[Jap. J. Parasit., Vol. 31, No. 1, 33-46, Feb., 1982]

Ultrastructure of the Tegument of *Diphyllobothrium latum* by Scanning Electron Microscopy

YUSUKE YAMANE,* AKIO NAKAGAWA,* YUMIKO MAKINO,*
SEIICHI YAZAKI† AND SOJI FUKUMOTO†

(Received for publication; November 9, 1981)

Key words: cestode, *Diphyllobothrium latum*, ultrastructure, SEM, tegument

Introduction

The function of the tegument of cestodes that have no digestive tracts has aroused increasing attentions of parasitologists. Literatures concerning with the fine structure of cestode tegument were reviewed by Lee (1966, 1972), Smyth (1969), Pappas and Read (1975), and Lumsden (1975a).

Many studies have been carried out by using transmission electron microscopy, and scanning electron microscopy has recently come to be used effectively for the study of morphology of the cestode tegument (Berger and Mettrick, 1971; Ubelaker *et al.*, 1973; Andersen, 1975; Yamane *et al.*, 1975; Hess and Guggenheim, 1977).

Some additional details of the tegument of *Diphyllobothrium latum* observed by scanning electron microscopy (SEM) were reported herein, and the relationship between the ultrastructure and absorption of nutrients through the body surface was discussed.

Materials and Methods

Several matured proglottids of *Diphyllobothrium latum* were obtained from a man who evacuated a strobila spontaneously. For SEM preparation, small pieces of proglottids were fixed for 2 hours at 4 C in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Following a buffer rinse, the specimens were post-fixed for 4 hours at 4 C in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4). Fixed specimens were dehydrated in a graded series of ethanol, soaked in amylacetate, transferred to critical point drying, sputter-coated with gold-palladium alloy, and examined with a Hitachi HFS-2ST scanning electron microscope.

Some fixed specimens were embedded in styrene after dehydration and substitution in ethanol-styrene mixture to get the cross fractures of the tegument. After polymerized for 48 hours at 60 C, the specimens were cracked under a stereoscope. The cracked specimens were soaked in propylene oxide to dissolve the polymerized styrene, and then substituted by amylacetate for critical point drying (Tanaka *et al.*, 1974).

Some proglottids were put in 2% pepsin solution to resolve the microtrichial layer and to expose the basal membrane complex directly, and were removed from the solution at each ten minutes to observe the process. These specimens were observed by SEM following the same procedure as mentioned above.

* *Department of Environmental Medicine, Shimane Medical University, Izumo 693, Japan.*
† *Department of Medical Zoology, Tottori University School of Medicine, Yonago 638, Japan.*

Results

Diphyllobothrium latum was similar to the other diphyllobothriid cestodes in the fundamental structure of the tegument. The genital papillae around the genital pore and the cirrus were covered with microtriches (Fig. 1). The microtriches (Fig. 1) over the cirrus were stout, short, and thick with a bulb-like swelling near the base, somewhat differing from those over the other part of the segment. Many small pores about 2 μm in diameter were distributed on the tegumental surface (Fig. 2). The distal cytoplasmic layer of the tegument was a continuous cytoplasmic syncytium with microtriches on the free surface (Fig. 3).

Each microthrix was thick and cylindrical proximally and slender distally. The surface of the proximal part of microtriches exhibited characteristic granular appearance in contrast with the distal smooth surface. Some short undeveloped microtriches (Fig. 4, arrows) grew among developing microtriches which budded out from the surface of distal cytoplasmic layer.

With the higher resolution, the outermost membrane surface of microtriches showed uneven, granular pattern with numerous grobular droplets (Fig. 5, arrowheads). Many pores (Fig. 5, arrow) were observed in the distal cytoplasm, especially at the roots of microtriches. There were numerous vesicles or vacuoles (Fig. 6, arrows) scattering throughout the distal cytoplasmic layer. Observation at the higher magnification revealed the complex membranous structures (Fig. 7, arrows) which were connected with numerous granules in the distal cytoplasmic layer.

The multitubular complex and pore canals (Fig. 8, arrow and arrowheads) were observed at the bottom of the distal cytoplasmic layer. Excretory canals ran underneath the basal membrane complex (Fig. 9). The subtegumental cells were connected with the distal cytoplasm by the cytoplasmic bridge, in which numerous vesicles (Fig. 9, arrowheads) were observed.

The surface of the basal membrane complex of the specimens which were treated in pepsin solution revealed many pores which were about 0.5–1.0 μm in diameter, and looked like a sieve (Figs. 10a, 10b and 11). From some of these pores round globes about 0.3 μm in diameter and amorphous substances peeped out (Fig. 11).

