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Cytochemical Studies on the Effects of Starvation in the Gastrodermis of the Lung Fluke, *Paragonimus ohirai*

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Abstract

The gastrodermis of the adult lung fluke, *Paragonimus ohirai*, was examined to determine the effects of starvation *in vitro*. Acid phosphatase (AcPase) and thiamine pyrophosphatase (TPPase) activities were used for labeling Golgi complexes and lysosomes. During the early stage of starvation, secretory bodies are disappeared and Golgi complexes markedly increased in number with the production of primary lysosomes from the lateral edges of their saccules. Autophagic vacuoles with identifiable organelles such as mitochondria and endoplasmic reticulum appeared during early stages of starvation, and then cytosomes and vacuolations including membranous or myelin-like elements were evident as starvation time increased. Lysosomal enzymes such as AcPase were released into the autophagic vacuoles. Membrane of autophagic vacuoles originated presumably from the basal infoldings or the lateral plasma membranes. Elongate filamentous structures, which have not been reported in trematodes, occurred in ribosomes and endoplasmic reticulum during the later stages of starvation. The ultrastructural and cytochemical changes following starvation in *P. ohirai* were similar to those of the worms, which have hitherto been reported from different host sites.

Key words: *Paragonimus ohirai*, gastrodermis, cytochemistry, ultrastructure, starvation effects

Introduction

The study of ultrastructural and cytochemical changes following starvation, has provided an understanding of the function of the organelles associated with the gastrodermis of the frog rectal worm, *Megalodiscus temperatus* (Bogitsh, 1973), the salamander intestinal worm, *Brachycoelium salamandrae* (Bogitsh and Ryckman, 1982) and *Schistosoma mansoni* (Bogitsh, 1975). A change in localization of worm enzyme activity and increased gastrodermal autophagy occurred during starvation (Bogitsh, 1973, 1975; Bogitsh and Ryckman, 1982). Additional trematodes from other host sites should be examined during starvation to accumulate more information on the gastrodermal cell function.

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This study examines gastrodermal ultrastructure and the distribution of acid phosphatase (AcPase) and thiamine pyrophosphatase (TPPase) of starved lung worms of *Paragonimus ohirai*. Results of this study were compared with those on the above-mentioned species.

Materials and Methods

Adults of *Paragonimus ohirai* were removed to Ringer's saline from the lungs of experimentally infected albino rats. The worms were separated into three groups. One group of worms was fixed immediately after removal from the host, and the other groups, 3 worms each, were incubated at 37°C in 20 ml of Ringer's saline for 10 and 40 hr, respectively. The incubation was carried out with several changes of saline. After incubation, worms were washed in 0.1 M cacodylate buffer (pH 7.4), fixed for 40 min in 2% paraformaldehyde plus 2.5% glutaraldehyde containing 0.025%

$CaCl_2$ and 3% sucrose at 4°C. The worms were then rinsed overnight in 0.1 M cacodylate buffer, and sectioned at 20–30 μm using a McIlwain Tissue Chopper. The sections were collected in the buffer and then placed in the incubation medium at 37°C for 40 min for AcPase and for 100 min for TPPase. Incubation was done following the modified Gomori (1952) method for AcPase and in the Novikoff and Goldfischer (1961) medium for TPPase. Control sections were incubated in a medium without substrate, or, in the case of AcPase, in a medium to which sodium fluoride (10 mM) was added. After a brief rinse in buffer the sections were postfixed for 90 min in cacodylate-buffered 1% OsO_4 at 4°C. After dehydration in ethanol series the sections were embedded in Epon 812. Thin sections cut in the ultramicrotome were either unstained or stained with uranyl acetate and were viewed under a Hitachi HS-9 electron microscope at 75 kV.

Results

Gastrodermis of nonstarved worms

Most abundant cells of the gastrodermis of nonstarved worms were the tall, columnar with blunt or rounded apices in the secretory phase. These cells were characterized by numerous secretory granules of various sizes (Fig. 1). Lipid droplets were also seen among the secretory granules. The lamellar cytoplasmic projections were moderately numerous and long with minute reaction deposits for AcPase on the surface. Reaction product was also seen on secretory granules and endoplasmic reticulum. Dense deposits lay on the lateral plasma membranes bordering the cells. Enzyme activity in each cell appeared to be different to some extent, probably reflecting physiological conditions of absorption-secretion cycle in the cells. Reaction sites for TPPase were a few Golgi complexes and multivesicular bodies (not shown in the figure).

