〔Jap. J. Parasit., Vol. 31. No. 2, 89-97, April, 1982〕

The Ultrastructural Study of the Excretory Canal of the Cestode, *Diphyllobothrium latum*

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(Received for publication; December 4, 1981)

Key words: cestode, *Diphyllobothrium latum*, excretory canal, exocytotic activity, functional morphology

Introduction

Recently many workers have accumulated special attention to the structure and function of the tegument of cestodes which lack digestive tracts, yet have shown little interest in the excretory system of cestodes. The excretory system is often referred to as "osmoregulatory" or "protonephridial" system, and it has been presumed that the system might serve for excretion or osmoregulation (Smyth, 1969; Wilson and Webster, 1974; Schmidt and Roberts, 1977).

The functions of the excretory system may possibly include active transport of excretory wastes, resorption of substances such as ions from the excretory fluid (Baron, 1968; Webster and Wilson, 1970; Slais *et al.*, 1971; Webster, 1972; Swiderski *et al.*, 1975; Parshad and Guraya, 1977).

Meanwhile, the functional morphology of the duct wall of excretory system has rarely been documented. It is still under debate whether surface projections on the wall are secreted substances or microvilli (Morseth, 1967; Dougherty *et al.*, 1975; Yamane *et al.*, 1978). Some investigators described the morphological similarity of the particles on the duct wall to the C-type virus, relating the viral infection to the abnormal growth pattern of proliferating *Sparganum*, but the identity has not yet been proved (Mueller and Strano, 1974; Daly *et al.*, 1975; Dougherty *et al.*, 1975).

The purpose of the present paper is to determine the nature of granular substances on the duct wall and to define the function of the superficial cytoplasmic layer (duct wall) of the excretory duct of *Diphyllobothrium latum* with scanning and transmission electron microscopes.

Materials and Methods

Adult worm of *D. latum* was obtained from a man who spontaneously evacuated the strobilae. Fresh material was immersed in physiological saline, and dissected into small pieces. A few matured proglottids were fixed in 5% glutaraldehyde for 2 hrs, postfixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) for 4 hrs at 4 C, dehydrated in ethanol series, and finally embedded in styrene. After polymerization, the specimens were cracked under a stereoscope to observe the internal structure of the excretory system following the method of Tanaka *et al.* (1974). Some of the materials