The subtegumental cells showed a multiporous, sponge-like structure in the cross fracture (Figs. 12 and 13). The subtegmental cell had a relatively large nucleus which was generally situated at the proximal part of the cell. The whole nucleus was constituted of a fine network of nucleic substance and a large nucleolus which was observed as a conglomerate of fine granules (Fig. 13). Vesicles, 0.5–1.0 μm in diameter with thin capsule, were densely packed in the cytoplasm (Fig. 13).

Discussion

Cestodes, which have no digestive tract at all, have to absorb nutrients and all the other necessary substances through their tegument. Many studies revealed that the cestode tegument has a complex absorptive function and is engaged in such a high metabolic activity as enhancement or inhibition of host enzymes (Senturia, 1964; Smyth, 1972, 1973; Read, 1973; Threadgold and Arme, 1974; Lumsden *et al.*, 1974; Lumsden, 1975a, 1975b; McCracken and Lumsden, 1975; Pappas and Read, 1975; Starling, 1975; Uglem, 1976; Hopkins *et al.*, 1978; Oaks and Mueller, 1981; Schroeder *et al.*, 1981; Pappas, 1981; Gamble and Pappas, 1981; Threadgold and Hopkins, 1981).

The recent application of SEM has brought about a great advancement to the study of the function of the tegument and

the taxonomy. A few scanning electron microscopical observations have been carried out on the topography of diphyllobothriid cestodes (Andersen, 1975; Bylund, 1975; Yamane *et al.*, 1974).

Smyth (1973) described the host-parasite interface phenomena and suggested that various activities could theoretically occur at the brush border of the tegument, for instance, excretion (secretion), diffusion, active transport, pinocytosis, extra-cellular digestion, membrane digestion and molecular mimicry. Some modes of nutrient uptake through the tegument may be probable. The first is 'membrane digestion' or 'contact digestion' that Ugolov (1965) has described. It may be done at the plasma membrane of microtriches through glycocalyx. The second is 'pinocytosis' which can occur at the cytoplasmic membrane of the brush border. The third is the uptake through pore canals or the basal membrane complex.

The tegumental surface of *D. latum* was covered with microtriches which serve for absorptive function. The microtriches observed here resembled those of *Spirometra erinacei* (Yamane *et al.*, 1974). The bud form of short microtriches which Bråten (1968) supposed as 'budding formation' of microtriches were also observed. The branched microtriches that some other studies (Blitz and Smyth, 1973; Hayunga and Mackiewicz, 1975) have revealed were not observed herein.

The higher resolution images of the outermost layer of microthrix revealed an uneven pattern and fine granular protuberances, or exocytotic particles. This pattern of the microtricheal surface was seen especially clearly on the cylindrical proximal part. Threadgold and Befus (1977) confirmed that the immunoglobulin-binding sites were localized on the surface of the microtriches. Belton (1977) demonstrated that there were numerous particles on freeze fracture faces of the cylindrical proximal part of the microtriches while there were few particles on the fracture faces of the distal part. McCracken and Lumsden (1975) described that receptor sites for lectin concanavalin A were decontinuously distributed on the plasma membrane of the tegumental surface and that the majority of these sites were located along cylindrical proximal part of microtriches. Morphological features observed here seem to have some connection with the above-mentioned possibility of digestive-absorptive activity through the cytoplasmic membrane of microtriches.

High molecules such as protein and glycogen have been suggested to be taken into the tegument by pinocytosis, not by membrane digestion (Hopkins *et al.*, 1978; Threadgold and Hopkins, 1981). Some authors (Threadgold, 1962; Jha and Smyth, 1969; Rothman, 1963) described that evaginations of the limiting membrane were suggestive of formation of pinocytotic vesicles. However, Pinocytosis has not yet been unequivocally demonstrated in cestodes. Hopkins *et al.* (1978) recently demonstrated macromolecular uptake by micropinocytosis in *Schistocephalus solidus*. He exhibited how numerous electron-lucid, membrane-limited vesicles (pinosomes) were formed in the distal cytoplasmic layer by pinocytosis, and that contents of vesicles appeared to be released into the underlying interstitial material by exocytosis. The porous structure observed here along the superficial plasma membrane of the tegument may reveal the dynamic pinocytotic activity. Threadgold and Hopkins (1981) demonstrated that endocytosis occurred in the plerocercoid and the adult worm of *Schistocephalus solidus* and *Ligula intestinalis*, and also suggested that nutrition uptake and defence were two possible functions of endocytosis.

