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Further Studies on the Fine Structure of the Gastrodermal Lamellar Projections in *Fasciola hepatica* and *Paragonimus ohirai*

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Abstract

A high resolution scanning electron microscope (SEM) was used to compare the surface ultrastructure of the gastrodermal cells in *Fasciola hepatica* versus *Paragonimus ohirai*. Such detailed observations would be difficult with conventional SEM. In *F. hepatica*, the lamellae are broad and rhomboidal with tapered tips. They are divided or notched deeply into two or more. Long, slender pseudopodium-like or filamentous elongations extend from the tip and margin of the lamellae, and occasionally form small spherical swellings. These elongations are denser in the main ceca than in the lateral diverticula. In *P. ohirai*, the lamellae are triangular with smooth or indented margins without divisions or digitiform elongations. Numerous linear and irregularly arranged tubular structures of various length were observed on the lamellar surface. Tests with ruthenium red and cationized ferritin showed that these structures are of glycosaminoglycans and probably contain sialic acid as the major component.

Key words: SEM, TEM, ultrastructure, *Fasciola hepatica, Paragonimus ohirai,* gastrodermal cells

Introduction

Scanning electron microscopic observations on the luminal surface of the gastrodermal cells in some trematodes have provided 3-dimensional images of the cells and their cytoplasmic or lamellar projections on the free surface (Fujino and Ishii, 1978, 1979; Threadgold, 1978). The lamellar projections differed from microvilli in certain features. Fujino and Ishii (1978, 1979) described differences in the shape of the lamellae in several species. Threadgold (1978) showed by transmission electron microscopy that the gastrodermal lamellae of *Fasciola hepatica* have an internal skeleton consisting of laterally oriented 'fibres', which may be responsible for the shape and flexibility of the lamellae.

Further observations with a field emission SEM on the gastrodermal cells of *F. hepatica* and *Paragonimus ohirai* revealed features not previously observed by conventional SEM and are reported in this paper.

Materials and Methods

Scanning electron microscopy. *Fasciola hepatica* from livers of cattle and *Paragonimus ohirai* from lungs of experimentally infected albino rats were used. The specimens just after dissection from the host tissue were pinned on a small silicone board. The length of the gut of *P. ohirai* and the main ceca and diverticula of *F. hepatica* were cut open with a fine needle in physiological saline. The gut surface was washed by gentle flow of saline followed by buffer to remove debris. Fixing, dehydration and drying of the specimens followed the method described by Fujino and Ishii (1978). After drying, the specimens were coated with gold and observed in a field emission SEM JEOL F15.

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The preparation of freeze cracking followed the method by Tokunaga *et al.* (1974). The specimens were fixed for 2 h in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2. After washing in the buffer the specimens were immersed in ascending series of dimethyl sulfoxide (DMSO) up to 50%. The material was frozen in liquid nitrogen and cracked with a knife blade. The cracked specimens were processed through a descending series of DMSO from 50 to 15% and then distilled water. The specimens were post-fixed for 1 h in 1% osmium tetroxide, dehydrated with ethanol, dried and observed as described above.

Transmission electron microscopy. The flukes, *F. hepatica* and *P. ohirai* were obtained as described for SEM preparation. After fixation in glutaraldehyde and osmium tetroxide and dehydration with ethanol the material was embedded in Epon 812. The sections were stained with both uranyl acetate and lead acetate, and viewed in a Hitachi HS-9 electron microscope set to operate at 75 kV.

For labeling anionic groups on the gastrodermal cell surface, the gut of *P. ohirai* was dissected and incubated for 30 min in 0.1 M phosphate buffer (pH 7.2) including cationized ferritin (1.15 mg/ml) at room temperature. After washing in the same buffer, the procedure followed the method described above. For enzymatic treatment, the material was incubated for 1 h at 37°C in the presence of 20 U/ml neuraminidase, pH 5.5, in acetate buffer.

For the detection of acid mucopolysaccharide, ruthenium red was used. The tissue was fixed for 1 h in 1.2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 500 ppm ruthenium red and postosmicated.

