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Scanning Electron Microscopic Observations of Flame Cell and Associated Excretory Systems in *Diphyllobothrium*

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Abstract

The terminal structures in excretory system of flatworms, namely *Diphyllobothrium nihonkaiense* and *D. cameroni*, were three-dimensionally observed.

A scanning electron microscopic observation of the "flame cells" and associated structures revealed certain characteristic components as follows: antlered processes of a flame cell, two rows of forty-five rods, internal leptotriches from the inner rods connected with a bundle of cilia from the flame cell, short external leptotriches from the outer rods leading from the infundibulum, and a few flame cells associated with the bulged joint of the primary excretory duct.

Key words: Scanning electron microscopy, Cestoda, *Diphyllobothrium*, Flame cell, Excretory system, Infundibulum

Introduction

Examination of the excretory system of parasitic flatworms with the transmission electron microscope (TEM) has been done by a number of investigators. The basic terminal structure of the excretory system is called the flame cell, which has been assumed to act as a filter for the entrance of extracellular fluid into the system. In TEM observations, the terminal excretory system is shown to be composed of an irregularly shaped uninucleate flame cell bearing a bunch of hexagonally arranged cilia as well as a filter which is the infundibular portion of the primary excretory duct. The wall of upper quarter of the infundibulum is composed of a double line of longitudinal rod structures. In general, studies thus far have shown flame cells and the infundibulum to be common structures in flatworms.

A remarkable difference in the flame cells between cestodes and trematodes appears to exist in the structure of external leptotriches which arise from the outer rods. The flame cells of both the larval and adult trematodes have internal leptotriches leading from the inner rods in addition to the previously mentioned external leptotriches (Inatomi, 1960; Senft *et al.*, 1961; Rees, 1967; Gallagher and Threadgold, 1967; Willson, 1969; Tongu *et al.*, 1970; Rohde, 1970, 1973; Ebrahimzadeh and Kraft, 1971; Bennett and Threadgold, 1973; Bennett, 1977). However, external leptotriches were not recognized in any of the previous studies dealing with the flame cells of cestodes (Race *et al.*, 1965; Bonsdorff and Telakka, 1966; Morseth, 1967; Nieland and Weinbach, 1968; Howells, 1969; Sakamoto and Sugimura, 1969; Slais *et al.*, 1971; Yamane *et al.*, 1978; Lumsden and Specian, 1980). In addition, there have been no observations of the flame cell structure using a scanning electron microscope (SEM). Thus the aim of the present paper is to ascertain whether the above mentioned difference between cestodes and trematodes shows to be true by SEM observation.

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Materials and Methods

An adult Japanese broad tapeworm, the *Diphyllobothrium nihonkaiense* Yamane *et al.* 1986, was obtained from a man in Tottori Pre-

fecture and a marine species, *D. cameroni* Rausch, 1969, was obtained from a man in the Kyushu District.

Mature segments sliced off from these materials were fixed for 4 hr at 4°C in 2.5% glutaraldehyde, after washing with distilled water. Following a washing in 0.1M phosphate buffer (pH 7.2), the specimens were dehydrated in a graded series of ethanol and then embedded in stylene. After polymerization for 24 hr at 60°C, the specimens were cracked and soaked in propylene oxide in order to dissolve the polymerized styrene. The cracked specimens were substituted by amylacetate, critical point dried, coated with gold in an ion-sputtering apparatus, and examined in a Hitachi S-450 scanning electron microscope.

Results

The flame cell was irregular in shape, and embedded in the peripheral reticular structure (Figs. 1–4). Many irregular processes were also observed on the surface of the flame cell. In addition, it was observed that the tapering ends of antler-like or arboraceous processes disappear into peripheral reticulations.

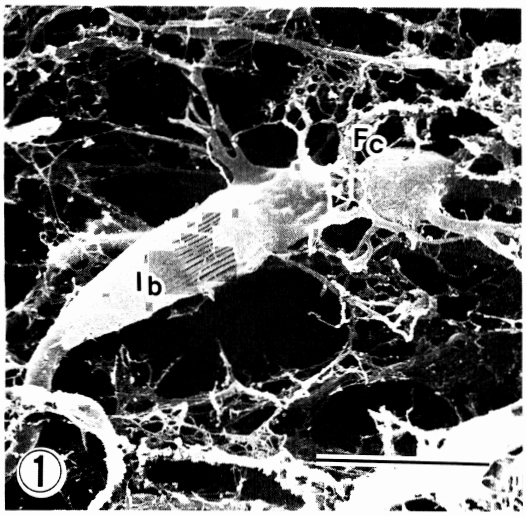
The terminal of the primary excretory duct adjoining to the flame cell was distended, forming an infundibulum which measured 5–6 μm in length by 2–3 μm in width. The wider portion of the infundibular duct was surrounded by a row of rod-like structures leading from the duct wall (Fig. 5). At the level of the rod-like structures (Figs. 6–7), the wall of infundibular chamber, housing a bundle of cilia, was composed of a double line of alternately adjoining rods (Figs. 8–12). About 45 outer rods were observed in each infundibulum.

