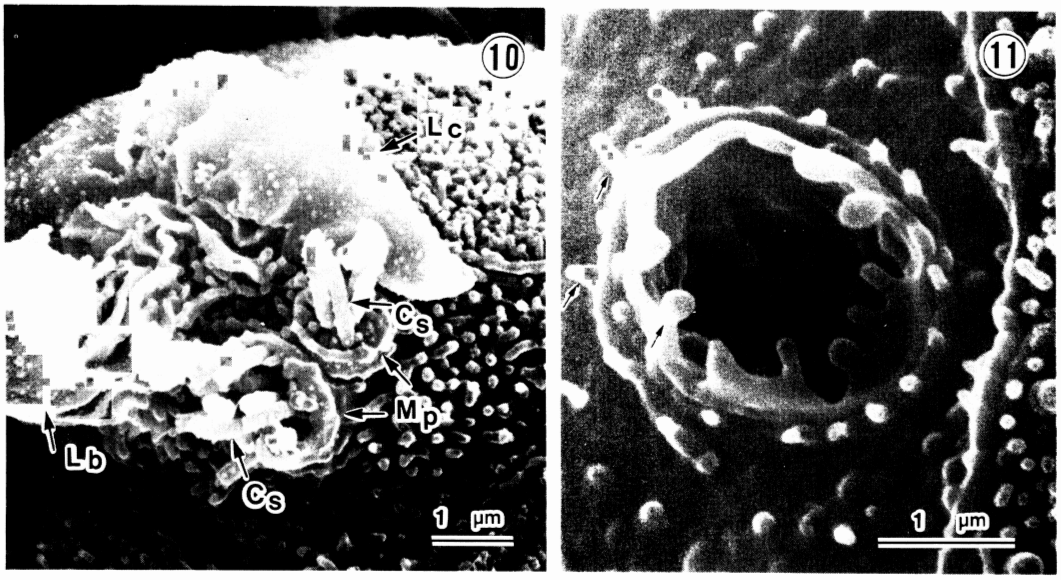


Fig. 10. A crescent lateral papilla (Lc) adjoining just above a bulbous lateral papilla (Lb) and multiciliated nerve endings (Mp) with broken cilia (Cs).

Fig. 11. An excretory pore surrounded by double circular brims of the cytoplasmic evaginations with fine microvilli (arrows).

species of trematodes, the narrow intercellular ridges form three transverse rings. The miracidium of *G. sturniae* resembles rather *S. japonicum* than the above mentioned species in the following characteristics: 1) the intercellular ridges cover a large area on the surface of the body except for the ridge between the first and second tiers of the epidermal plates and 2) the sensory organs are found only on the ridge of anterior ring.

A pair of the lateral papillae in schistosome miracidia appear to be nerve endings connecting with the central nerve mass (Køie *et al.*, 1976). On the other hand, under LM observation two pairs of lateral papillae have been observed on the miracidia of *G. sturniae* (Iwasaki, 1960), *T. brevis* (Suzuki and Kawanaka, 1980), *S. japonicum* (Ozaki, 1952) and *S. margrebowiei* (Southgate and Knowles, 1977). The lateral bulbous papillae of *G. sturniae* by SEM resemble those of *F. hepatica* (Køie *et al.*, 1976) and of schistosomes (Køie and Frandsen, 1976; Eklu -Natey *et al.* 1985). In SEM investigations of the trematode miracidia, a characteristic crescent-shaped structure has been shown only in *S. mansoni* miracidia, however, this structure also located just above the bulbous lateral papilla is explained as the ridge folds by Køie and Frand-

Fig. 5. Epidermal plates removed the cilia by an ultra-sonication, showing those of the first (I) and second (II) tiers. Lateral papillae (large arrows) and multiciliated nerve endings with broken cilia (small arrows) are seen on the transverse intercellular ridge forming the anterior ring.

Fig. 6. Posterior half of the body, showing the epidermal plates of the third (III) and fourth (IV) tiers and an excretory pore located on the longitudinal ridge between the plates of third tier (Ep).

