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Phagocytosis and Autophagy in the Apical Gastrodermis of the Lung Fluke, *Paragonimus ohirai*

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Abstract

Phagocytosis was observed at the apical gastrodermis of *Paragonimus ohirai* adults by electron microscopy. Phagocytosed vacuoles containing luminal foodstuff were found in the cytoplasm. Autophagic vacuoles were observed in gastrodermal cells of senescent worms taken from albino rats at 170 days postinfection. These vacuoles were occasionally found in gastrodermal cells that were in a secretory phase of physiological activity, in spite of previous reports that their occurrence was limited primarily to cells in an absorptive phase. Some unusual autophagic vacuoles were also observed.

Key words: phagocytosis, autophagy, gastrodermis, *Paragonimus ohirai*, TEM

Introduction

Investigations of the ultrastructure and function of the gastrodermis of *Paragonimus* species have led to the idea that the epithelium undergoes morphological alterations that are related to secretion and absorption (Fujino and Ishii, 1988b). Regional differentiation of the gastrodermis has also been suggested by Ernst (1975) and Fujino and Ishii (1988b).

In the present study, phagocytosis and autophagy in the gastrodermis of *P. ohirai* is described by electron microscopy.

Materials and Methods

Paragonimus ohirai adults (170 days post-infection) were removed from the lungs of experimentally infected albino rats. Transverse slices of the worms were fixed for 2 hr at 4°C in 4% glutaraldehyde buffered to pH 7.3 with 0.1 M phosphate buffer. After postfixation with osmium tetroxide, dehydration with ethanol and embedding in Epon 812, the tissue was sectioned, stained with uranyl acetate and lead acetate, and viewed at 75 kV in a Hitachi HS-9 electron microscope.

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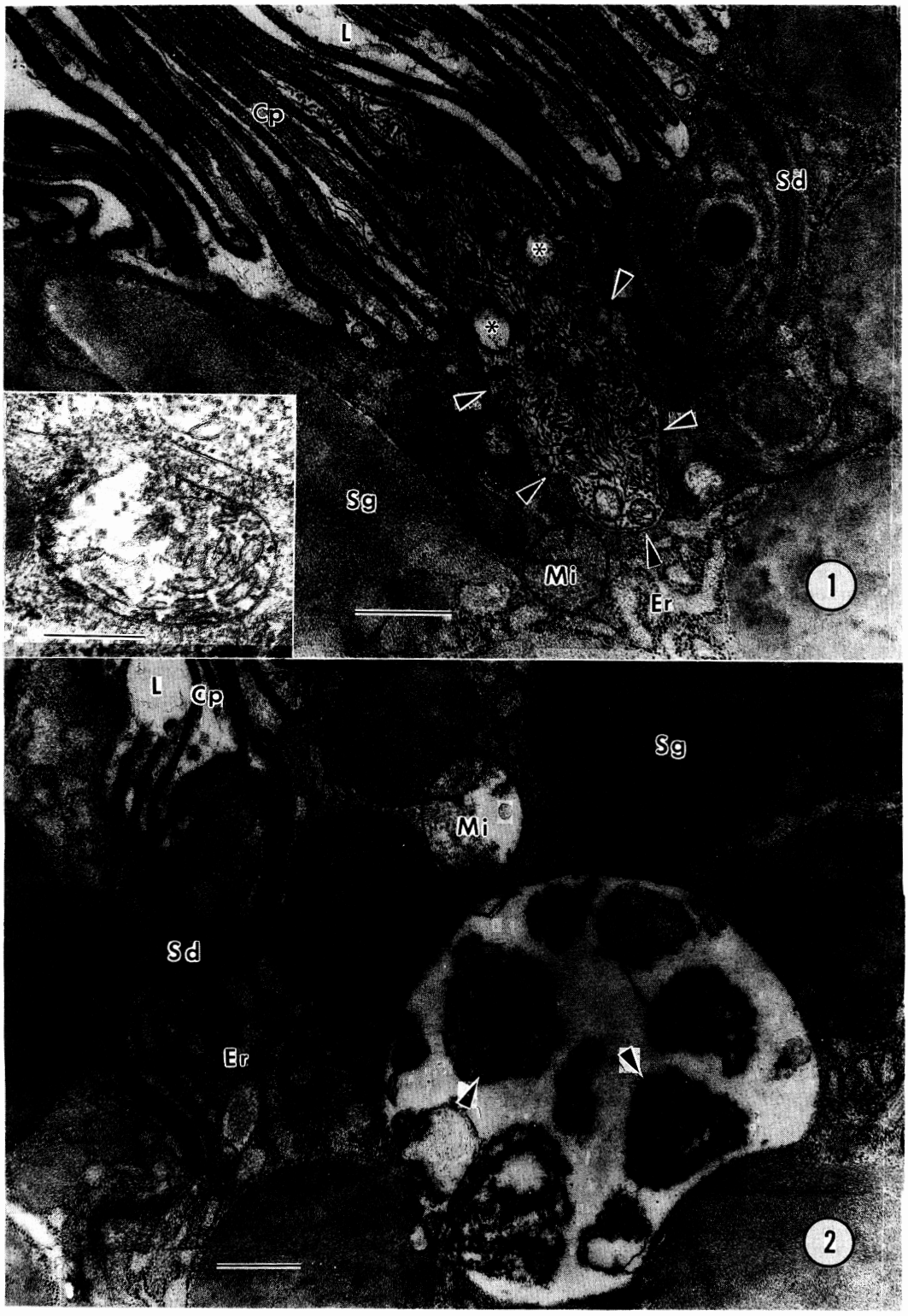
Results and Discussion

