[Jpn. J. Parasitol., Vol. 36, No. 4, 227–238, August, 1988]

Secretion, Absorption and Lipid Excretion in the Gastrodermis of the Lung Flukes, *Paragonimus ohirai* and *P. westermani:* Ultrastructural Observations

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(Received for publication; March 26, 1988)

Abstract

Secretion, absorption and lipid excretion by the gastrodermis of the lung flukes, *Paragonimus ohirai* and *P. westermani,* were studied by light and electron microscopy and enzyme histochemistry. No distinctive differences in the above-aspects were found between *P. ohirai* and *P. westermani.* Ultrastructural and light microscopical observations indicated that the gastrodermal cells represent a continuing variation of a single cell as was described in the gut diverticula of *Fasciola hepatica* by Robinson and Threadgold (1975). Changes in cellular morphology occurred due to secretion and absorption. A cytochemical test for acid phosphatase showed variation in the activity of gastrodermal cells, probably reflecting localized, physiological differences. Uptake of cationized ferritin by the gastrodermis occurred by phagocytosis. Numerous lipid droplets were released from the gastrodermis, especially by cells in the late secretory phase. These lipids were identified histochemically as neutral lipid and released as described by Kurosumi (1961) by a process defined as 'macroapocrine' secretion.

Key words: *Paragonimus ohirai*; *P. westermani*; gastrodermis; secretion; absorption; ultrastructure

Introduction

Early studies on the structure and function of the digenean gastrodermis have been reviewed by Erasmus (1977) and Smyth and Halton (1983). In brief, there are two types of gastrodermis in digenetic trematodes: the syncytial gastrodermis which occurs in *Gorgodera amplicava* (Dike, 1967), *Gorgoderina attenuata* (Davis and Bogitsh, 1971), *Megalodiscus tempelatus* (Morris, 1973) and schistosomes (Morris, 1968; Shannon and Bogitsh, 1969; Spence and Silk, 1970; Bruce *et al.*, 1971; Ernst, 1975), and the cellular gastrodermis as seen in *Fasciola hepatica* (Robinson and Threadgold, 1975), *Haematoloechus medioplexus* (Davis *et al.*, 1968) and *Paragonimus* species (Dike, 1969; Fujino and Ishii, 1978). A regional differentiation in both morphology and cytochemical reactivity occurs in the syncytial gastrodermis of *Schistosoma mansoni* (Ernst, 1975). Robinson and Threadgold (1975) noted that each gastrodermal cell of the gut diverticula in *F. hepatica* has a cyclical transformation between secretory and absorptive forms. Secretion and absorption occur more or less simultaneously and continuously throughout the diverticula.

The gastrodermal structure of *Paragonimus* species has been studied by Dike (1969), Fujino and Ishii (1978) and Fujino *et al.* (1987). Dike (1969) described acid phosphatase activity in *P. kellikotti,* and the other authors examined the surface topography of some Japanese species by scanning electron microscopy.

This paper describes the functional morphology relative to secretion and absorption in the gastrodermis of *P. ohirai* and *P. westermani* which have simple, tubular guts unlike the diverticulated gut of *F. hepatica.* Reports of lipid excretion from the gastrodermis of digenetic trematodes have been few (Harris and

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Cheng, 1973), and such excretion is also examined in the present study.

Materials and Methods

Source of worms

Paragonimus ohirai (60 days-old) and *P. westermani* (100 days-old) adults were removed from lung cysts of experimentally infected albino rats and dogs, respectively. The worms were washed in Ringer's saline and phosphate buffer and then processed as follows.

Scanning electron microscopy (SEM)

The length of the guts of the worms pinnned on a silicone board was cut open with a fine needle in Ringer's saline. After washing with gentle flow of saline and buffer to remove debris the specimens were processed by the method of Fujino and Ishii (1978). The specimens were critical point dried, coated with gold and observed in a field emission SEM JEOL F15.

Transmission electron microscopy (TEM)

Worms were removed from the host tissue, cut into small pieces, fixed with 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, and postfixed in 1% osmium tetroxide. Some specimens were dissected as above and separated into anterior, middle and posterior regions before fixation. The tissue was dehydrated, embedded in Epon 812, sectioned, stained with both uranyl acetate and lead acetate, and viewed in a Hitachi HS-9 electron microscope operated at 75 kV. For light microscopic observations (LM), thick sections were cut and stained with Toluidine Blue 0.