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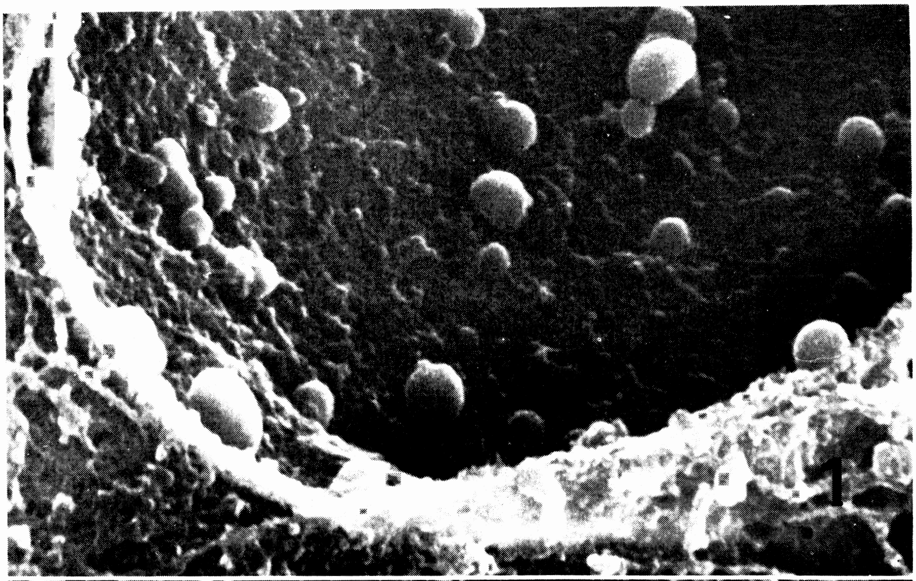
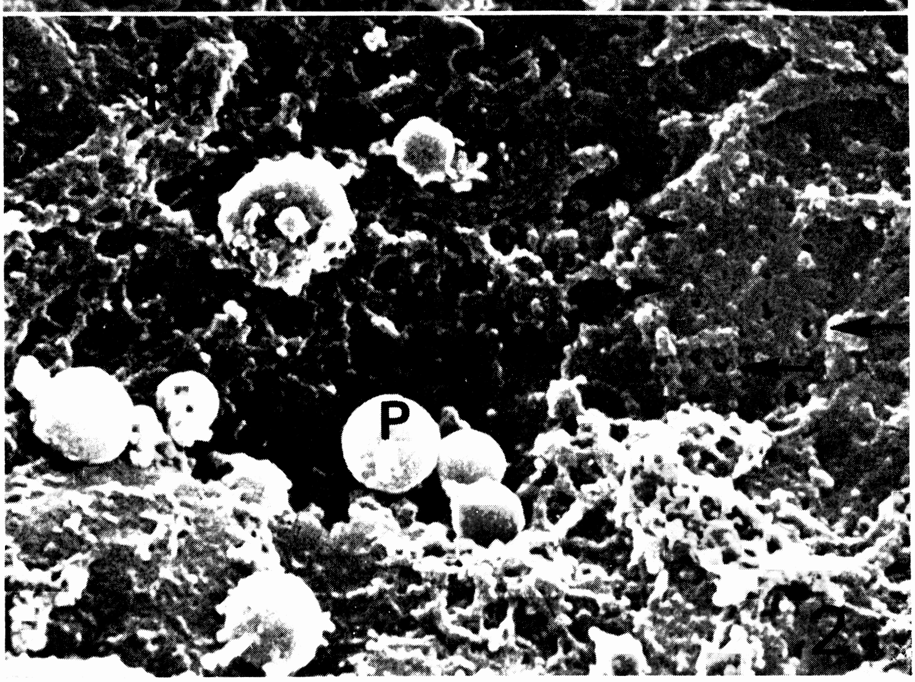
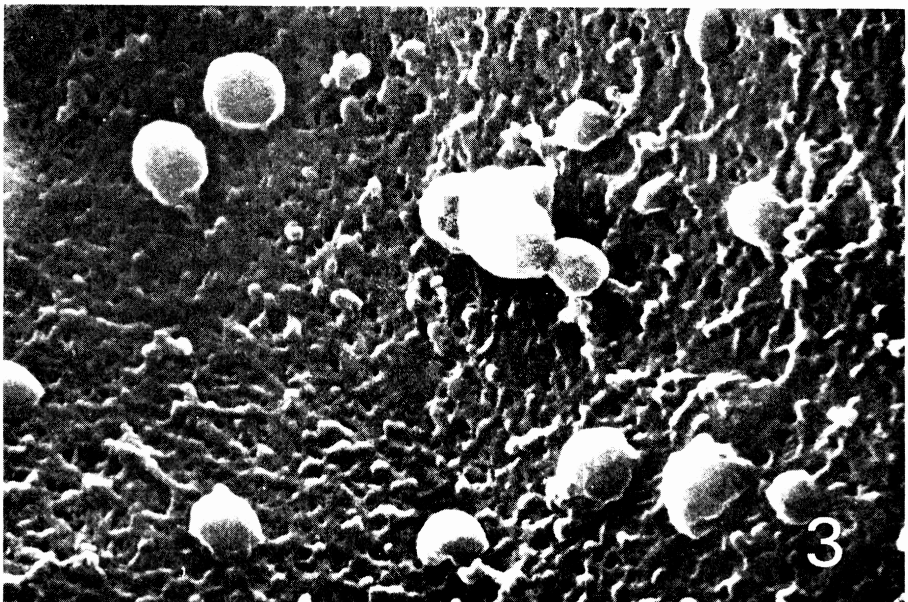




Explanation of Figures

Fig. 1 Scanning electron micrograph (SEM) of cross fracture of the excretory duct showing the various-sized particles on the wall. (×42,000).