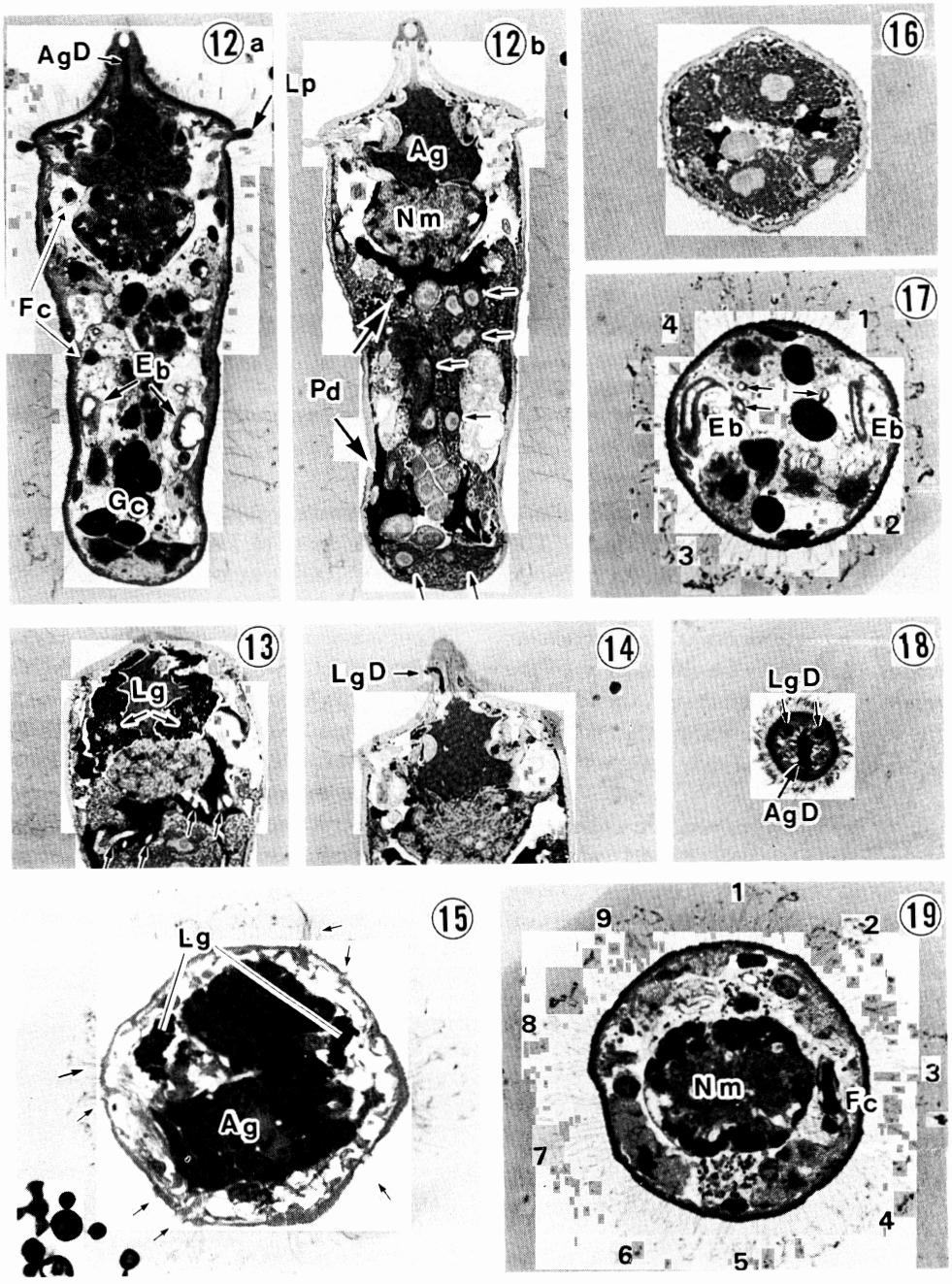
Fig. 7. Body surface of a miracidium, showing the fine microvilli (Mv) and the cilia (C) on the epidermal plate.

Fig. 8. Body surface of a miracidium from which the cilia are removed by an ultrasonication, showing the wavy evagination (large arrows) of the cytoplasmic folds separating the plates, and the cilium stubs (Cs) on the epidermal plates.

Fig. 9. A multiciliated nerve ending between the first and second tiers (arrow).

sen (1976).

The secretions of the gland cells in trematode miracidia is presumed to have adhesive or cytolytic properties (Dunn, *et al.*, 1987). Wajdi (1966) suggests that the secretions of the apical gland of *S. mansoni* help to digest the epithelial cells of the snail tissue, and that the lateral glands (adhesive glands) help the miracidium to adhere to the host tissue. Conversely, Wikel and Bogitsh (1974) report that most of the contents of the lateral penetration glands are released after penetration. In the cercaria of *G. sturniae,* the



adhesive glands are stained intensely with PAS, while the penetration glands are not (Maejima *et al.*, 1988). The apical gland in the miracidium of *S. japonicum* and also the penetration gland in the cercariae of *S. japonicum* and *S. mansoni* are stained with Arizalin S (Miura, 1955; Lewert *et al.*, 1966). From these resemblances in the histochemical natures of the gland cells in both cercarial and miracidial stages, it is reasonable to assume that the apical gland corresponds to the penetration gland, and the lateral glands to the adhesive glands.

As is generally known, the excretory bladder exists in both cercarial and adult stages of digenean trematodes, while the existence of it in any miracidia has not been recognized. The data presented here, however, show that the miracidium of *G. sturniae* possesses a pair of thick-walled excretory bladders stained intensely with TB as in the case of the cercarial stage (Maejima *et al.*, 1988). The PAS-positive deposits surrounding the cluster of the excretory ducts may be correlated more or less with the excretory functions.

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Fig. 12a. Horizontal section stained with TB, showing the apical gland duct (AgD), two flame cells (Fc), a pair of bolbous lateral papillae (Lp), a pair of the excretory bladders (Eb) and several germ cells (Gc).

Fig. 12b. The same specimen in Fig. 12a stained with PAS after washing in distilled water, showing the localities of an apical gland (Ag), a nerve mass (Nm), several nucleate cells (small arrows) scattered between the germ cells and the PAS-positive deposits (Pd).

Fig. 13. Horizontal section stained with PAS, showing a pair of lateral glands (Lg) and the excretory ducts (small arrows) adhering the PAS-positive deposits.

Fig. 14. Horizontal section stained with PAS, showing the opening of lateral gland duct (LgD) at the base of the apical papilla.

Fig. 15. Transverse section stained with TB and PAS at the level of the anterior ring, showing an apical (Ag) and two lateral (Lg) gland cells and several multiciliated nerve endings (arrows).

Fig. 16. Transverse section at the level of non-ciliated ridge between second and third tiers of the plates, showing a hexagonal-shaped body.

Fig. 17. Transverse section stained with TB at the level of the third tier of the plates, showing four zones of cilia, a pair of the elongated excretory bladders (Eb) and excretory ducts (arrows).

Fig. 18. Transverse section at the level of the first tier of the plates with the short cilia, showing a pair of lateral gland ducts (Lgd) stained with PAS and an apical gland duct (AgD) stained with TB.

Fig. 19. Transverse section stained with TB at the level of the second tier of the plates, showing nine zones of cilia, a nerve mass (Nm) and a flame cell (Fc).

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