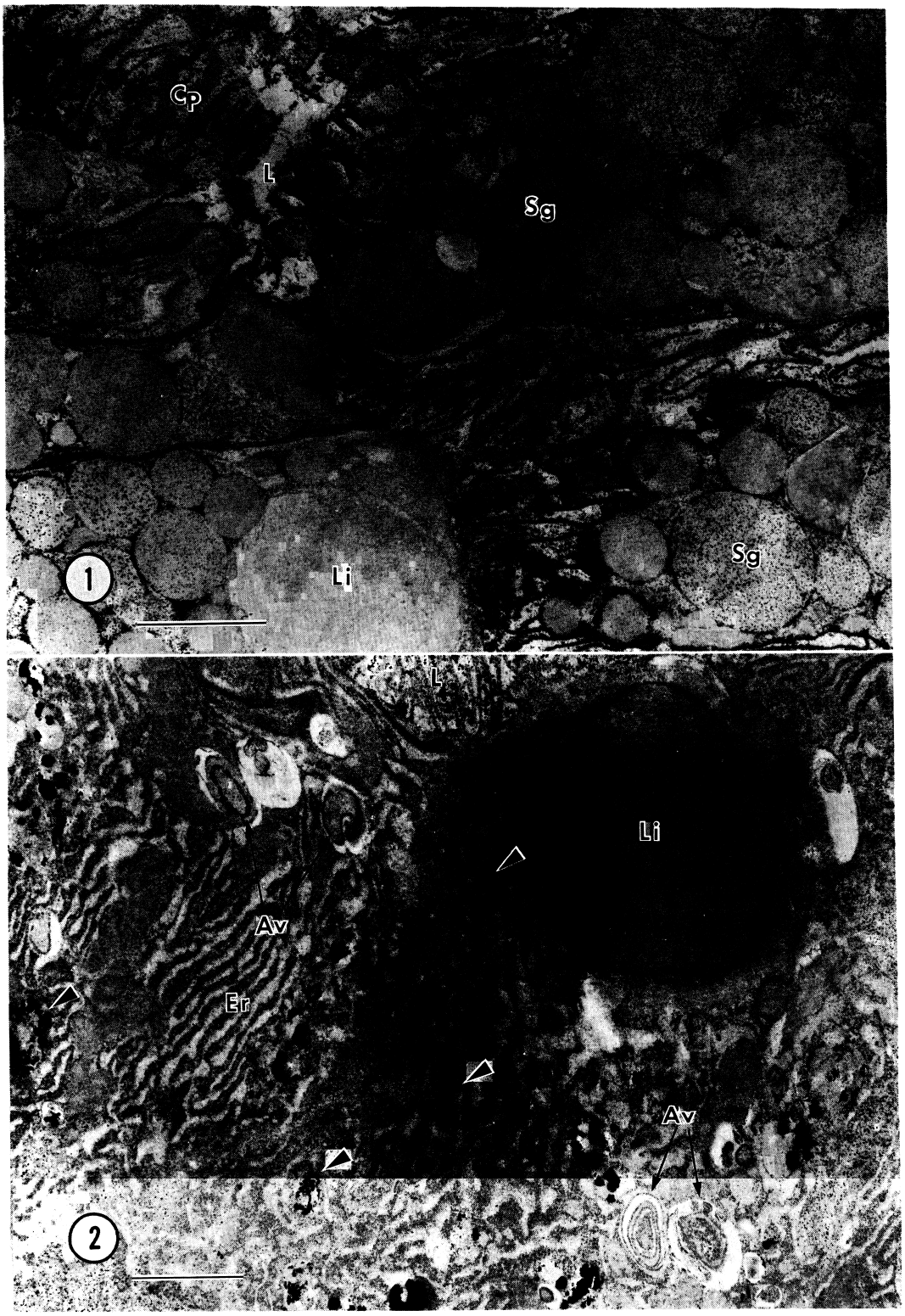
Gastrodermis of starved worms

The gastrodermis of worms starved for 10 hr decreased in height and appeared flat. Most of all secretory granules disappeared, leaving some lipid droplets in the cells. Enzyme activity for AcPase on the cytoplasmic projections and luminal contents was much weaker than in nonstarved worms. The reaction in the endoplasmic reticulum was also very weak. The gastrodermal cells were marked by a large number of Golgi complexes which displayed AcPase activity and autophagic vacuoles scattered throughout the cells (Fig. 2). Golgi complexes consisted of two to several saccules with numerous vesicles or occasionally of cisternal and tubular structures (Fig. 3). Vesicles derived from Golgi complexes, probably primary lysosomes, were positive for AcPase. Numerous autophagic vacuoles containing identifiable organelles such as the endoplasmic reticulum and mitochondria (Fig. 4) were observed in the gastrodermis. TPPase activity was observed in saccules and vesicles of Golgi complexes (Fig. 5), but did not occur in most