Fig. 2 SEM micrograph of duct wall showing round particles (P) and the pseudopodia-like cytoplasmic projections (PR). Note the bleb-like protuberances (arrowheads) and minute pores (arrows). (×52,000).



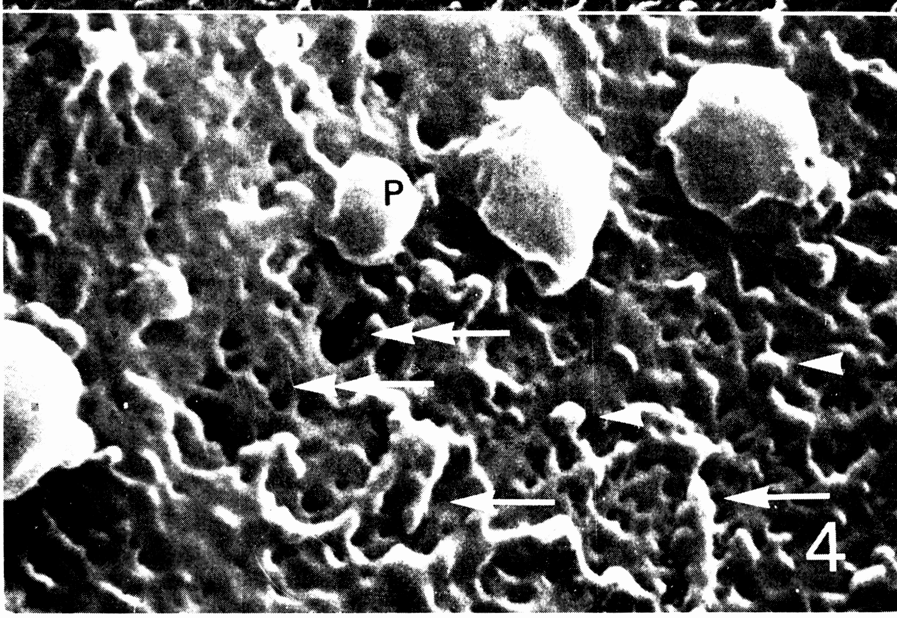


Fig. 3 SEM micrograph of the duct wall at a higher magnification showing many particles on the superficial cytoplasmic membrane. (×54,000).

Fig. 4 SEM micrograph of the duct wall at a higher magnification showing secreted particles (P), pseudopodia-like cytoplasmic projections (arrows), bleb-like protuberances (arrowheads) and minute pores (double arrows). (×108,000).