Cytochemistry

Dissected worms that were stained for lipids were fixed for 12 hr at room temperature in buffered 4% formalin, frozen in dry ice-acetone, and then embedded in the water-soluble medium, O.C.T. compound Tissue TEK II (Lab-Tek Products, USA). Sections were cut on a cryostat at 10 μm and stained with Oil Red O or Sudan Black B.

Worms that were stained for acid phosphate (AcPase) activity were fixed for 2 hr at 4°C in 1% formaldehyde and 0.5% glutaraldehyde buffered with 0.1 M sodium cacodylate to pH 7.4. The specimens were rinsed overnight in the same buffer with 7% sucrose, and then sectioned at 20–30 μm thick using a McIlwain Tissue Chopper. The tissue was incubated in modified Gomori (1952) medium. Control sections were incubated in a substrate-free medium or in a medium to which 10mM sodium fluoride was added. After a brief rinse in the buffer, the tissue was postfixed for 1 hr in cacodylate-buffered 1% osmium tetroxide at 4°C and processed for TEM as described previously.

Tracer studies

For study of the uptake of high molecular weight compounds, some specimens of *P. ohirai* were dissected and incubated for 30 min at room temperature in 0.1 M phosphate buffered saline (pH 7.2) containing cationized ferritin (1.15 mg/ml). After the tissues were washed in the same buffer, they were fixed and embedded for TEM as described earlier.

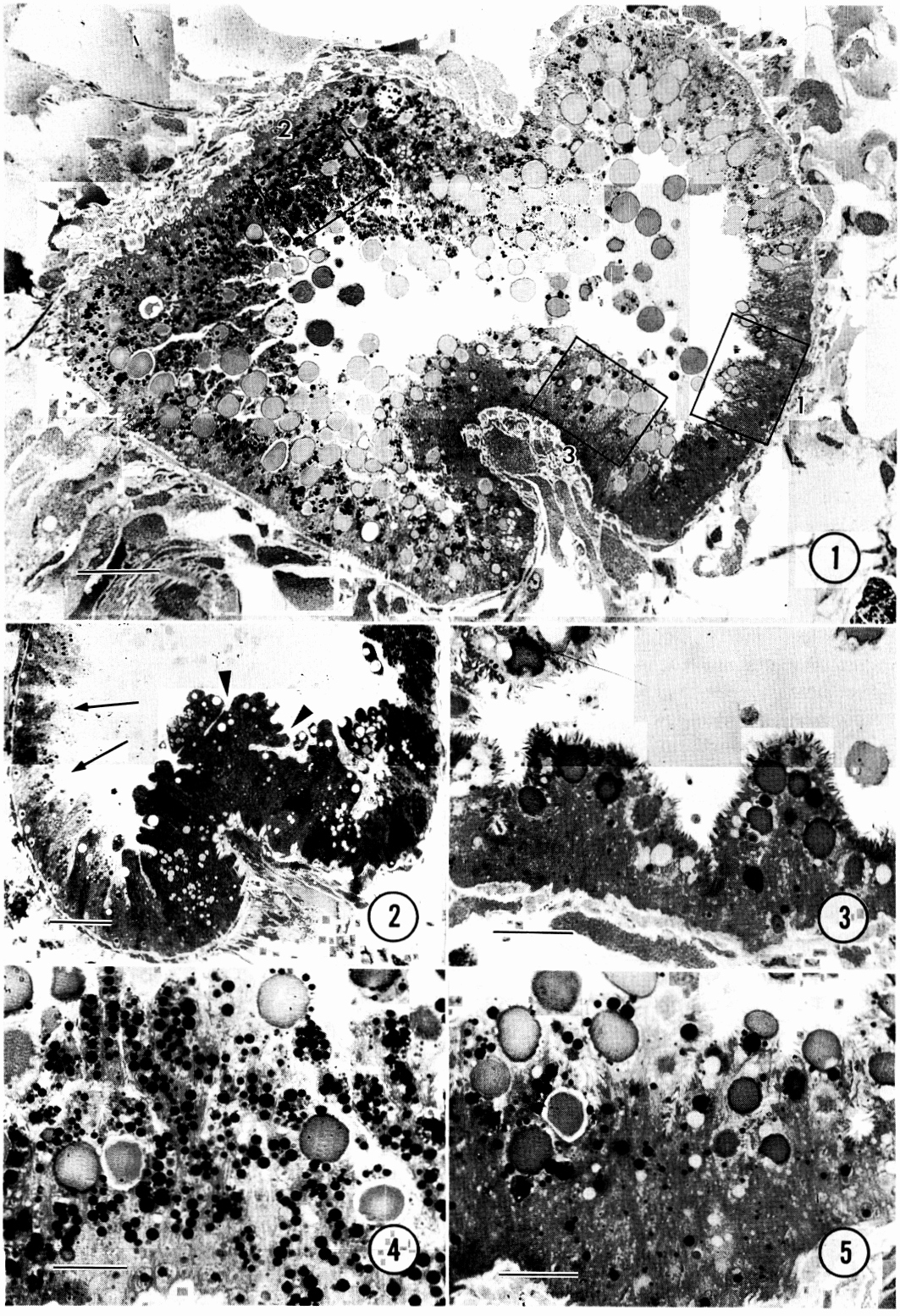
Fig. 1. Cross section of the middle region of the gut of *Paragonimus ohirai,* stained with Toluidine Blue O. The gastrodermis is composed of columnar and pyramid-shaped epithelial cells which differ locally in height. Small dense secretory granules and lucent lipid droplets are prominent. Rectangular areas indicate where enlarged pictures of Figs. 3-5 are taken. Bar = 50 μm