autophagic vacuoles and secretory granules. In some cells, multivesicular bodies appeared (Fig. 5 inset). These bodies were occasionally found in close contact with Golgi complexes, and other unidentifiable organelles. The lateral plasma membranes bordering the adjacent cells were tortuous and reacted weakly for TPPase.

The number of autophogic vacuoles increased in the gastrodermis of worms starved for 40 hr (Fig. 6). Few reaction deposits for

Fig. 1. Apical part of the gastrodermis of a nonstarved worm, fixed just after removal from the host. The tissue was incubated to demonstrate AcPase. The cells are filled with secretory granules of various sizes (Sg). The enzyme reaction occurs on the surface of the lamellar cytoplasmic projections (Cp) and in the luminal contents. Reaction deposits are also localized on secretory granules and endoplasmic reticulum. L: Lumen; Li: Lipid droplet. No counterstain. Bar = 3.0 μm.

Fig. 2. The gastrodermis of worm starved for 10 hr and reacted for AcPase. Reaction product is on the Golgi complexes (arrowheads) and autophagic vacuoles (Av). Reaction deposits are also observed associated with cytoplasmic projections and the luminal contents to a lesser extent. L: Lumen; Li: Lipid droplet. No counterstain. Bar = 3.0 μm.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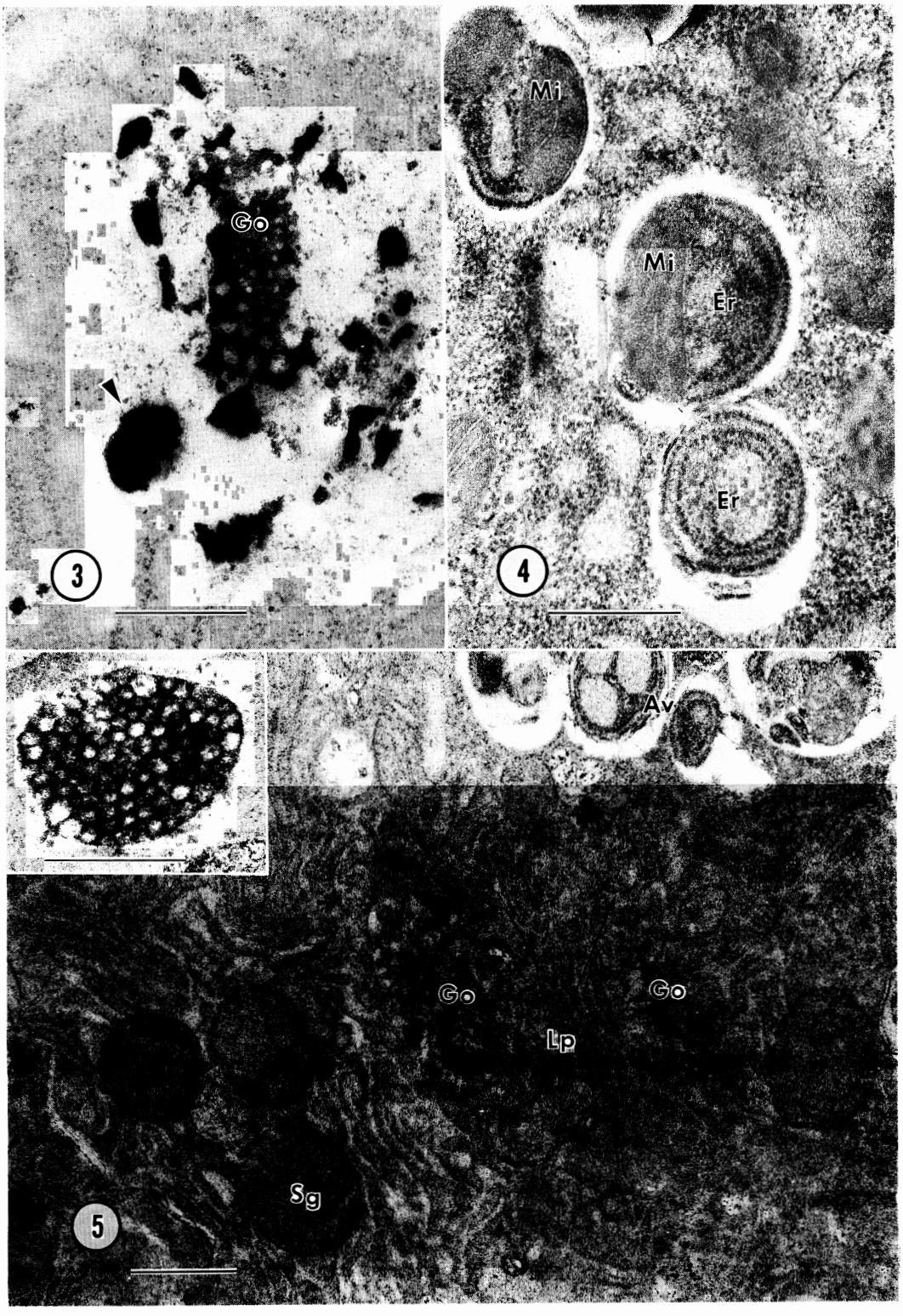
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AcPase were left in the cytoplasmic projections. There remained only a few Golgi complexes or parts of Golgi saccules which reacted for TPPase (Fig. 7). Some autophagic vacuoles were surrounded by round or lamellar vesicles with TPPase activity (Fig. 7 inset). AcPase released from the primary lysosomes appeared mixed with the segregated portion of the gastrodermis to form cytosomes (Fig. 8). Cytosomes including myelin-like whorl or unidentifiable elements also increased in number. Some of the cytosomes have their outer membrane which reacted discretely for AcPase as in the lateral plasma membranes (Fig. 10). Near the apical portion of the gastrodermis elongate filamentous structures bordering ribosomes or endoplasmic reticulum appeared (Fig. 9).