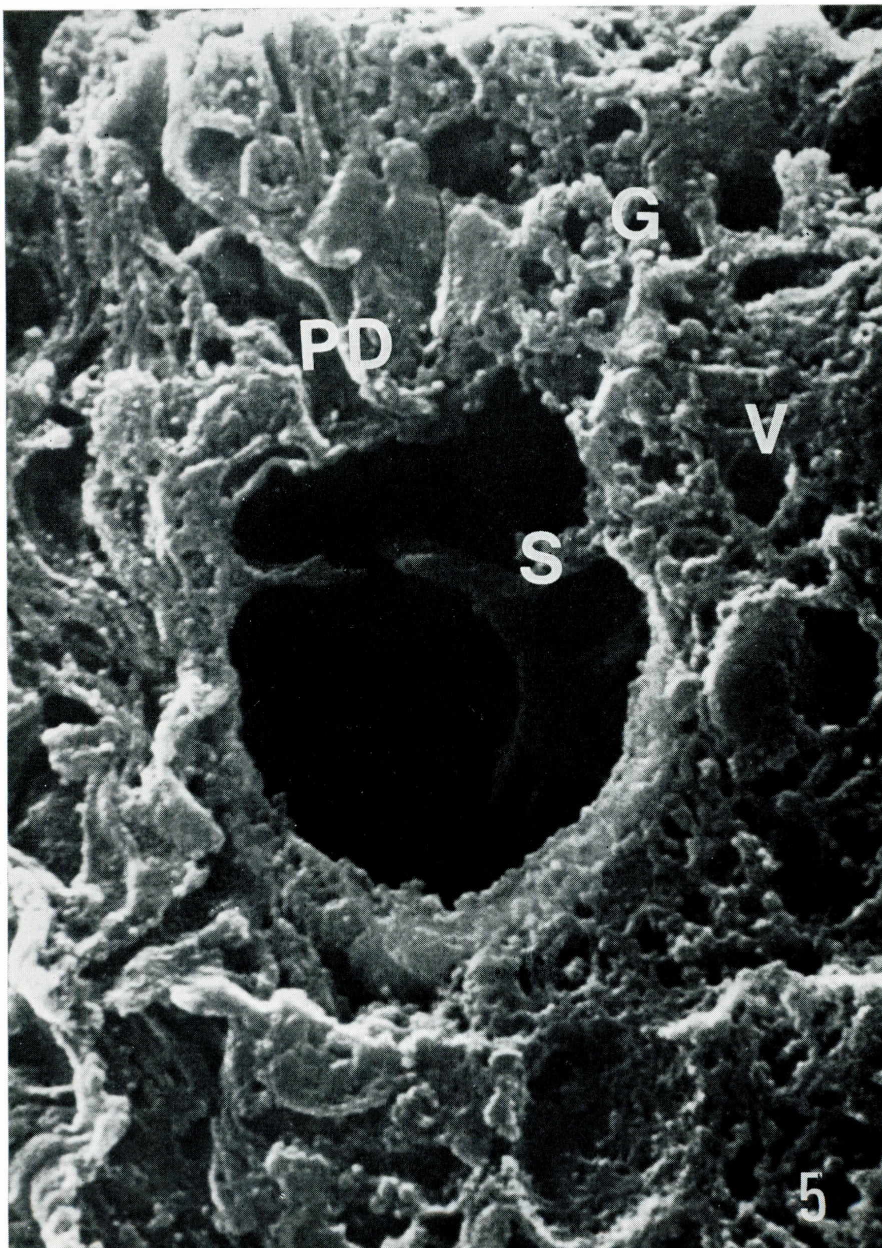
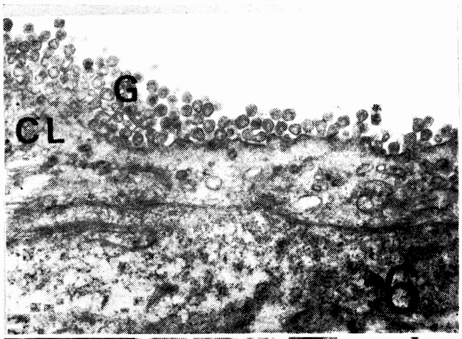
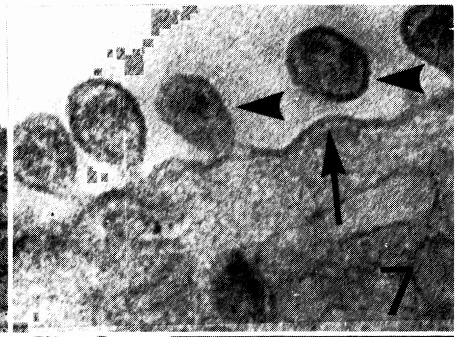
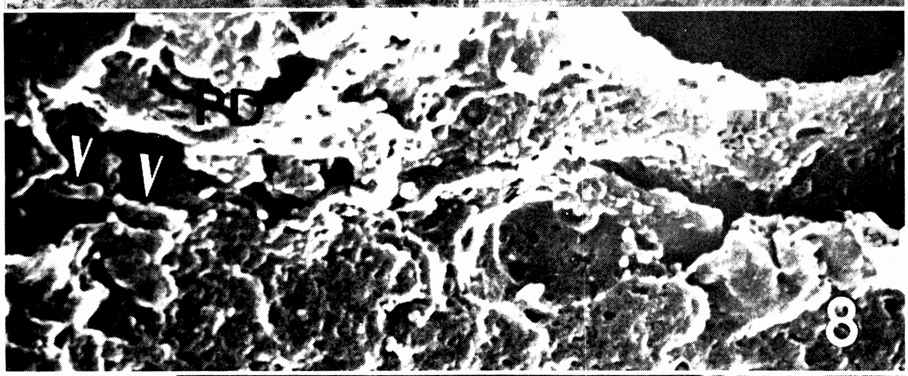