Results

Fasciola hepatica (Figs. 1–3)

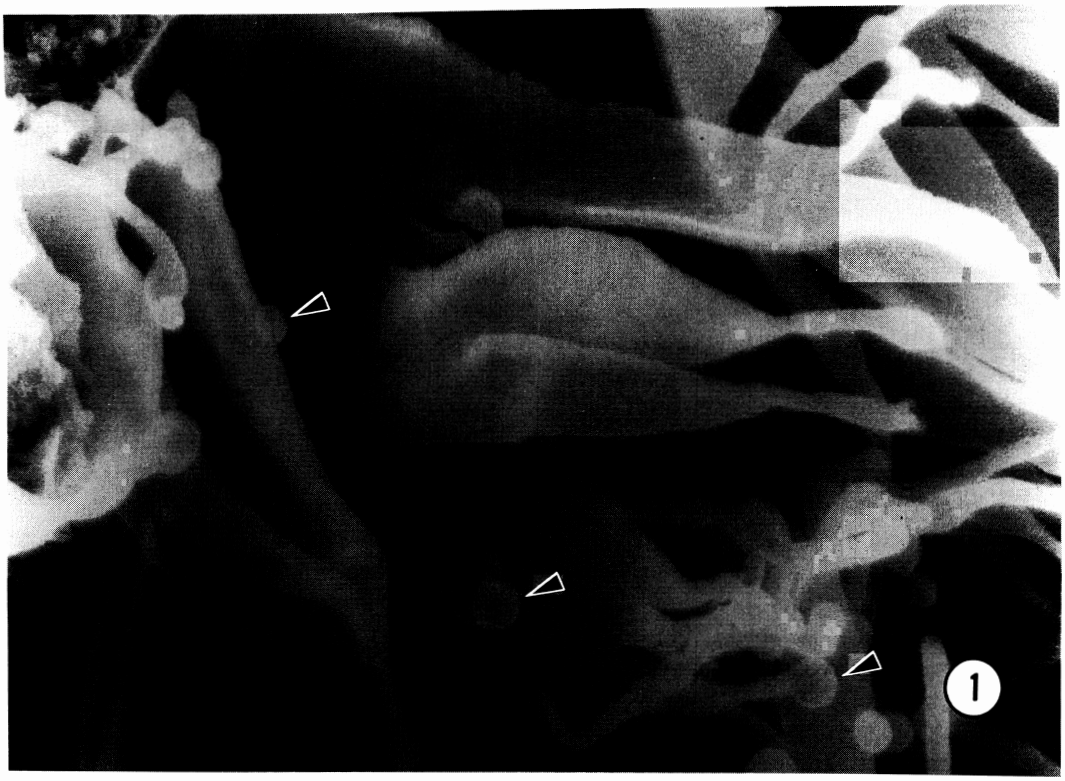
The gastrodermal epithelium was characterized by thickly grown, numerous leaf-like lamellae. Individual lamellae varied in shape, but were roughly triangular or rhomboid with their broad bases. The lamellar surface was smooth. The lamellae were usually divided or deeply notched into two or more and formed long and slender digitiform or pseudopodium-like elongations (Figs. 1 and 2). These elongations were round in cross section, and often formed small spherical swellings (Fig. 2). The elongations appeared denser in the main ceca than in the lateral diverticula. There were occasional small blisters at the tip of the lamellae or on the margin of the lamellae (Fig. 1).