Under SEM investigation, it was observed that the pointed ends of outer rods were connected with peripheral reticulations (Figs. 10–13, arrows). These connections were usually broken as a result of tearing out when the specimen was cracked. A few, short, secondary projections were observed on the outer rod (Fig. 13). In the longitudinally fractured section of the infundibular chamber, the following structures were clearly observed: a row of inner rods arising from the flame cell, the internal leptotriches connected on the one hand with each of the bundled cilia, and on the other with the smooth inner surface of infundibulum (Figs. 13–15).

The primary excretory duct was connected at a joint of the bulged duct (Figs. 16–17). Two or three flame cells, situated in the cortical parenchymal layer, were often grouped together (Fig. 18). The peripheral network systems were also scattered slightly outside of vitelline glands in the cortical parenchyma (Figs. 19–20). Many longitudinal peripheral ducts were observed in the sections cracked transversely (Fig. 21) or longitudinally (Fig. 22). The longitudinal duct, 10–20 μm in diameter, was connected with a number of small transverse ducts of 2–6 μm in diameter. On the inner surface of the peripheral excretory ducts, i.e., the longitudinal, transverse and primary ducts, there were numerous nodular protrusions existing in a variety of sizes and numbers (Figs. 23–26). In contrast, there were no protrusions on the inner surface of central ducts which ran longitudinally in the medullary parenchymal layer (Figs. 27–28). A diagram of the terminal excretory system of *Diphyllobothrium* is illustrated in Fig. 29.