Fig. 5 SEM micrograph of the cross fracture of the excretory duct with the septa (S) in the lumen and the primary excretory duct (PD). The vacuoles (V) and many glycogen granules (G) distributed around the duct. (×11,000).







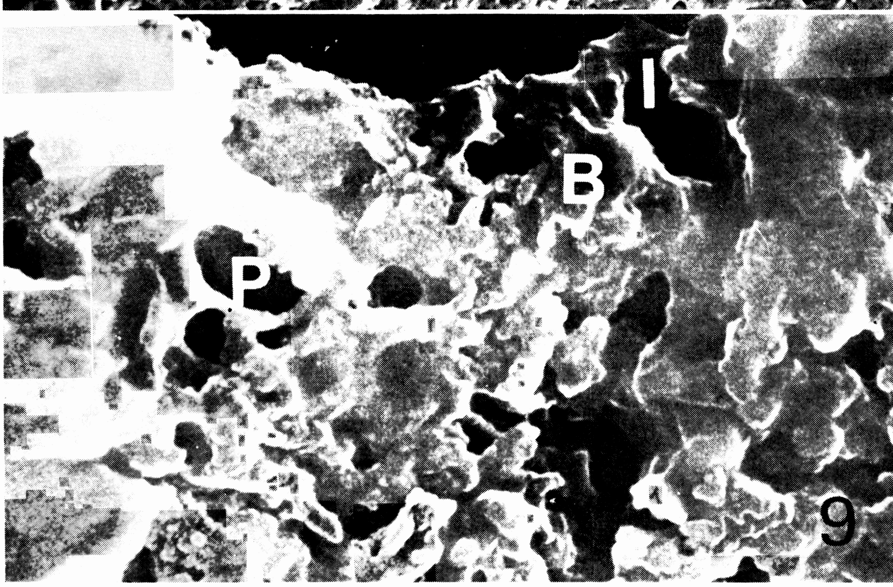


Fig. 6 Transmission electron micrograph (TEM) of the superficial cytoplasmic layer (CL) of the excretory duct showing the conglomeration of numerous globules (G). (×12,000).

Fig. 7 TEM micrograph of globules (arrowheads) budding from the surface of the cytoplasmic membrane (arrow). (×56,000).

Fig. 8 SEM micrograph of the cross fracture of the wall of the excretory duct, showing the porous structure which is similar to the tegument. A part of the primary excretory (PD) showed cytoplasmic projections (arrowheads) lining on the duct wall. (×17,500).

Fig. 9 Higher magnification of the cross fracture of the superficial cytoplasmic layer of the excretory duct, showing pores (P), invaginations (I) and bleb-like protuberances (B). (×32,000).

were freeze-fractured, then dried in a critical point drying apparatus, immediately coated with Pt-Pd alloy, and examined under a MSM-V and a HFS-2ST scanning electron microscope (SEM).