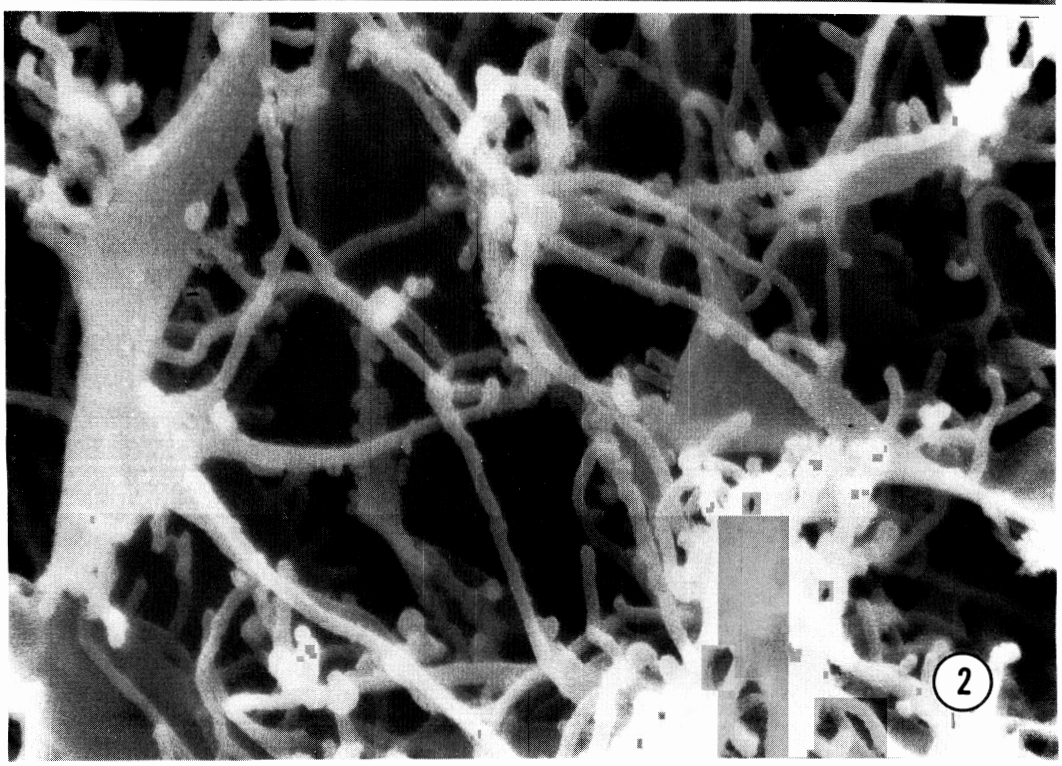
The basal region of the lamellae were viewed clearly by using the freeze-cracking method (Fig. 3). The lamellae at the bases were expanded broadly and sheet-like with a thickness of about 90 nm. The lamellae occurred at regular intervals on the epithelial surface, and the individual lamellae were folded partly and anastomosed occasionally. The cracked surface of the lamellae appeared rough and no core fibres were seen at the edge.

Paragonimus ohirai (Figs. 4–7)

The gastrodermal cell surface was covered thickly with lamellar projections. The broad, flat and rhomboidal lamellae with their expanded bases tapered to blunt ends or further extended to narrow projections (Fig. 4). The edge of the lamellae was smooth or indented occasionally, and marginal divisions and digitiform extensions as seen in *F. hepatica* were not observed. Small blisters occasionally occurred at the tip or on the margin of the lamellae. Some of the lamellae were curled and contained luminal contents (Fig. 4). In TEM observations, the lamellae, 60 nm thick, had central core fibres seen as a succession of small dense dots which were arranged at a regular interval of 34 nm. Amorphous material were attached to the fibres and also to the plasma membrane (Fig. 6 inset).

Numerous linear structures of various length were found on the surface of the lamellae under a high magnification (Fig. 5). They ran mostly parallel to the long axis of the lamellae, but were arranged irregularly in some area or lacking partly. Cross sections showed tubular structures just above the surface of the lamellae, which correspond to the linear structures observed in SEM (Figs. 6 and 6 inset). These





tubules, 13 nm in diameter, were apart from the surface of the lamellae at a distance of approximately 10 nm. Some tubules were separated partly from the lamellae and extended deeply into the lumen. The lamellae were observed occasionally to be devoid of these tubules due probably to the absence of tubules or to their running parallel to the sectioned surface. These tubules stained strongly with ruthenium red (Fig. 6), and were covered with a single layer of cationized ferritin particles (Fig. 7). Few particles were seen over the surface of the lamellae.

Discussion

The observations reported here confirm and extend previous knowledge on the apical surface of the gastrodermal cells (Fujino and Ishii, 1978, 1979; Threadgold, 1978). A field emission SEM with high resolution is useful for detailed observations of the lamellar surface structures, which are difficult to study with conventional SEM.