Discussion

As is generally known, the terminal excretory system of flatworms is presumed not only to have an excretory function for waste material but also a regulating function for osmotic pressure. The whole figure of the flame cell joining to be infundibulum was visible with SEM. The antlered processes leading from the surface of the flame cell were tapered, and associated with peripheral reticulations just like numerous anchors. The joint of bulged duct was associated with two or three terminal excretory systems. These structures have yet to be described in any flatworms. Thus may be due to the difficulties involved in demonstrating a three dimensional structure by TEM only. Through TEM observation, the flame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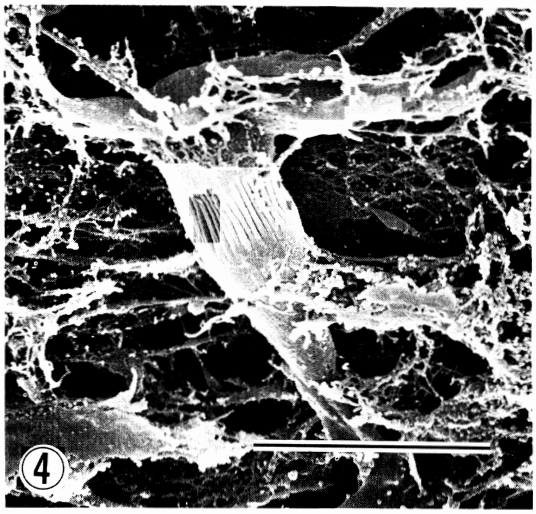