Fig. 2. Cross section of the posterior region of the gut of *P. ohirai* stained with Toluidine Blue O. The gastrodermis consists of groups of tall cells that contain numerous lucent secretory granules (arrowheads) and columnar cells that contain dense granules (arrows). Bar = 50 μm

Fig. 3. Enlarged view of rectangular area 1 in Fig. 1. Early secretory phase, in which the cells are pyramidal with many cytoplasmic projections, a few secretory granules and lipid droplets. Bar = 20 μm

Fig. 4. Enlarged view of rectangular area 2 in Fig. 1. Intermediate secretory phase, which is marked by the presence of dense secretory granules in the tall cells. Bar = 20 μm

Fig. 5. Enlarged view of rectangular area 3 in Fig. 1. Absorptive phase. The cells are tall with a few secretory granules and many lipid droplets. Bar = 20 μm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Results

Light microscopy

Cross sections of the gut were usually round with a few indentations that varied in depth. The gastrodermis was composed of both columnar and pyramidal epithelial cells. In cross sections these cells varied locally in height from one cell to another (Fig. 1). Some regional morphological differences were also observed in the gastrodermal cells. Tall columnar cells with few secretory granules occurred in the anterior most end of the gastrodermis near the esophagus. The posterior region of the gastrodermis consisted of tall groups of cells that were packed with lucent secretory granules along with cells with dense granules (Fig. 2).

Sections of the gastrodermis from the middle of the gut contained both low and pyramid-shaped cells. Cells which appeared to be in the early secretory phase, were in a part of the gastrodermis (Fig. 3). These cells had many cytoplasmic projections along their luminal edges and contained a few dense secretory granules and lucent lipid droplets. Tall, columnar cells had abundant dense secretory granules with few cytoplasmic projections and appeared in the intermediate secretory phase (Fig. 4). Groups of tall cells having only a few secretory granules and showing active excretion of lipid were in an absorptive phase (Fig. 5). When the lumen of the gut was filled with food, the gastrodermal cells were extended and flattened.

Electron microscopy

The tall, columnar cells with rounded apices corresponded to the early-intermediate secretory phase defined by Robinson and Threadgold (1975) (Fig. 6 and inset). Two types of secretory granules with different densities were seen. Rough endoplasmic reticulum was well developed with distended cisternae, some of which included small dense secretory granules that had been recently synthesized. Round or oval and rather dense mitochondria with short cristae were located basally. Short cytoplasmic projections on the apical end of columnar cells were few in number, while long cytoplasmic projections on the basal end of cells were moderately numerous.