Bråten (1968) reported the presence of disc-like bodies and the absence of lamellated bodies in the tegument of *D. latum*

adult. Grammeltvedt (1973) also found numerous disc-like bodies in the distal cytoplasmic layer and scanty lamellated bodies in *D. dendriticum* adult. Conway-Jones and Rothman (1978) suggested that electron-opaque bodies and tegumentary discs or disc-like bodies were a part of the system of continuous stacks which meander in the distal cytoplasmic layer. The present study showed numerous membrane-bounded vesicles and spherical or ovoid bodies which exhibit a close connection with meander or net-work of membranous structure. It is probable that the lamellated bodies often observed by transmission electron microscope may be a part of the meander of membranous structures, and that the meander of membranous structures which originate from the basal membrane complex may serve for taking pinosomes from the tegument actively and transporting them.

The third mode of nutrients absorption is concerned with the pore-canal system. The presence of pore-canal or similar structures have been demonstrated about several species of cestodes (Lee, 1966), but it is still disputable whether they are real pore-canals or other structures such as nerve endings, pinocytotic vesicles or some pit-organelles. Tubular structures, infoldings of the basal plasma membrane, often have been reported as an unusual laminar structure or a unique membrane specialization in the tegument (Reissig, 1970; Threadgold and Read, 1970). We could not confirm the total pore-canal system and tubular structures, but often find small vesicles, probably pinosomes, which were connected with the basal plasma membrane. Threadgold and Hopkins (1981) recently described that the basal membrane had a special function of exocytosis, and was connected with the uptake and transportation through the tegument.

The porous, sieve-like structure en face of the basal membrane complex may coincide with the pores demonstrated by Wright and Lumsden (1969) or Graeber and Storch (1978). Wright and Lumsden (1969) interpreted these pores as the representation of cross sections of tubular canals which are parts of the tegumentary pore-canal system. In the present study, we suppose that these pores may be cytoplasmic bridges which connect the distal cytoplasmic layer with the subtegumental cells.

Summary

Ultrastructuarl observation of the tegument of *Diphyllobothrium latum* adult was carried out by using SEM from the view point of functional morphology. The outermost surface of the proximal part of microtriches showed uneven, granular pattern with numerous grobular droplets. Many pores were observed in the distal cytoplasmic layer, especially at the roots of microtriches. The complex membranous structures which were connected with numerous granules were observed in the distal cytoplasmic layer. These structures suggest a relationship with the mode of nutrients absorption and transportation, namely, 'membrane digestion' at the superficial membrane of microtriches, and 'pinocytosis' at the tegumental surface.

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広節裂頭条虫 (*Diphyllobothrium latum*) の外皮の電子顕微鏡的観察

山根洋右　中川昭生　牧野由美子
(島根医科大学環境保健医学教室)
矢崎誠一　福本宗嗣
(鳥取大学医学部医動物学教室)

広節裂頭条虫の外皮を，機能形態学の観点から，走査電子顕微鏡により観察した．

体表面に密生する絨毛近位端の表面構造は，凹凸不整で多くの微細顆粒様突出がみられた．微絨毛の基部，外皮の最表層には，表面の細胞膜の陥凹やそれと関連した多くの小孔が観察された．表皮の遠位細胞質層（外層）は，複雑な膜様構造，微細顆粒，小胞などで構成されていた．これらの諸構造は，絨毛最表面膜における「膜消化」や，外皮表層の細胞質膜の「pinocytosis」という二つの栄養吸収及び輸送機能との関連を示唆した．

Explanation of Figures

Fig. 1 Microtriches with the bulb-like roots (arrows) on the cirrus surface. ×18,800.

Fig. 2 Opening of the excretory canal (EC) on the tegumental surface, being surrounded by densely growing microtriches. ×15,000.

Fig. 3 Cross fracture through the tegument, showing high density of microtriches (M) and the distal cytoplasmic layer (DC). ×2,500.

Fig. 4 Dense growth of microtriches, showing the cylindrical proximal part with whip-like slender distal part, and the undeveloped, short microtriches (arrows). ×25,000.

Fig. 5 Basal region of microtriches, showing numerous excavations (E) and pores (P); note the surface pattern of microtriches (arrowheads). ×44,000.

Fig. 6 Cross fracture through the distal cytoplasmic layer of the tegument, showing a porous structure (arrows). ×1,000.

Fig. 7 Distal cytoplasmic layer of the tegument, showing vesicles (V), spherical bodies (SB) and the meander of the membranous structure (arrows) near the basal membrane. ×43,000.

Fig. 8 Cross fracture through the tegument, showing vesicular pore (arrowheads) and peculiar complex membranous structure (arrow) which are connected with the basal membrane (B). ×7,000.