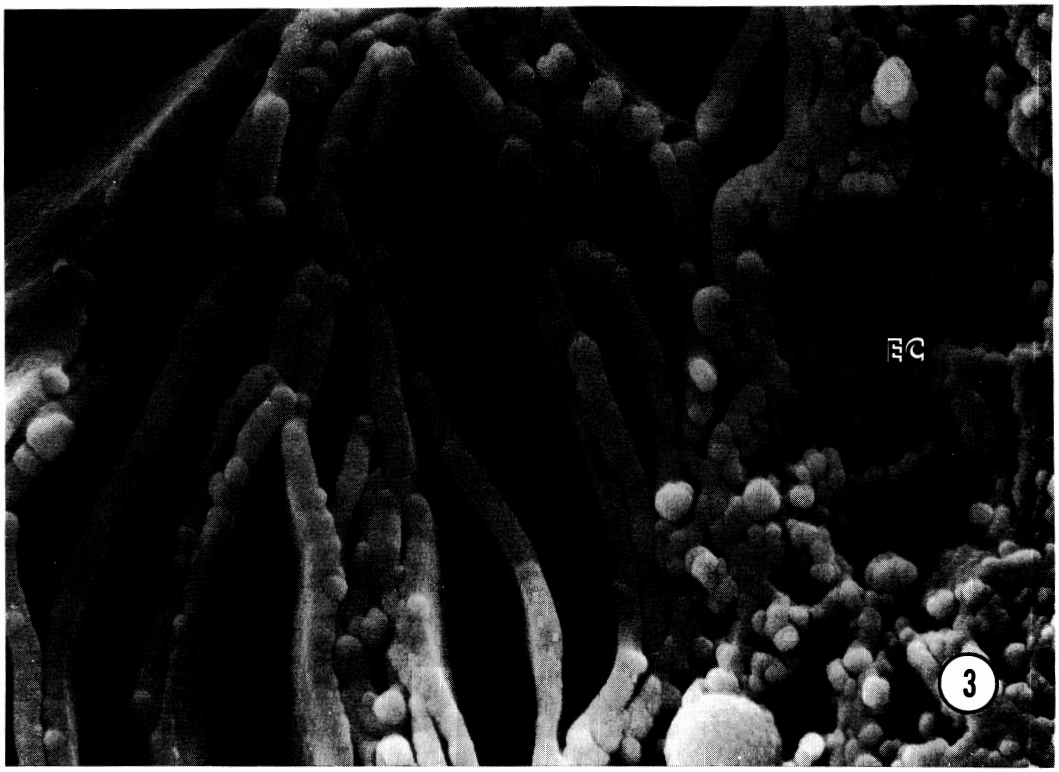
In *Fasciola hepatica*, the margin of the lamellae is divided or deeply notched to form slender projections, which extend into pseudopodium-like or elongate tubular structures with occasional transformation into small spherical swellings. These elongations are more densely developed in the main ceca than in the diverticula. It may be suggested that this structure functions to help convey foods with the peristaltic movement of the gut from the main ceca to the diverticula where active absorption and secretion are made. Such a modification of the lamellae in the part of the gastrodermis does not occur in *Paragonimus ohirai* that has a simple tubular gut. Blisters or oval bodies formed just on the edge or at the tip of the lamellae are seen occasionally in *F. hepatica* as well as *P. ohirai*. Threadgold (1978) observed the similar structure in *F. hepatica* and noted that these bodies appeared derived from lamellae by local swelling and eventual detachment. He also mentioned that this would provide the mechanism of recycling and conserving membrane components.

The basal part of the lamellae was well viewed with freeze cracking. The lamellae are uniform in thickness and raised in layers as seen in the cracked surface, this feature corresponding well with the image given by TEM. The internal structure of the lamellae, 'core fibres' and amorphous material attached laterally to the plasma membrane, has been observed in the transverse section by TEM, but not in the cracked edge of the lamellae by SEM. Threadgold (1978) described that "the 'fibres' are not solid structures but are composed of from 2 to 6 very small tubules in a dense matrix". It is possible that this arrangement provides some stiffening and still allows the lamellae certain flexibility (Threadgold, 1978), and is involved in the more active movement of engulfing food particles, although it is not known whether these 'fibres' are of actin filaments and have motility as in microvilli (Mooseker and Tilney, 1975; Mooseker *et al.*, 1978). This hypothesis may be supported by the observation that the apical part of the lamellae occasionally curled and appeared to entrap or engulf luminal contents in *P. ohirai*. Davis *et al.* (1968) and Bogitsh *et al.* (1968) also observed 'a superficial vacuole' that is made by the lamellae on the epithelial surface in *Haematoloechus medioplexus* gut, and noted that this vacuole is the place where engulfed food is digested with the aid of digestive enzymes released into it.