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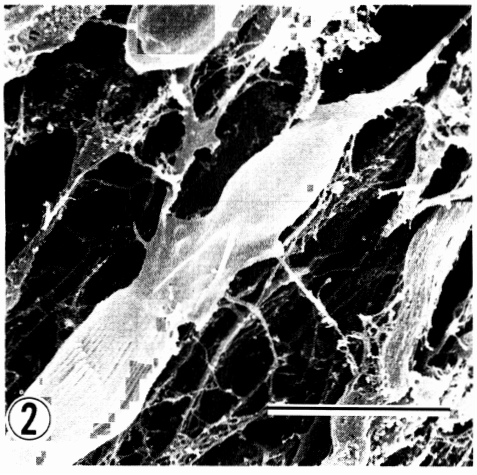
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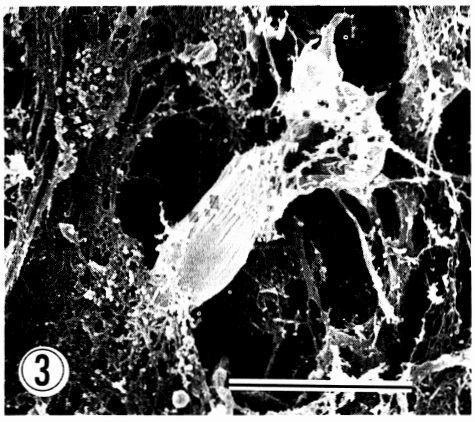
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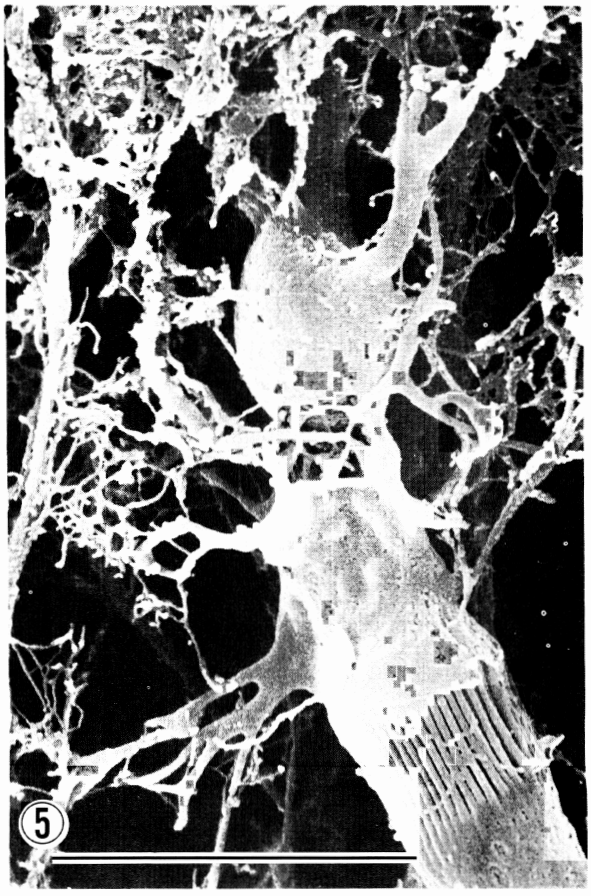
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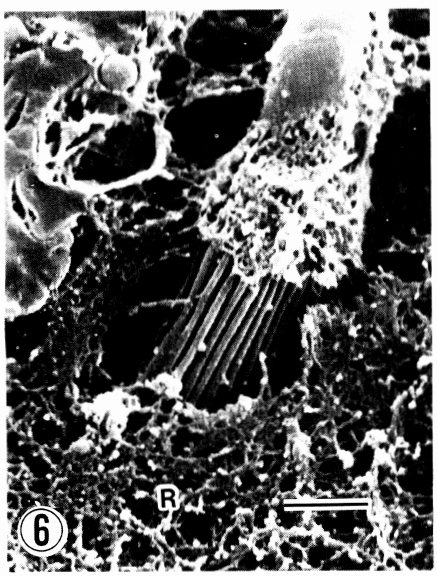
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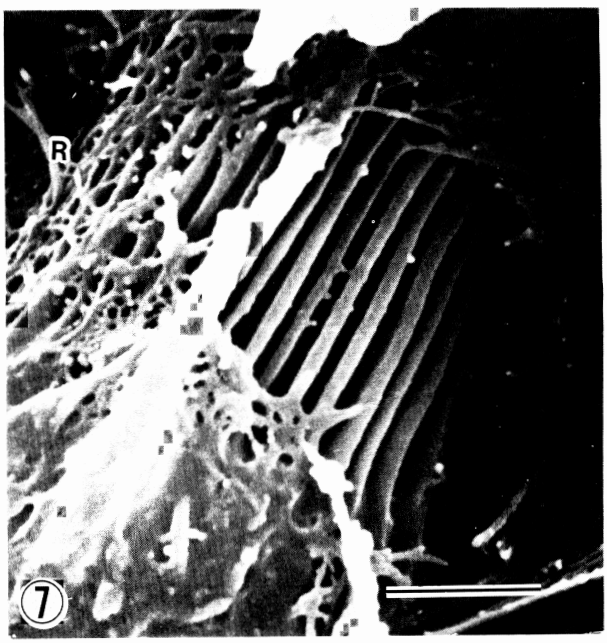
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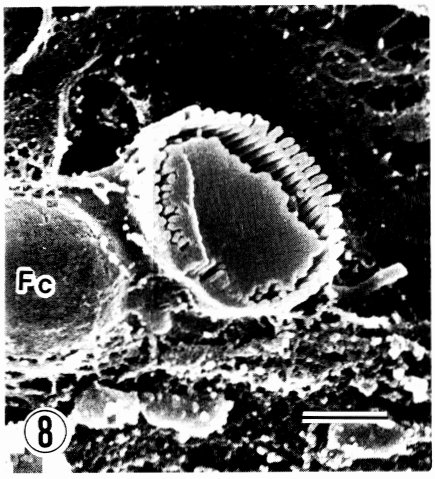
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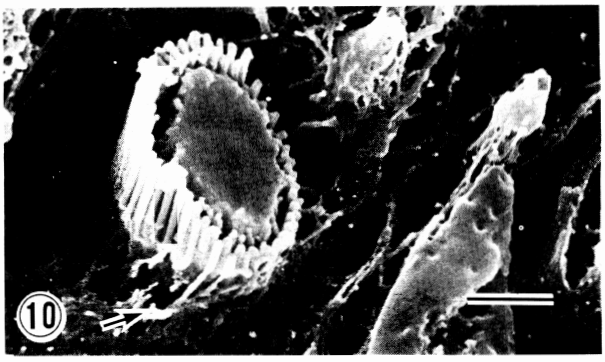
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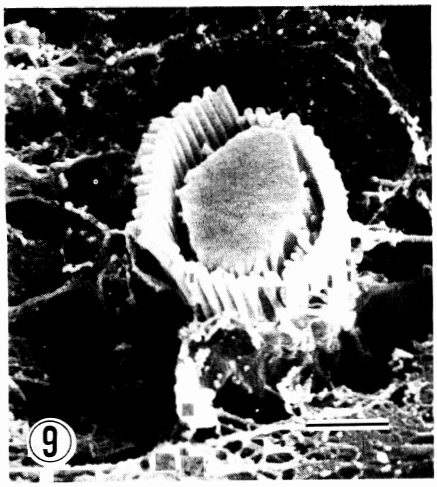
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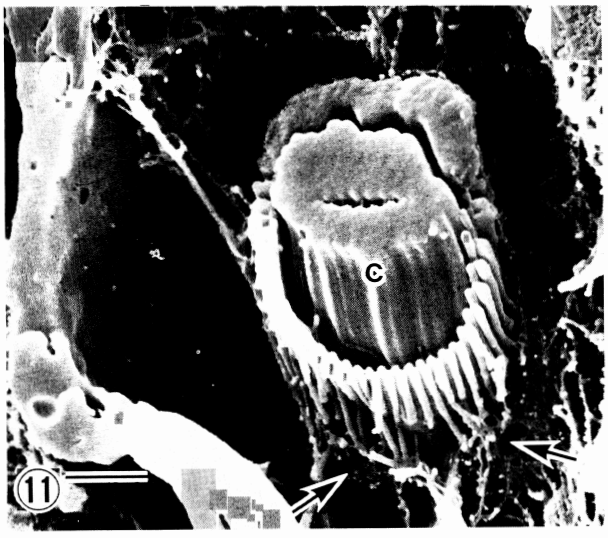