For transmission electron microscopy (TEM) some pieces of specimens were embedded in Epon 812. Ultrathin sections were cut on a Sorvall MT-II B ultramicrotome, were double-stained with uranyl acetate and lead citrate, and examined under a Hitachi HU-12A transmission electron microscope operated at an accelerating voltage of 70 kV.

Results

Many round particles, 0.3–1.0 μm in diameter, were observed on the superficial cytoplasmic layer (duct wall) of the excretory duct in matured proglottids of *Diphyllobothrium latum*. The surface of particles was relatively smooth (Fig. 1).

The bleb-like protuberances were budding just from the cytoplasmic membrane (Fig. 2, arrowheads), and small pores on the superficial layer of the excretory duct (Fig. 2, arrows) showed a sieve-like pattern. The round particles and the pseudopodia-like projections were also observed on the surface (Fig. 2). A higher magnification showed that the particles had some connection with pores on the duct wall (Fig. 3). Many minute pores of various sizes and irregular forms (Fig. 4, double arrows) were found around the particles. Pseudopodia-like projections (Fig. 4, arrowheads) were budding from the marginal part of the pores. The superficial cytoplasmic layer showed a wrinkled, waved pattern with numerous cytoplasmic projections (Fig. 4, arrows).

The cross fracture of the excretory duct showed that the thin septa separated the lumen of the duct. The wall of the excretory duct was about 6–10 μm in thickness. The vacuoles and many glycogen granules were scattered around the duct. The primary excretory canal joined to the secondary excretory duct in the cross fracture (Fig. 5).

TEM micrographs revealed that numerous globules were observed on the superficial cytoplasmic layer of the excretory duct (Fig. 6). These globules (Fig. 7, arrowheads) appeared quite different from the typical tubular microvilli in structure and occurred from the superficial cytoplasmic membrane (Fig. 7, arrow) showing the budding or the exocytotic activity at a higher magnification.

Cross fracture of the duct wall showed many minute pores or vacuoles distributed in the superficial cytoplasmic duct wall (Fig. 8). Cytoplasmic projections (Fig. 8, arrowheads), small granules attached on their tips, developed especially on the wall of the primary excretory duct and showed microvillous appearance.

A higher magnification showed many invaginations, pores and protuberances in the cross fracture of the superficial cytoplasmic layer of the excretory duct (Fig. 9).

Discussion

The excretory system of *Diphyllobothrium latum* is similar to those of the other cestodes in the fundamental structure. A complex network of the system is composed of flame cells and primary, secondary and collecting excretory canals. Although the structure of the excretory system has been investigated by many workers (Smyth, 1969; Wilson and Webster, 1974; Schmidt and Roberts, 1977), the physiology and the functional morphology of the system have not yet been completely defined.

Many observations have been made on the ultrastructure of the duct wall, which is suggestive of metabolic activities, but it is still in controversy whether particles on the duct wall are microvilli or secreted globules. Moreover, the function and the surface structure of the superficial cytoplasmic layer anucleate lining the duct wall is still debatable.

One interpretation of the microvillous structure on the duct wall is that the increased surface area may be related to the reabsorption of solutes from the lumen or that the microvilli may assist the flow of fluid towards the openings of the excretory canal in order to facilitate the transport of waste materials (Wilson and Webster, 1974). The resorption of glucose, lactate and urea from the fluid in the excretory duct of *Hymenolepis diminuta* has been demonstrated (Webster, 1972).

There is another interpretation of the surface structure of the duct wall; typical bleb-like projections on the inner surface of the ducts are not microvilli, but seem to be a process of secernent. Baron (1968) and Slais *et al.* (1971) suggested that the ducts take an active part in the excretion of waste materials other than water. Howells (1969) also postulated that the protonephridial canals of *Moniezia expansa* might function not only in secretion but also in reabsorption. The existence of phosphatases in the wall of excretory canals, which may be connected with selective resorption and excretory function, is simultaneously reported (Erasmus, 1957; Threadgold, 1968).