SEM observations at high magnification de-

Fig. 1 *Fasciola hepatica*, a SEM micrograph showing the lamellae in a lateral diverticula. The lamellae are roughly triangular or rhomboidal and are divided into two or more at their margin. The subdivided ends extend into pseudopodium-like projections. There are occasional small blisters at the tip or on the margin of the lamellae (arrow heads). The lamellar surface is smooth. × 53,000

Fig. 2 *Fasciola hepatica*, a SEM micrograph showing the lamellae in a main cecum. The lamellae extend irregularly into long, slender and tubular projections which frequently form small spherical swellings. × 53,000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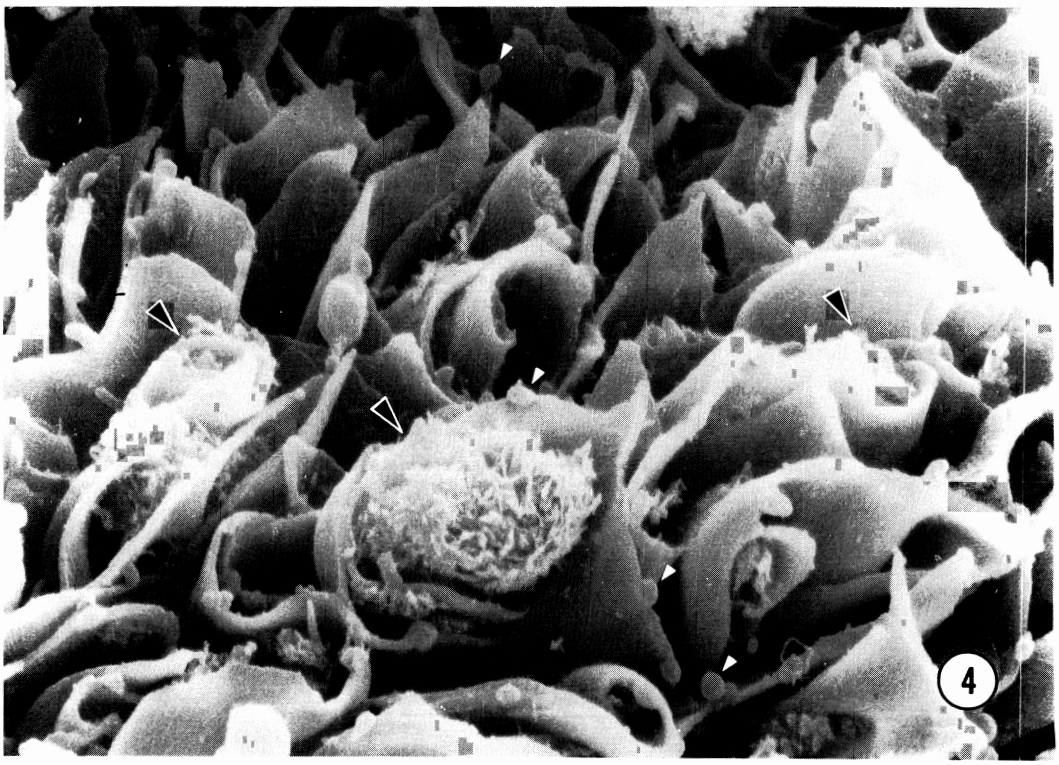
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monstrated numerous linear structures of various length on the lamellar surface in *P. ohirai*, which were hardly viewed with conventional SEM. Under TEM observations these structures appeared as tubules with a uniform thickness above the lamellar surface. This unique structure has been observed in some other *Paragonimus* species, but not in *F. hepatica*, the lamellae of which are covered with ladder-like connections of glycocalyx (Fujino and Ishii, 1978, 1979). Davis and Bogitsh (1971) also observed different features in the lamellar glycocalyx between *Gorgoderina attenuata* and *Haematoloechus medioplexus*. Although the enteric surface of the mammalian intestinal microvilli is known to be coated with a conspicuous filamentous layer of glycocalyx (Ito, 1965, 1969), the coat having tubular structures as in *Paragonimus* species is not known. Tests with cationized ferritin and cationic dye, ruthenium red, indicate that these tubules are negatively charged and consist of glycosaminoglycans. It is known that the negative surface charge of eucaryotic cells is caused by the presence of carboxyl, sulfate, and phosphate groups exposed on the cell surface (Danon *et al.*, 1972; Burry and Wood, 1979). It has also been shown that the carboxyl group of sialic acid is mainly responsible for the negative surface charge (Cook *et al.*, 1961; Eylar *et al.*, 1962; Wallach and Kamat, 1966). Reduction of labeled cationized ferritin by neuraminidase digestion showed that these structures contain sialic acid as the major component, and are probably secreted originally from the gastrodermal cells. It is possible that these structures serve as an adsorbing surface for the digestion and absorption of particulate materials taken into the gut, although they are poorly developed in most trematodes except *Paragonimus* species.