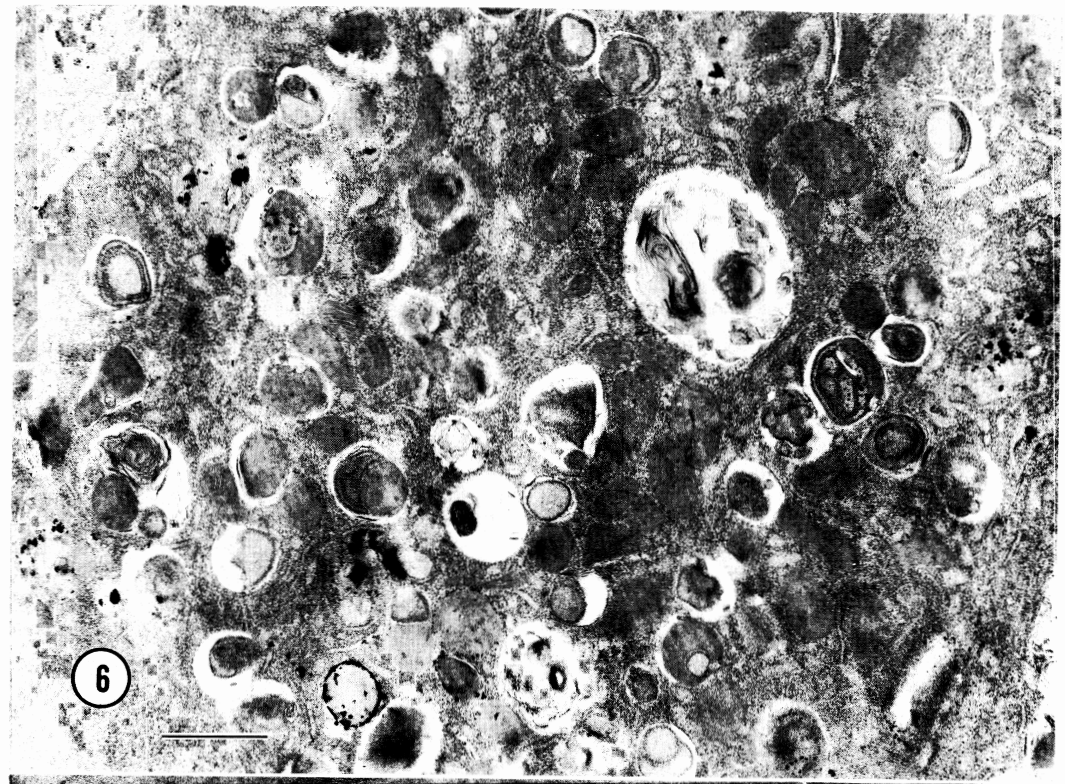
Discussion

The most characteristic ultrastructural changes in the gastrodermis of *Paragonimus ohirai* following starvation was the flattened cells and the disappearance of the secretory granules. Dike (1969) reported AcPase activity in numerous membrane limited bodies in nonstarved *P. kellikotti*. These bodies may correspond to secretory granules or bodies in *P. ohirai*, where reaction products for AcPase were observed. In *Brachycoelium salamandrae*, disintegration of whole cells occurred following starvation (Bogitsh and Ryckman, 1982), although no such dramatic changes were seen in *Megalodiscus temperatus* and *Schistosoma mansoni* (Bogitsh, 1973, 1975).

Cytochemically, AcPase activity on the surface of the cytoplasmic projections and endoplasmic reticulum in *P. ohirai* decreased during worm starvation. Golgi complexes, which reacted for AcPase and TPPase, showed a marked increase in number during early phases of starvation, and a decrease in the later stages of starvation. An increase in the number of Golgi complexes following starvation was also observed in the gastrodermis of *S. mansoni* (Bogitsh, 1975). Bogitsh (1972, 1973), however, failed to detect AcPase activity associated with Golgi complexes of the gastrodermis of *Haematoloechus medioplexus* and *M. temperatus*. In *P. ohirai*, Golgi complexes appeared closely associated with the lysosome system in that they produced primary lysosomes emanating from lateral edges of saccules of Golgi complexes (Ericsson and Trump, 1966). Lysosomal enzymes such as AcPase are synthesized in ribosomes of the rough endoplasmic reticulum and transported to Golgi complexes as are most proteins (Jamieson and Palade, 1967; Novikoff *et al.*, 1966). Bogitsh (1982) hypothesized that a functional bipolarity may be demonstrated by Golgi complex in *S. mansoni*. He speculated that "Under certain stress conditions, the forming face of the Golgi may package lysosomal enzymes while the emitting region of the Golgi appears to be responsible for the packaging of the secretory granules".

Multivesicular bodies reacted for TPPase, and only a few were found in nonstarved worms. These bodies showed a marked increase in number in starved worms of *P. ohirai*. *Brachycoelium salamandrae*, however, had some multivesicular bodies even in nonstarved worms (Bogitsh and Ryckman, 1982). Multivesicular bodies function as either being involved in intracellular transport or phagolysosomes (De Duve and Wattiaux, 1966; Ericsson and Trump, 1966). TPPase activity of these bodies suggests