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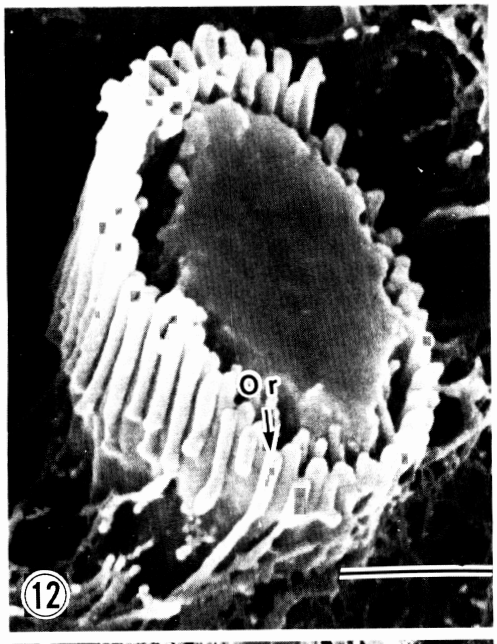
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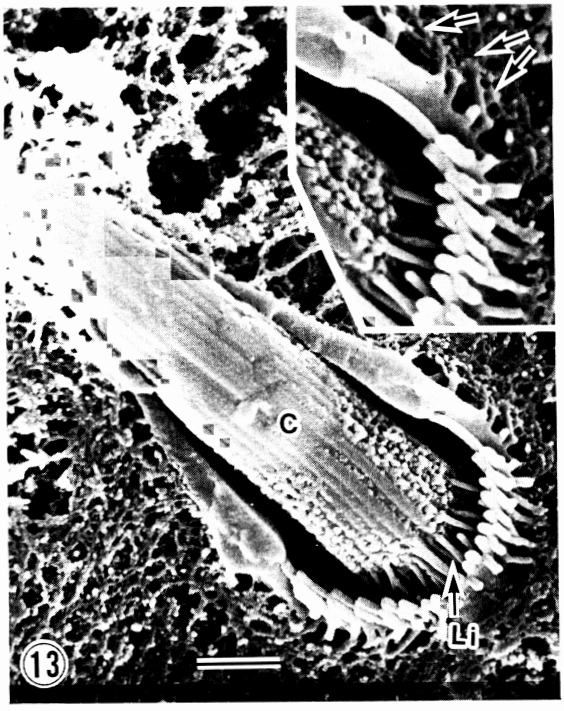
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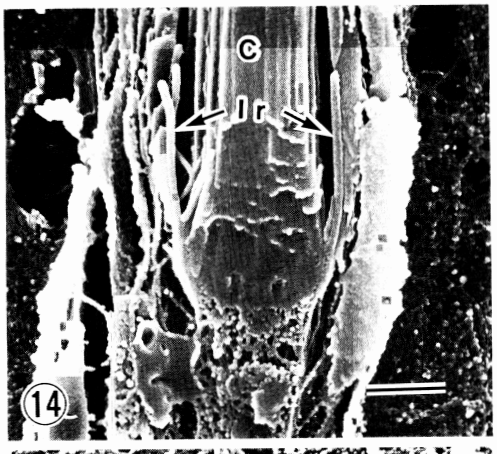
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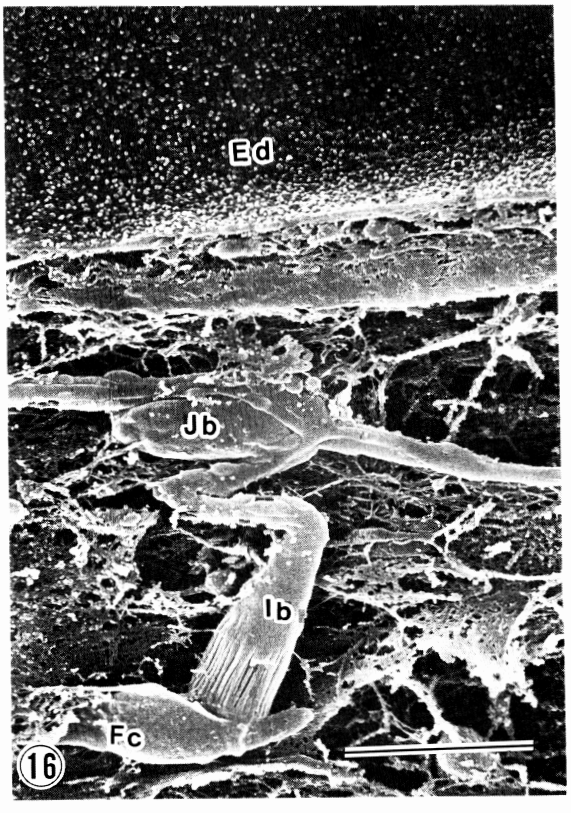
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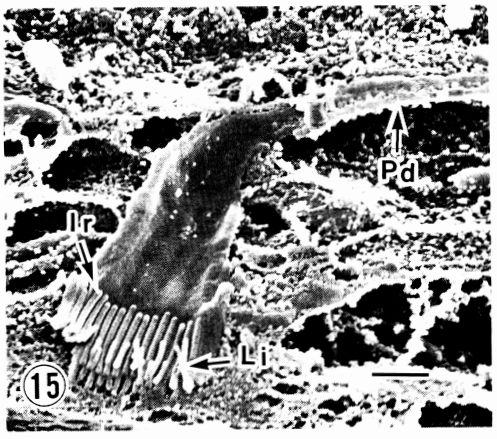
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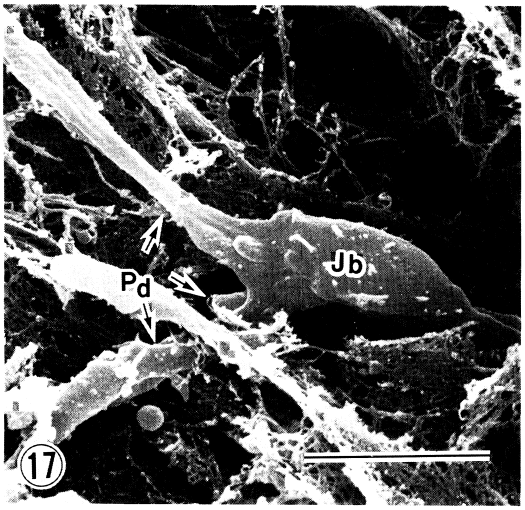
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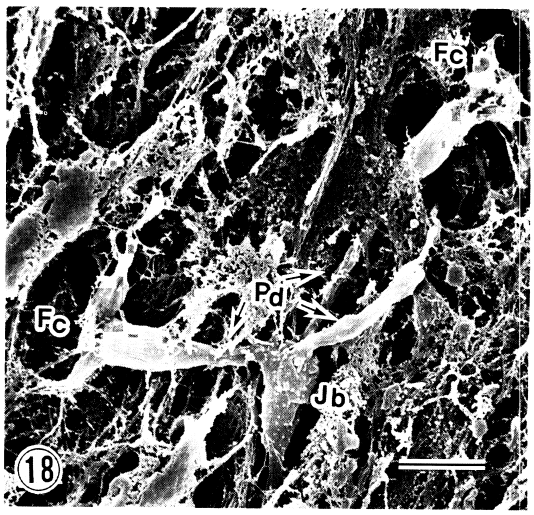
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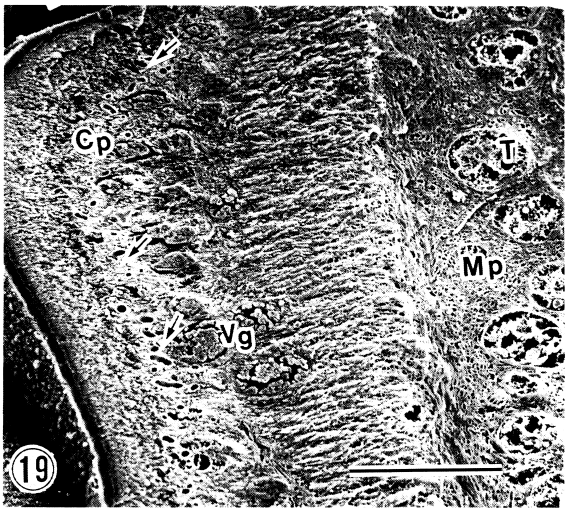
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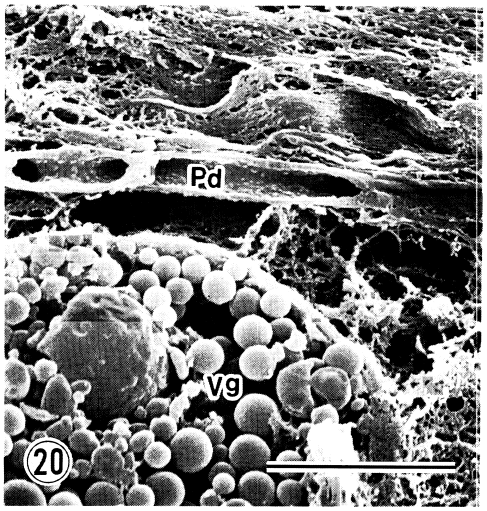