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Fig. 3 *Fasciola hepatica*, a SEM micrograph of the freeze-cracked lamellae in a lateral diverticula. The cracked edge of the lamellae has uniform thickness of about 90 nm. The lamellae are sheet-like and growing in layers basally. EC: epithelial cell. × 53,000

Fig. 4 *Paragonimus ohirai*, a SEM micrograph of the thickly grown triangular lamellae. Some of the lamellae appear curled and contain luminal contents (large arrow heads). Blisters occur at the tip or edge of the lamellae (small arrow heads). × 27,000


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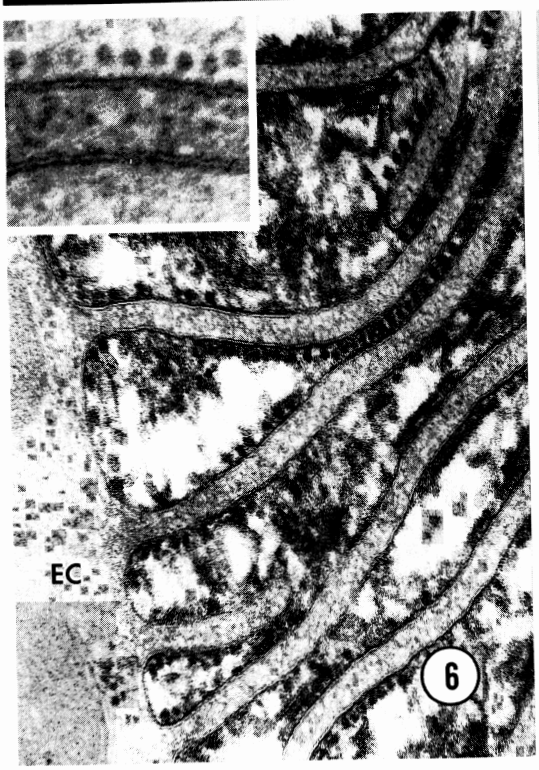
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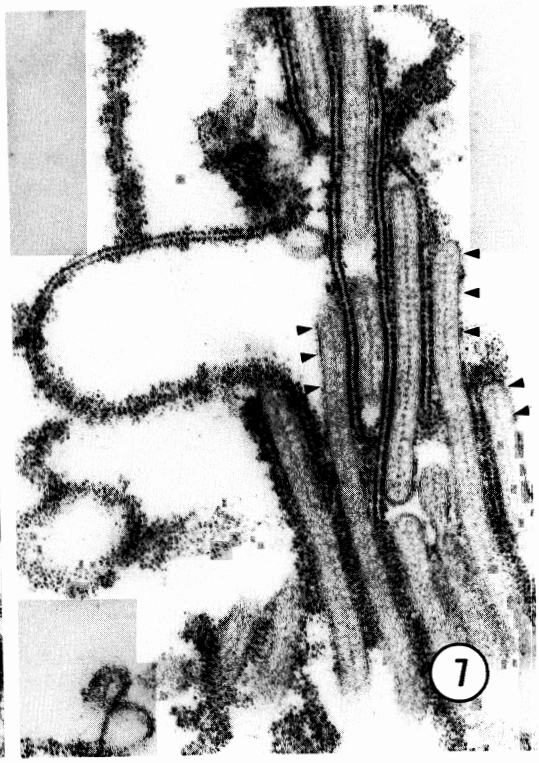
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Fig. 5 *Paragonimus ohirai,* a high magnification SEM micrograph of the lamellae. A number of fine rods or linear structures of various length, which are arranged partly in parallel or random direction, are seen on the lamellar surface. x 130,000

Fig. 6 *Paragonimus ohirai,* a TEM micrograph of the lamellae and apical part of the epithelial cell stained with ruthenium red. Tubular structures, which are sectioned in various directions, on and between the lamellae stain specifically. EC: epithelial cell. x 63,000 Inset, a high magnification TEM micrograph of the lamellae sectioned transversely showing rows of dense 'core fibres' in the lamellae and outer small tubules with the diameter of 18 nm on the side of the lamellae, which are apart from the surface of the lamellae at a distance of about 10 nm. x 175,000

Fig. 7 *Paragonimus ohirai,* a TEM micrograph of the lamellae showing cationized ferritin labelled on the anionic surface. A single layer of ferritin particles is seen over tubules on the surface of the lamellae and also in the lumen. Note the ferritin-free surface of the lamellae (arrow heads). x 63,000