Fig. 3. The gastrodermis of worm starved for 10 hr and reacted for AcPase. Enzyme activity occurs in cisternae and vesicles of the Golgi complex (Go). Arrowhead indicates a primary lysosome. No counterstain. Bar = 0.5 μm.
Fig. 4. The gastrodermis of worm starved for 10 hr and reacted for AcPase. Either mitochondrion (Mi) or endoplasmic reticulum (Er) or both are enclosed by the membrane of autophagic vacuoles. Stained with uranyl acetate. Bar = 0.5 μm.
Fig. 5. The gastrodermis of worm starved for 10 hr and reacted for TPPase. Reaction deposits are seen in the Golgi complexes (Go). Autophagic vacuoles (Av) are observed. The lateral plasma membrane (Lp) is indicated by arrows. Sg: Secretory granule. No counterstain. Bar = 1.0 μm. Inset: Multivesicular body reacted for TPPase. No counterstain. Bar = 0.5 μm.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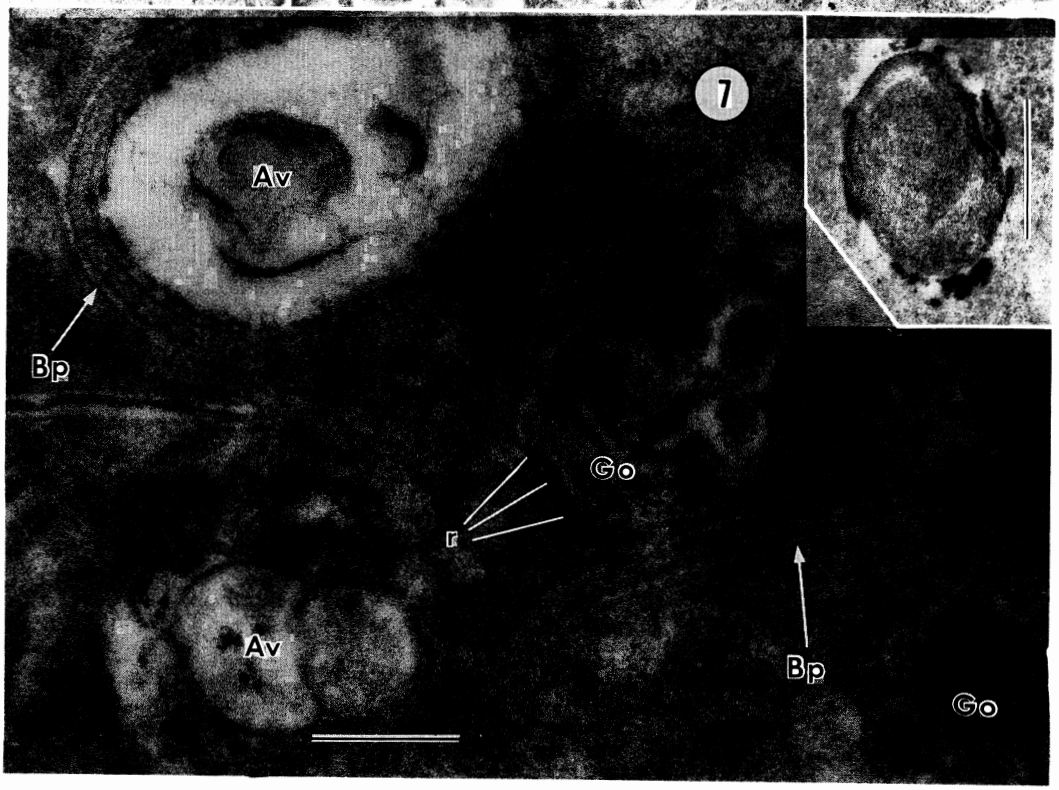
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that they originate from Golgi complexes. The multivesicular bodies in *P. ohirai* are presumably involved in the lysosome system because of the observation that some are in close contact with cytoplasmic substances in the gastrodermis as seen in *B. salamandrae* (Bogitsh and Ryckman, 1982).

The other feature that characterizes the gastrodermis of starved *P. ohirai* is the appearance of numerous autophagic vacuoles containing mitochondria and endoplasmic reticulum. During the later stages of starvation, unidentifiable membranous or myelin-like elements, showing AcPase activity, are found in most of the autophagic vacuoles. Bogitsh (1975) noted in *S. mansoni* that autophagic vacuoles were formed by the sequestration of a portion of the gastrodermis by the infolding of the basal plasma membrane. In the parenchymal cells of *Fasciola hepatica*, autophagic process starts with 'budding off' of membranes by mitochondria to form small vesicles which finally wrap around areas of cytoplasm (Threadgold and Arme, 1974). Levy and Elliott (1968) suggested that the membrane of autophagic vacuoles in the ciliate, *Tetrahymena pyriformis*, arises from the endoplasmic reticulum, or by sequestration of the cytoplasm or by nuclear blebbing. According to Brandes *et al.* (1964), Golgi-type vacuoles and multivesicular bodies in starved *Euglena* probably involved in the sequestration of mitochondria and other cytoplasmic structures. In *P. ohirai*, double membranes surrounded autophagic vacuoles (Fig. 7). The origin of these membranes is uncertain. Some micrographs of *P. ohirai* suggest that the lateral plasma membranes are closely associated with autophagic vacuoles. This appears to be the case in Fig. 10, where the lateral plasma membranes with discrete AcPase activity are in close contact with the vacuoles, and the vacuole membrane also possess AcPase activity.

In *P. ohirai*, elongate filamentous structures emerge in the ribosomes and endoplasmic reticulum of the gastrodermis during later stages of starvation. Such inclusions have not yet been reported in any trematode, and the origin and function of these structures are uncertain. Bogitsh (1975) described the appearance of striated rootlets in the gastrodermis of *S. mansoni* and speculated that this structure might be "a sensory network associated with ceca by which an impulse may be transmitted." In *M. temperatus*, ciliated sensory endings were reported associated with the foregut region of the digestive tract (Bogitsh, 1972).