The present study revealed that the particles on the duct wall were secreted from the superficial cytoplasmic layer. The so-called microvillous projections were not microvilli in the strict sense but might be protoplasmic projections which often occur in the process of the budding or the exocytotic secretion. Pseudopodia-like projections and small pores may take part in the active secretion, and transport of materials into the canals, or selective resorption from the fluid in the canal. The cross fracture of the cytoplasmic layer of the duct wall also revealed complicated structures of this layer made of bulging projections, bleb-like protrusions and invaginations, suggesting a kind of the endocytotic and/or exocytotic activities in connection with secretive and/or reabsorptive functions of this layer.

Concerning the absorptive mechanisms of the excretory canals, Webster (1972) suggested a filtration-resorption mechanism in the protonephridial canals of *Hymenolepis diminuta*. Wilson (1967) also pointed that the flame cell was a site of filtration. Howells (1969) described that the ration of sodium to potassium in the canal fluid of *Moniezia expansa* was indicative of an intercellular origin of the fluid, and that this suggests the function of the filtration of the duct wall.

Nevertheless, an active exocytotic activity or buddings on the duct wall observed in the present study. Available evidence may indicate that the functions of the duct wall differ at each part of the excretory system: The flame cells may take part in the filtration mechanism to absorb water and ion, and serve for osmoregulatory function. The cilia of the flame cell presumably provide some motive force to the fluid in the canals. Meanwhile the duct wall may take share in the endocytotic and/or exocytotic activities of macromolecules and serve for secretive and/or reabsorptive functions. The evidence of the synthesis of macromolecules in the cells of the duct wall and their secretion into the duct lumen (Wilson and Webster, 1974) may give another support to this hypothesis.

Parshad and Guraya (1977) proved that the ingredients accumulated in the lipid around excretory canals were triglyceride, phospholipid and lipoprotein. Lipid storages and calcaneous corpuscles are often found in and around the duct wall (Yamane *et al.*, 1978; Makino *et al.*, 1981). Von Brand *et al.* (1960) suggested that the concretion material in the excretory canal contain calcium salts, proteins, mucopolysaccharide, glycogen and lipid. We must wait further studies to define the relationship between the exocytotic activity of the superficial cytoplasmic layer of the duct wall and the concretion of formation in the excretory canal.

Nieland and Weinbach (1968) suggested that the bladder of *Cysticercus fasciolaris* might function in the nutrients supply of the developing *Cysticercus*, and that the net-work of excretory ducts might play a role in the transport of these nutritive substances to the developing strobila. Particles and pseudopodia-like projections from the duct wall as the result of the endocytotic and/or exocytotic activities were demonstrated more often on the duct wall of the plerocercoids and of the neck region of adult worms, where the nutrients supply are most required for development, than in the matured proglottids of *Spirometra erinacei* (Yamane *et al.*, 1978). These suggested that the excretory system might be related not only with the transportation of excretory wastes, but also with the development of the worm.

Summary

The excretory canal of adult worms of *Diphyllobothrium latum* was studied by using transmission and scanning electron microscopy. Special attention was paid to the superficial structure of the excretory ducts with reference to functional morphology. Numerous particles and projections on the wall of excretory ducts were found and they were probably produced as the results of a kind of an active exocytotic activity in the superficial cytoplasmic layer of the duct wall. The significance of the exocytotic phenomenon was discussed.

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広節裂頭条虫（*Diphyllobothrium latum*）の排泄管の微細構造に関する研究

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広節裂頭条虫成虫の排泄管壁表面構造および断面構造を走査電子顕微鏡および透過型電子顕微鏡を用いて観察した．管壁表面には，従来報告されていた微絨毛はみられず，微細な顆粒，出芽状の原形質突起，微細な小孔がみられた．これらの特徴的構造は，管壁表面の原形質層の活溌な exocytosis の過程あるいは結果と考えられる．この exocytosis 現象と条虫の排泄管の機能との関連性について，機能形態学の立場から考察した．