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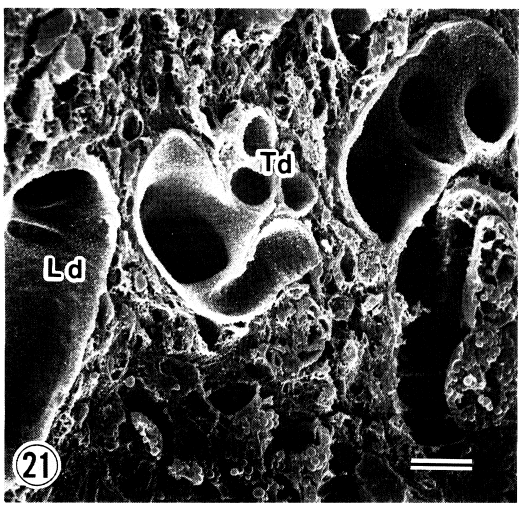
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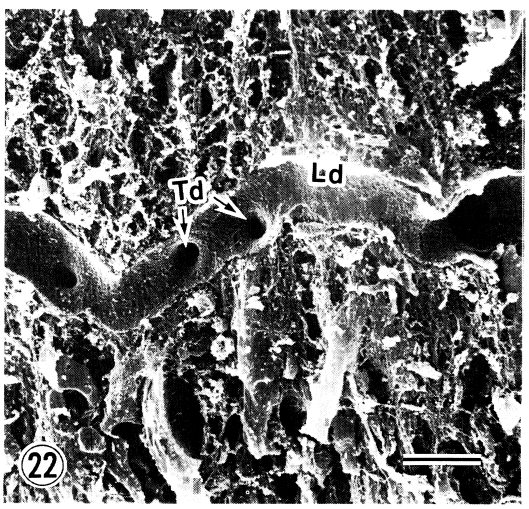
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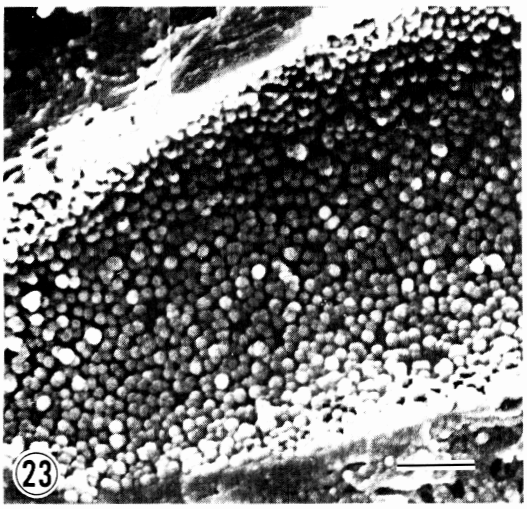
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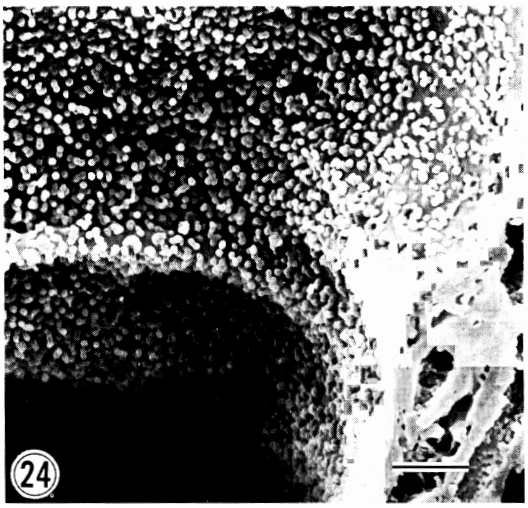
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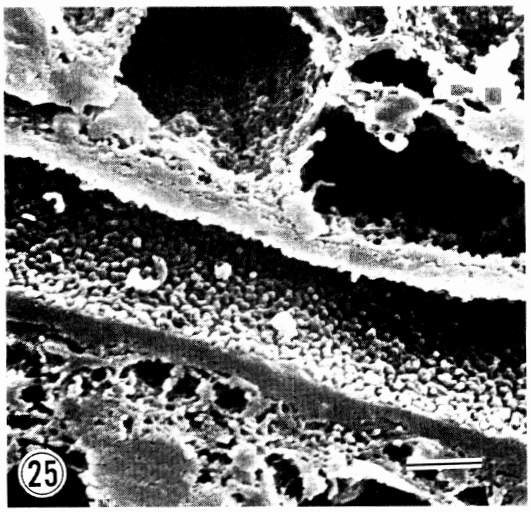
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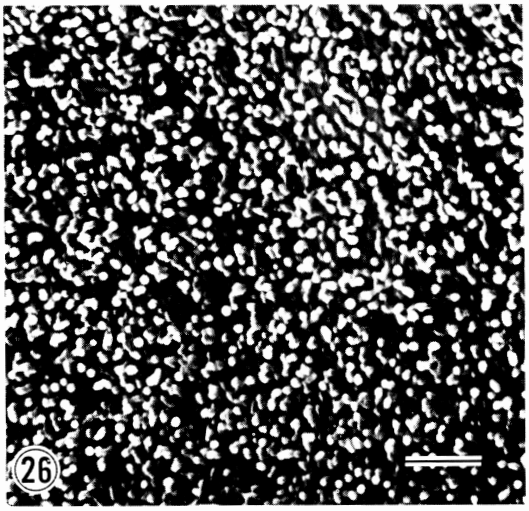
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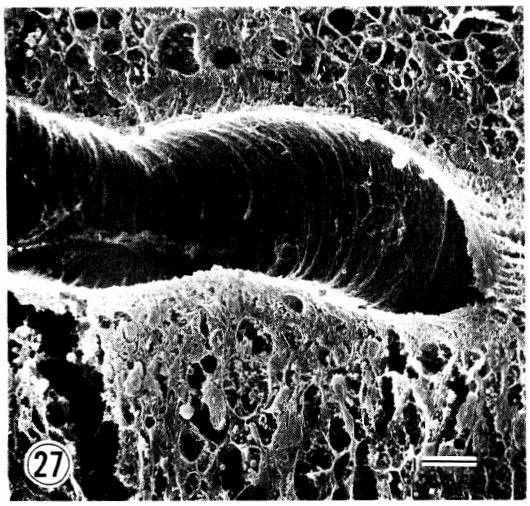
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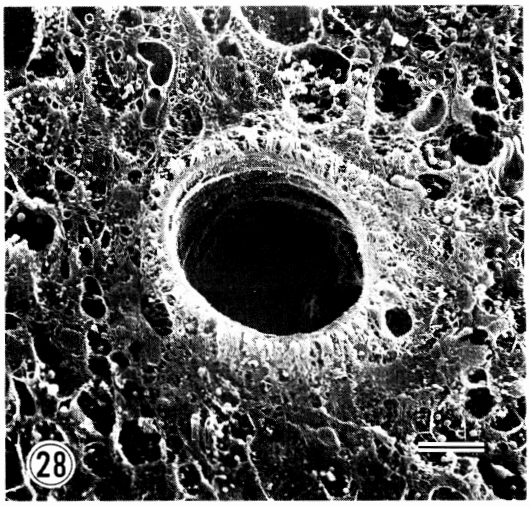
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Abbreviations in Figures