It seems difficult to conclude that the abovementioned ultrastructural differences following starvation between *P. ohirai* and the species from different host sites are due to physiological differences reflecting host sites.

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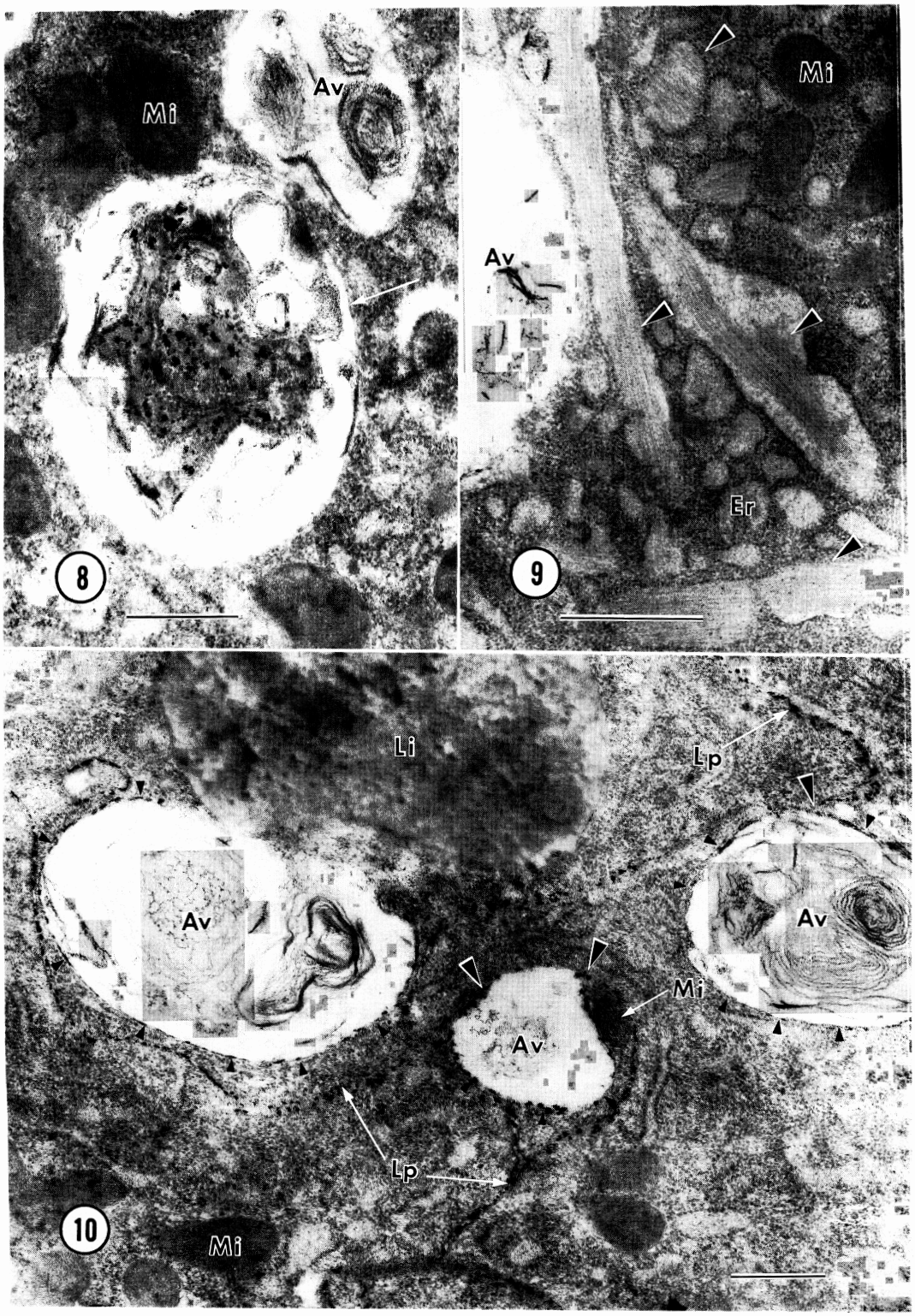
References

1) Bogitsh, B. J. (1972): Cytochemical and biochemical observations on the digestive tracts of digenetic trematodes. IX. *Megalodiscus temperatus*. Exp. Parasitol., 32, 244–260.
2) Bogitsh, B. J. (1973): Cytochemical and biochemical observations on the digestive tracts of digenetic trematodes. X. Starvation effects on *Megalodiscus temperatus*. J. Parasitol., 59, 94–100.
3) Bogitsh, B. J. (1975): Cytochemistry of gastrodermal autophagy following starvation in *Schistosoma mansoni*. J. Parasitol., 61, 237–248.
4) Bogitsh, B. J. (1982): *Schistosoma mansoni*: cytochemistry and morphology of the gastroder-

Fig. 6. The gastrodermis of worm starved for 40 hr and reacted for AcPase. Note autophagic vacuoles including various cytoplasmic elements. Only a few vacuoles show enzyme activity. Stained with uranyl acetate. Bar = 1.0 μm.