A sac-like invagination of the apical plasma membrane of the gastrodermis was observed (Fig. 1). This invagination formed at the base of cytoplasmic projections of the gastrodermis, and the apical plasma membrane was deeply invaginated into the cytoplasm. The invagination contained linear material and vesicular food substances, suggesting that the vacuole was developing by a phagocytic process. Similar linear material is commonly seen in the gastric lumen of *Paragonimus* species. It was tubular in shape, had a uniform thickness, varied in length and could be found over the lamellar surface of the cytoplasmic projections of gastrodermal cells (Fujino and Ishii, 1987). Membrane-bounded phagocytic vacuoles which contained luminal material were also observed (Fig. 1 inset).

Among digenetic trematodes, Dike (1969) suggested that phagocytosis occurs in the gastrodermis of *Haematoloechus medioplexus*, although Ernst (1975) and Thorsell and Björkman (1965) failed to demonstrate uptake of tracers by the gastrodermis of *Schistosoma mansoni* or *Fasciola hepatica*. Fujino and Ishii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(1988b) used cationized ferritin as a tracer for the investigation of the uptake of high molecular weight substances in *P. ohirai*. They demonstrated the incorporation of ferritin particles into a cytoplasmic vacuole. The present observations support the idea that gastrodermal cells of *P. ohirai* are capable of phagocytic activity. Køie (1971) also reported the occurrence of pinocytic vesicles or vacuoles in the apical intestinal cells of the redia of *Neophasis lageniformis*.

Autophagic vacuoles and/or linear or myelin-like structures have been reported in the gastrodermis of *F. hepatica* and *P. ohirai* during absorptive phases of the physiological cycle (Robinson and Threadgold, 1975; Fujino and Ishii, 1988b). Autophagic vacuoles may also be induced by starvation or some drugs (Bogitsh, 1973, 1975; Bogitsh and Ryckman, 1982; Clarkson and Erasmus, 1984; Fujino and Ishii, 1988a). In the present study, however, autophagic vacuoles were present even during the secretory phase (Figs. 2, 3, 5, 6). Residual material excreted from the cells appeared to be encircled by the cytoplasmic projections (Fig. 4). It is possible that these phenomena occur when worms reside in the host for a long time. It has been reported that encysted adult *P. ohirai* gradually degenerate and die within the lungs of albino rats, one of the definitive hosts, or leave their cysts when the length of infection exceeds 100 days (Miyazaki, 1946; Mera, 1951). The 170-day-old infections in the present study were probably long enough for encysted worms to become senescent. Therefore, it is possible that the autolysis occurs even during secretory phases of the gastrodermal cells.

Autophagic vacuoles usually contain identifiable material such as mitochondria or endoplasmic reticulum (Robinson and Threadgold, 1975; Bogitsh, 1973, 1975; Levy and Elliott, 1968; de Duve and Wattiaux, 1966; Fujino and Ishii, 1988a). Autophagic vacuoles containing secretory granules besides endoplasmic reticuclum or mitochondria were also observed in *P. ohirai* (Fig. 5). Little is known about the function of the Golgi-derived secretory granules in the gastrodermis (Bogitsh, 1982), although they are probably chiefly enzymatic in function (Smyth and Halton, 1983) or a major source of exportable polysaccharide (Wilson and Barnes, 1979). It is possible that autophagic vacuoles incorporate various kinds of cellular organelles as well as secretory granules into their contents. Unusual secretory granules which contained the remnants of embedded autophagic vacuoles were also observed (Fig. 6).