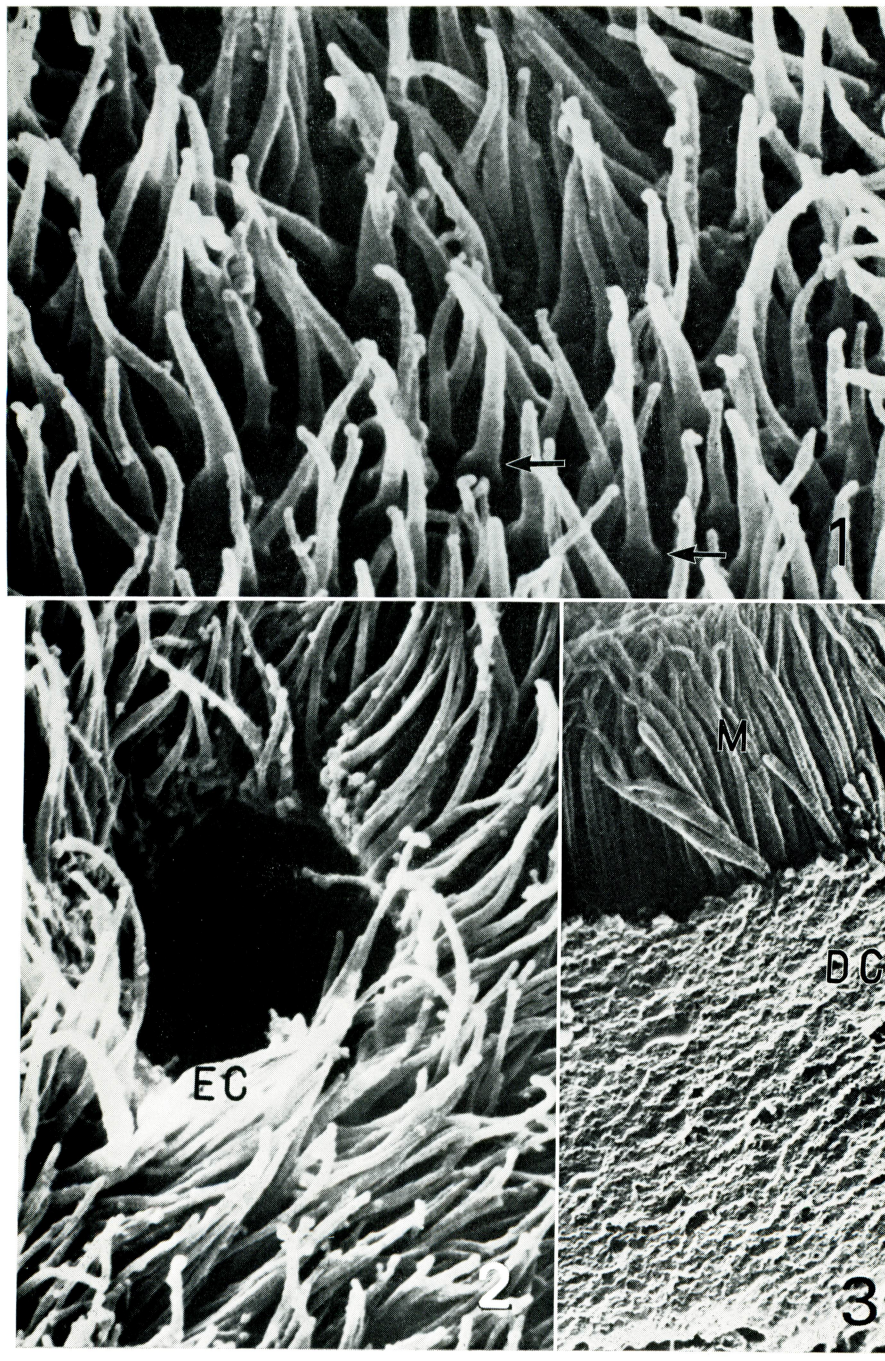
Fig. 9 Cross fracture of the basal membrane complex (B), muscle layer (M), excretory canals (EC) and vesicles (arrowheads) in the cytoplasmic bridge (CB) of subtegumental cells. ×10,500.

Fig. 10 Porous sieve-like structure of the basal membrane which was eliminated from the superficial distal cytoplasmic layer by the pepsin treatment; note the miliar pores (arrowheads) on the surface of the basal membrane. (10a. ×5,000; 10b. ×10,000).

Fig. 11 Pores on the surface of the basal membrane; note the spherical bodies (SB) and amorphous substance (arrow) in the pores. ×16,200.

Fig. 12 Cross fracture of the perinuclear cytoplasm, showing the nuclei (N) of the subtegumental cells in the porous, sponge-like structure. ×3,400.