Fig. 7. The gastrodermis of worm starved for 40 hr and reacted for TPPase. The Golgi complexes (Go) showing different enzyme activity. Reaction product (r) is seen in a portion of the Golgi complex. Arrows indicate infoldings of the basal plasma membrane (Bp) close to autophagic vacuole (Av). Stained with uranyl acetate. Bar = 0.3 μm. Inset: An autophagic vacuole surrounded by vesicles with TPPase activity. Stained with uranyl acetate. Bar = 0.5 μm.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mal Golgi apparatus. Exp. Parasitol., 53, 57–67.
5) Bogitsh, B. J. and Ryckman, C. S. (1982): Ultrastructure of *Brachycoelium salamandrae* gastrodermis with observations on the effects of starvation. J. Parasitol., 68, 824–833.
6) Brandes, D., Buetow, D. W., Bertini, F. and Malkoff, D. B. (1964): Role of lysosomes in cellular lytic processes. 1. Effect of carbon starvation in *Euglena gracilis*. Exp. Molec. Pathol., 3, 583–609.
7) Dike, S. C. (1969): Acid phosphatase activity and ferritin incorporation in the ceca of digenetic trematodes. J. Parasitol., 55, 111–123.
8) De Duve, C. and Wattiaux, R. (1966): Functions of lysosomes. Ann. Rev. Physiol., 28, 435–492.
9) Ericsson, J. L. E. and Trump, B. F. (1966): Electron microscopic studies of the epithelium of the proximal tubule of the rat kidney. III. Microbodies, multivesicular bodies, and the Golgi apparatus. Lab. Invest., 15, 1610–1633.
10) Gomori, G. (1952): In "Microscopic histochemistry. Principles and practice", Univ. Chicago Press, Chicago, 189pp.
11) Jamieson, J. D. and Palade, G. E. (1967): Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. J. Cell Biol., 34, 577–596.
12) Levy, M. R. and Elliott, A. M. (1968): Biochemical and ultrastructural changes in *Tetrahymena pyriformis* during starvation. J. Protozool., 15, 208–222.
13) Novikoff, A. B. and Goldfisher, S. (1961): Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. Proc. Natl. Acad. Sci. USA, 47, 802–810.
14) Novikoff, A. B., Roheim, P. S. and Quintana, H. (1966): Changes in rat liver cells induced by orotic acid feeding. Lab. Invest., 15, 27–49.
15) Threadgold, L. T. and Arme, C. (1974): Electron microscope studies on *Fasciola hepatica*. XI. Autophagy and parenchymal cell function. Exp. Parasitol., 35, 389–405.

Fig. 8. The gastrodermis of worm starved for 40 hr and reacted for AcPase. A vacuole including unidentifiable content which shows AcPase activity and another vacuole with myelin-like material (Av). An arrow indicates the plasma membrane of the vacuole. Mi: Mitochondrion. Stained with uranyl acetate. Bar = 0.5 μm.

Fig. 9 The gastrodermis of worm starved for 40hr and reacted for AcPase. Some filamentous structures (arrowheads) occur in ribosomes and endoplasmic reticulum (Er). Av: Autophagic vacuole; Mi: Mitochondrion. Stained with uranyl acetate. Bar = 1.0 μm.

Fig. 10. The gastrodermis of worm starved for 40hr and reacted for AcPase. Autophagic vacuole (Av) includes concentric myelin-like structures. The lateral plasma membrane (Lp) with discrete enzyme deposits is seen close to the vacuoles. The membrane of the vacuoles has similar enzyme activity (small arrowheads) to that of the lateral plasma membrane. Large arrowheads indicate where the lateral plasma membrane appears to fuse with the vacuole membrane. Li: Lipid droplet; Mi: Mitochondrion. Stained with uranyl acetate. Bar = 0.5 μm.