Ap : Antlered process
Cp : Cortical parenchyma
Fc : Flame cell
Ir : Inner rod
Ld : Longitudinal duct
Li : Internal leptotriches
Np : Nodular protrusion
Pd : Primary duct
T : Testis
Vg : Vitelline gland
C : Cilium
Ed : Excretory duct
Ib : Infundibulum
Jb : Joint of bulged duct
Le : External leptotriches
Mp : Medullary parenchyma
Or : Outer rod
R : Peripheral reticulation
Td : Transverse duct

Figs. 1–4. Terminal excretory system embedded in reticulations, showing an elongated infundibulum attached to an irregularly shaped flame cell. (*D. cameroni*) Bar = 5 μm.

Fig. 5. Enlargement of a flame cell in Figure 1, showing antlered or arboraceous processes, which gradually tapered towards the end. (*D. cameroni*) Bar = 5 μm.

Figs. 6–7. External appearance of a filtration chamber. Slit-like openings, which were constituted by rod-structures, were embedded in reticulations. (*D. nihonkaiense*) Bar = 1 μm.

Figs. 8–11. Transverse sections of the infundibulum at the level of rod-structures, showing two rows of rods as well as the pointed end of outer rods which connected with reticulations (arrows). (*D. cameroni*; Fig. 8, *D. nihonkaiense*; Figs. 9–11) Bar = 1 μm.

Fig. 12. Enlargement of the infundibulum in Figure 10, showing 45 rods. (*D. nihonkaiense*) Bar = 1 μm.

Figs. 13–15. Longitudinal section of the infundibulum with tightly packed cilia, showing thick wall, internal leptotriches and an inner rod of a flame cell. Short leptotriches from an outer rod is enlarged in the inset (arrows). (*D. nihonkaiense*) Bar = 1 μm.

Fig. 15. Inside of the infundibulum which was connected with primary duct, showed a smooth surface, a row of inner rods and internal leptotriches. (*D. cameroni*) Bar = 1 μm.

Figs. 16–17. Joint of a bulged duct associated with primary ducts. (*D. cameroni*) Bar = 5 μm.

Fig. 18. Joint associated with grouped flame cells. (*D. cameroni*) Bar = 5 μm.

Fig. 19. Transverse section of a mature segment, showing peripheral network of the excretory system in cortical parenchyma (arrows). (*D. nihonkaiense*) Bar = 200 μm.

Fig. 20. Primary duct near the vitelline gland. (*D. cameroni*) Bar = 5 μm.

Fig. 21. Transverse section of a mature segment, showing longitudinal and transverse excretory ducts in the cortical parenchyma. (*D. nihonkaiense*) Bar = 5 μm.

Fig. 22. Sagittal section of a mature segment, showing connecting pores of the transverse ducts on the wall of the longitudinal excretory duct. (*D. nihonkaiense*) Bar = 5 μm.

Figs. 23–24. Nodular protrusions lining the inner surface of transverse (Fig. 23) and longitudinal ducts (Fig. 24). (*D. nihonkaiense*) Bar = 1 μm.

Figs. 25–26. Nodular protrusions lining the inner surface of transverse (Fig. 25) and longitudinal ducts (Fig. 26). (*D. cameroni*) Bar = 1 μm.