Fig. 13 Subtegumental cell, with a nucleus (N) and numerous vesicles (V) in the cytoplasm. ×20,300.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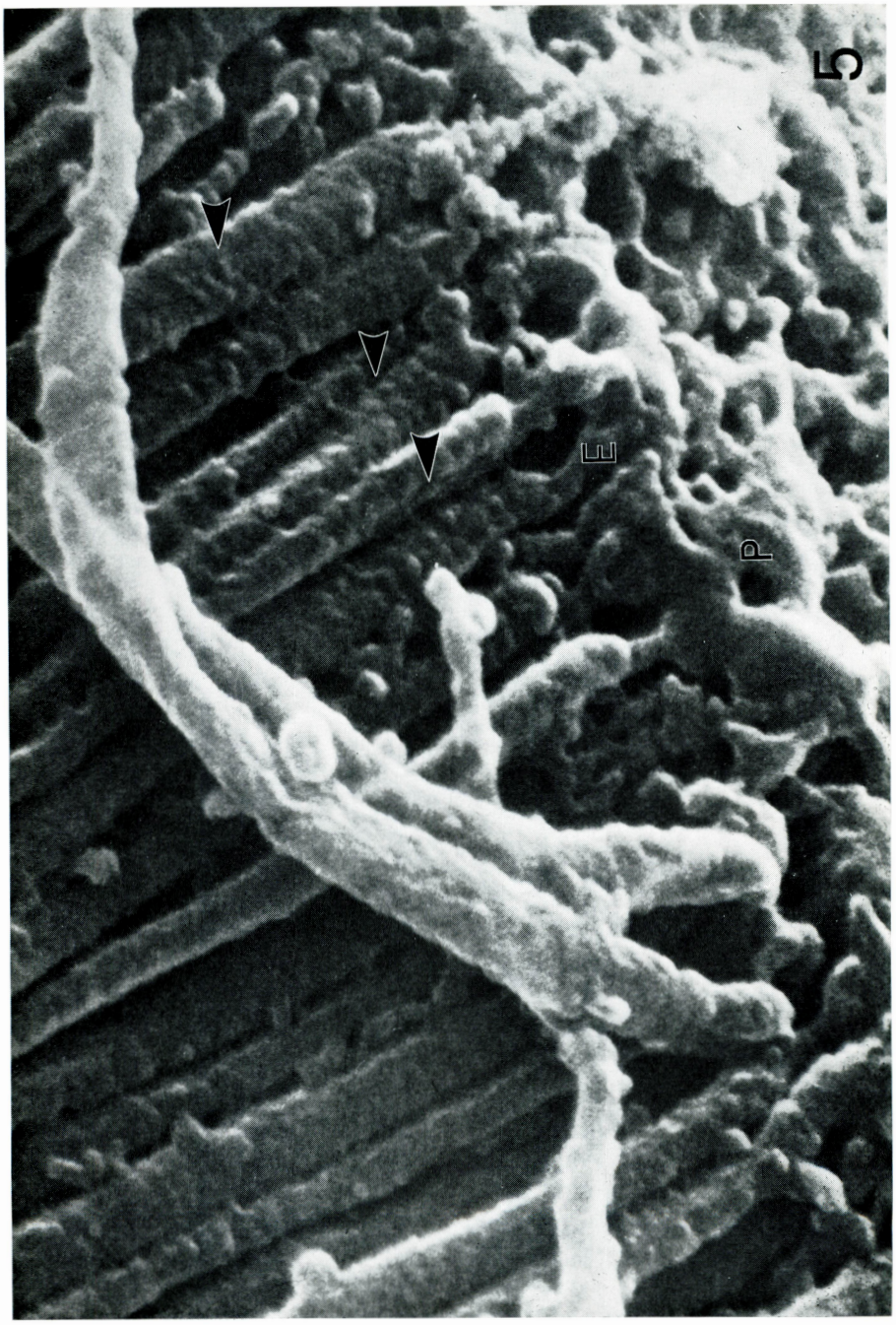
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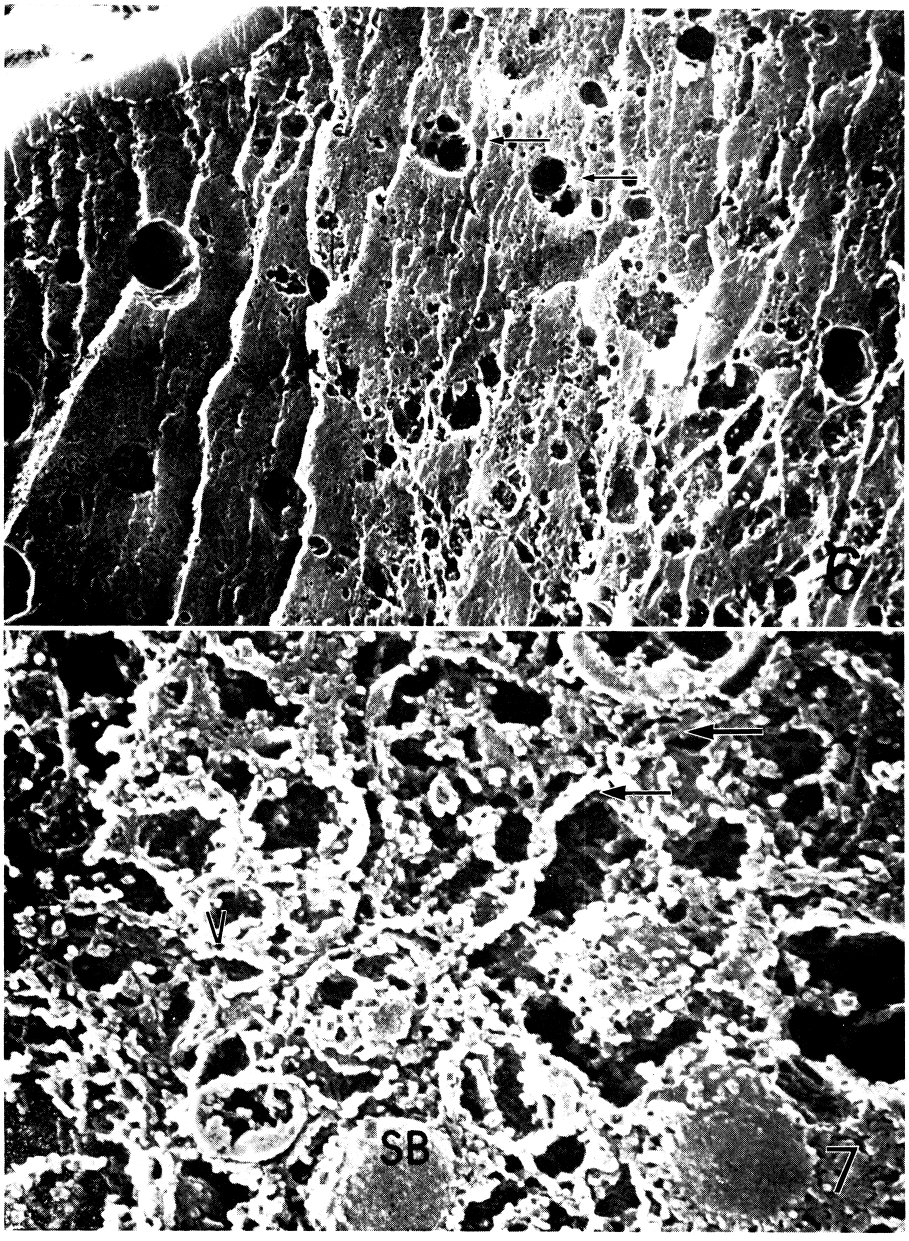