Acknowledgments

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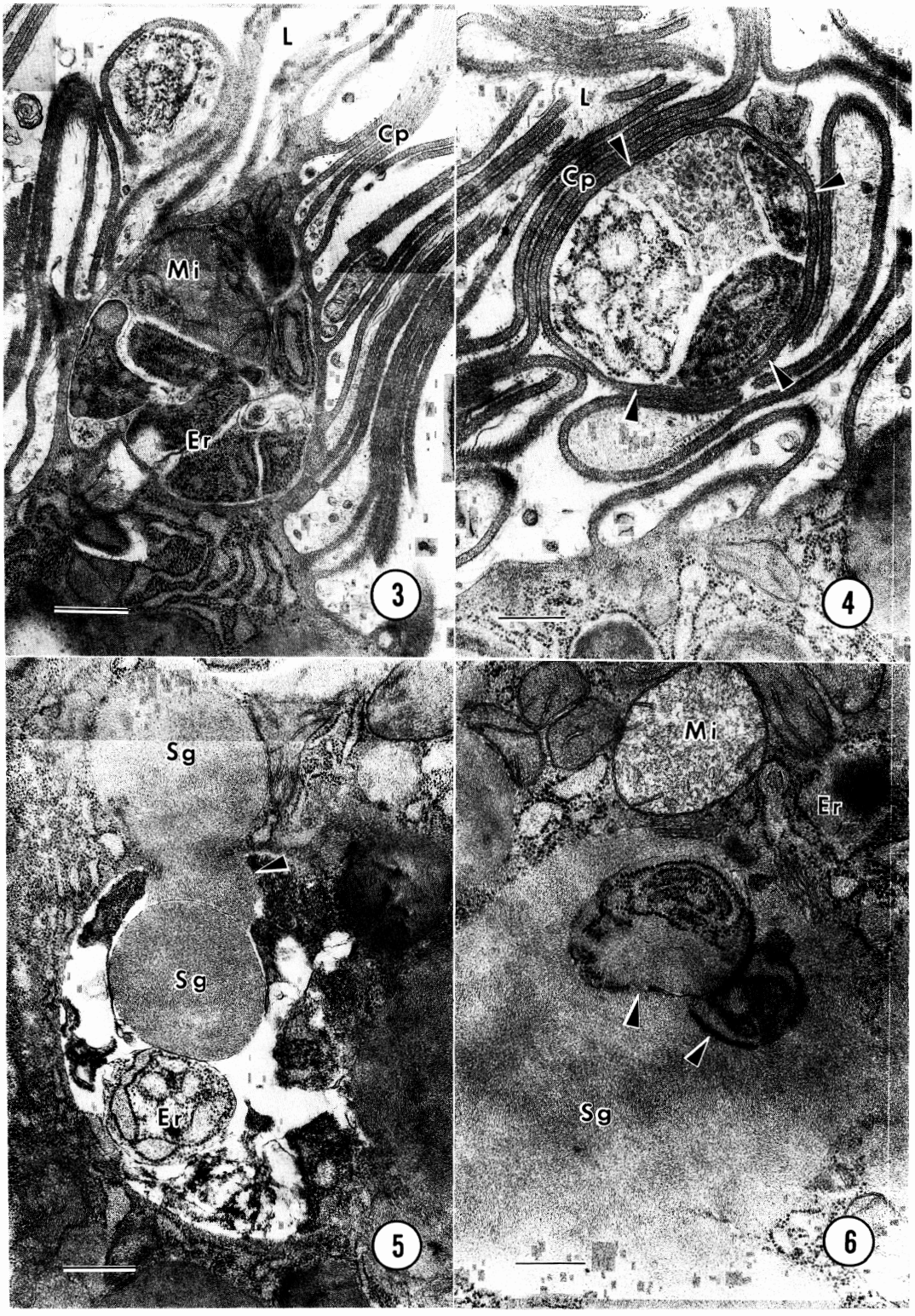
Fig. 1. Phagocytosis at the luminal surface of the gastrodermis. Arrowheads indicate invagination of the apical plasma membrane due to phagocytosis of foodstuff. Note that the same linear material (arrows) found between the bases of the lamellar cytoplasmic projections is packed together (double arrows) with vesicular food substances (*) in the invagination. Bar = 0.5 μm. Inset: Phagocytic vacuole formed in the apical area of the cell. Cp: Cytoplasmic projection; Er: Endoplasmic reticulum; L: Lumen; Mi: Mitochondrion; Sd: Septate desmosome; Sg: Secretory granule. Bar = 1.0 μm.

Fig. 2. Apical gastrodermis. A large autophagic vacuole contains unidentifiable material (arrowheads). Many secretory granules (Sg) and well-developed endoplasmic reticulum (Er) are also seen in the same cell. Cp: Cytoplasmic projection; L: Lumen; Mi: Mitochondrion; Sd: Septate desmosome. Bar = 0.5 μm.


L
Cp
Mi
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3
L
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4
Sg
Sg
Er
5
Mi
Er
Sg
6

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Fig. 3. An autophagic vacuole containing mitochondria (Mi) and rough endoplasmic reticulum (Er) at the apical end of the cell. Cp: Cytoplasmic projection; L: Lumen. Bar = 0.5 μm.

Fig. 4. Residual body (arrowheads) which is almost completely encircled by the cytoplasmic projections (Cp). L: Lumen. Bar = 0.5 μm.

Fig. 5. Autophagic vacuole in the apical gastrodermis. The vacuole already contained rough endoplasmic reticulum (Er) and a secretory granule (Sg). Another secretory granule (arrowhead) is being incorporated into the vacuole. Bar = 0.5 μm.

Fig. 6. Autophagic vacuoles in a secretory granule (arrowheads). Er: Endoplasmic reticulum; Mi: Mitochondrion; Sg: Secretory granule. Bar = 0.5 μm.