Fig. 27. Sagittal section of a mature segment, showing the central excretory duct in medullary parenchyma. (*D. nihonkaiense*) Bar = 10 μm.

Fig. 28. Transverse section of a mature segment, showing the smooth inner surface of a central excretory duct in medullary parenchyma. (*D. nihonkaiense*) Bar = 10 μm.

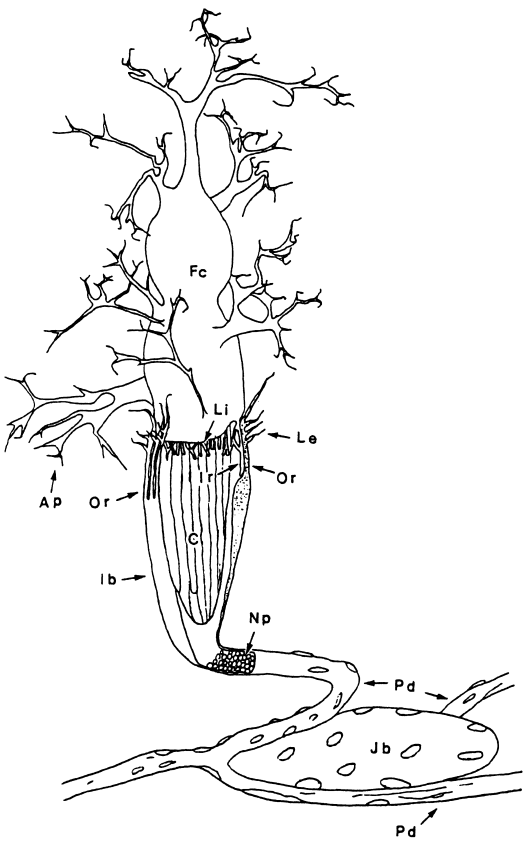

Fig. 29. Diagram of a terminal excretory system, showing flame cell, infundibulum, primary duct and joint of bulged duct.

cells have been expressed, in a variety of ways by previous authors, for various flatworms as follows: "many long and irregularly branched processes" in *Polystomoides renschi* (monogenea) by Rohde (1973), "elongated projection of cytoplasm" in *Multiceps seriaris* by Race *et al.* (1965), in *Multicotyle purvisi* (aspidogastrid) by Rohde (1970) and "deeply enfolded surface" in *Moniezia expansa* by Howells (1969). From these observations, it is natural to assume that these processes arise from the flame cells in many flatworms.

With TEM, about 39 rods have been recognized on the infundibulum of *Hymenolepis diminuta* by Lumsden and Specian (1980), and of *Fasciola hepatica* by Bennett and Threadgold, (1973). With SEM, however we recognized 45 rods on the infundibulum of *D. nihonkaiense.*

Howells (1969) suggested that the inwardly directed leptotriches might help maintain a clearance between the flame cell and the finger-like rods. Wilson (1969) suggested that the external leptotriches could perhaps serve to anchor the flame cell. In the area, where the cilia emerge from the flame cell body, the internal leptotriches and the cilia were certainly interconnected even though they both originate from the flame cell. Although the elongated external leptotriches observed in trematodes have not been demonstrated in cestodes, the short projections arising from its outer rods were reported in *H. diminuta* by Lumsden and Specian (1980) and in *Cysticercus bovis* by Slais *et al.* (1971). These projections, which may correspond to the external leptotriches in trematodes, were connected with the peripheral reticulations. The external leptotriches originating from the infundibulum may actually help to maintain the terminal excretory system. The antlered processes arising from a flame cell may also act to anchor it when extracellular fluid enters into the infundibulum from the openings between the separated double rows of rods.

The flame cell and the infundibulum of flatworms examined thus far seem to be similar in structure aside from some minor differences, such as the structure of the external leptotriches. However, one consistent difference between them appears to be the structure of the excretory duct. In trematodes, the inner surfaces of the ducts have cilia or lamellar structure arising from their walls (Senft *et al.*, 1961; Gallagher and Threadgold, 1967; Wilson, 1969; Powell, 1972; Bennett and Threadgold, 1973; Rohde, 1973; Bennett, 1977).

On the other hand, the nodular particles, which line the inner surface of the ducts, have been recognized in previously examined cestodes (Morseth, 1967; Baron, 1968; Nieland and Weinbach, 1968; Howells, 1968; Slais *et al.*, 1971; Dougherty *et al.*, 1975; Yamane *et al.*, 1978; Lumsden and Specian, 1980). Baron (1968) suggested that the purpose of the internal nodules in the ducts of *Cysticercus longicollis* was to ex-

crete other waste materials besides water. Yamane *et al.* (1978), showing the ultrastructure of *Spirometra erinacei,* described that the granular substances were vesicles produced by the excretory and secretory functions of the duct wall. However the nodular protrusions on the surface of peripheral excretory ducts many vary in sizes at any given place. In addition, the inner surfaces of the two central longitudinal ducts do not have any projections. It is difficult to justify such substances with these suggested functions.

Our scanning electron microscopic examinations did not reveal any differences between the structures of the excretory systems of *D. nihonkaiense* and *D. cameroni.*

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