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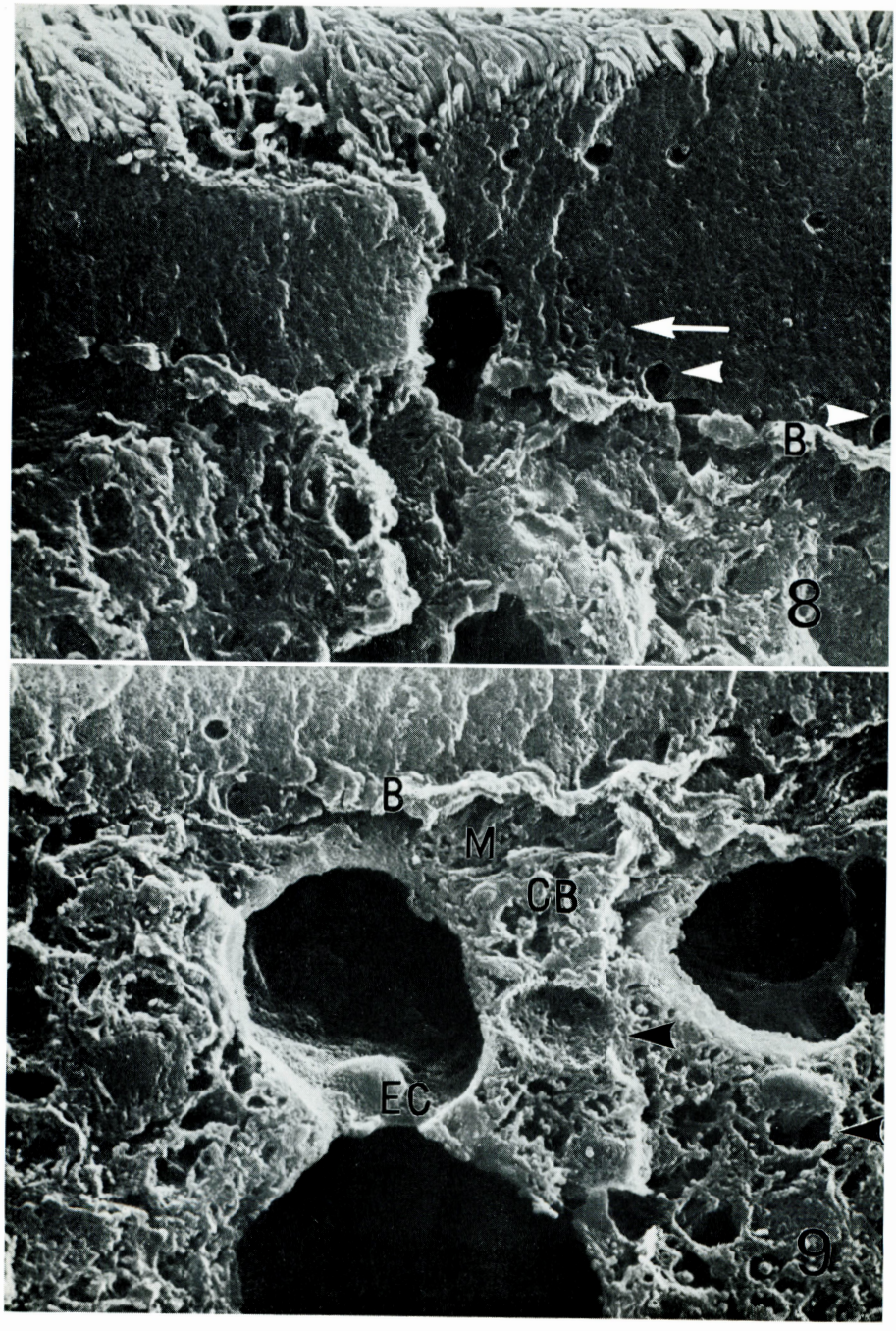
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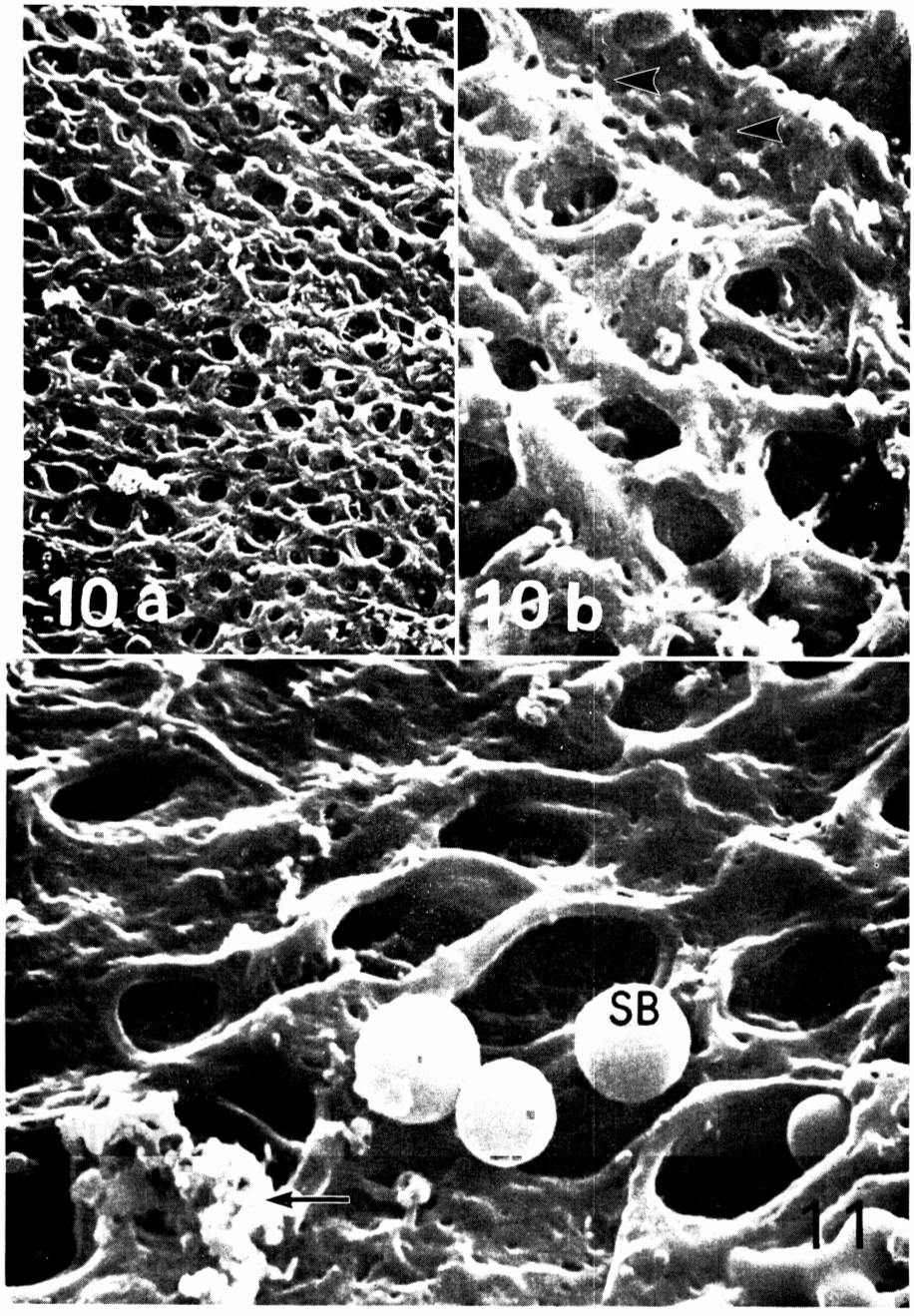
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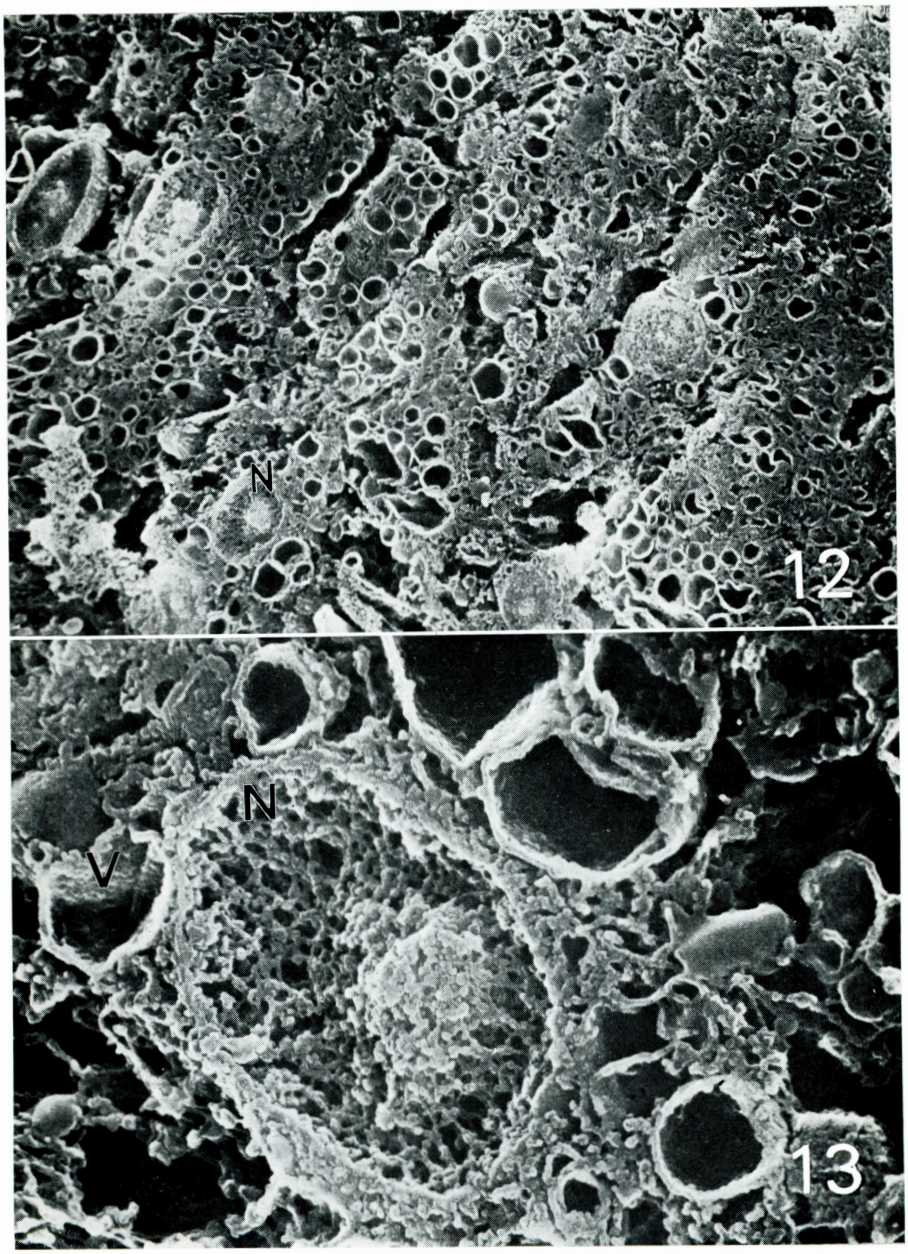
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