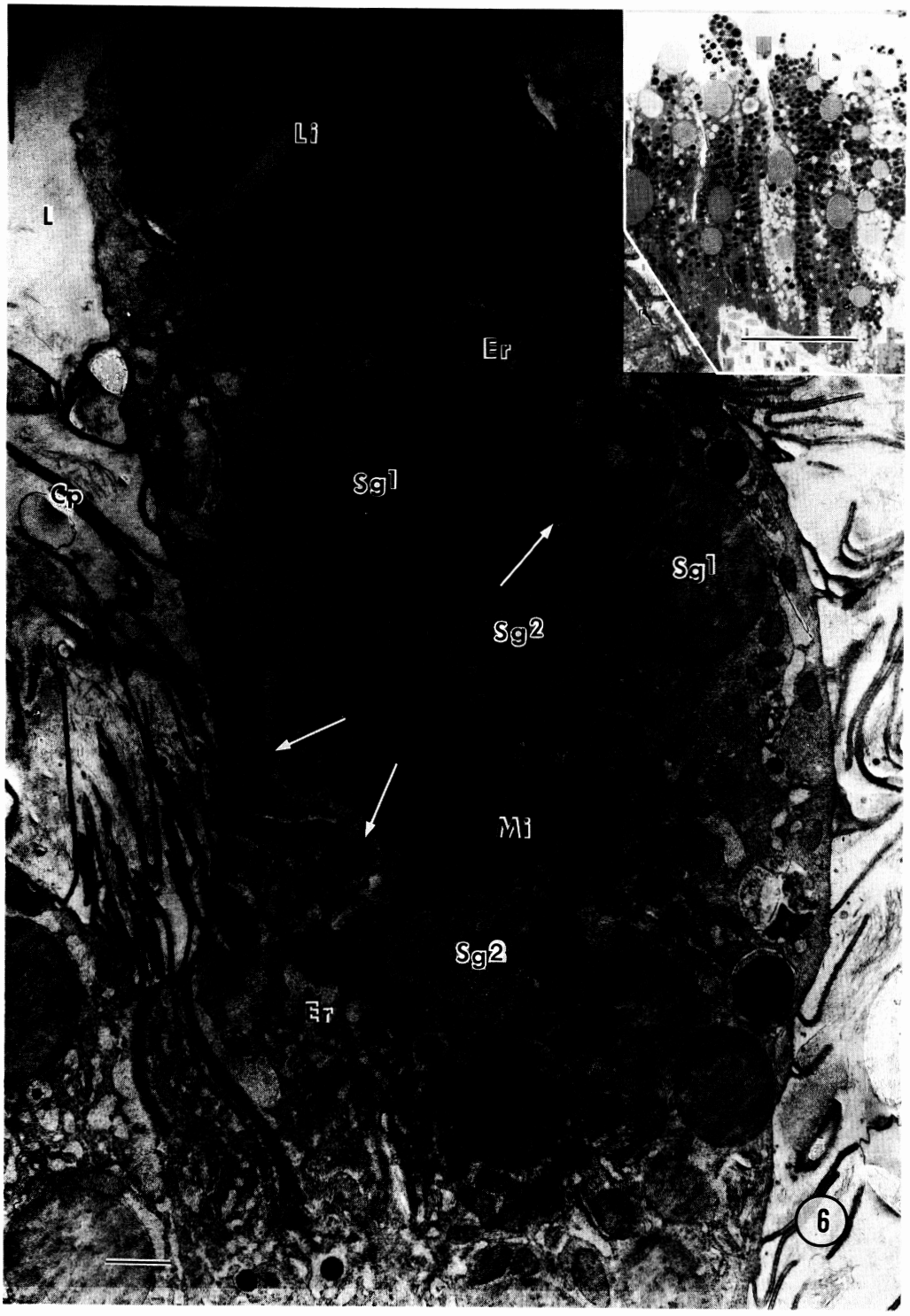
Groups of cells in an intermediate secretory phase were present (Fig. 7). These cells were filled with secretory granules of variable sizes. The cytoplasm contained a well-developed endoplasmic reticulum. The mitochondria were small, round, and dispersed throughout the cell.

In some cells in the late secretory or early absorptive phase, lysosomal or autophagic vacuoles were present in the apical region of the cytoplasm (Fig. 8). These bodies contained identifiable structures such as rough endoplasmic reticulum and mitochondria along with unidentifiable materials. Two kinds of secretory granules of different densities were identified. The mitochondria were elongate and dispersed throughout the cells. Some parts of the apical plasma membrane were invaginated into the cytoplasm to form phagocytic vesicles (Fig. 8). These phagocytic vesicles appeared to contain the same membranous food particles that were present in the lumen of the gut between the cytoplasmic projections.

Cells in an absorptive phase were low in height and had blunt or rounded apices and were characterized by a lack of secretory bodies and the presence of whorls or linear, sometimes myelin-like, membranous structures (Fig. 9). The mitochondria were round or elongate. Cytoplasmic projections were comparatively few and short.

SEM of the gastrodermal cell surface showed

Fig. 6. TEM of the gastrodermis of *P. ohirai*. A tall columnar cell in the early-intermediate secretory phase. Two types of secretory granules (Sgl and Sg2) are identified. Rough endoplasmic reticulum is well developed with distended cisternae, some of which contain small dense granules (arrows). Bar = 1 μm. Inset: light micrograph stained with Toluidine Blue O. Tall columnar gastrodermal cells contain two types of secretory granules with different densities. Cp: Cytoplasmic projection; Er: Endoplasmic reticulum; L: Lumen; Li: Lipid droplet; Mi: Mitochondrion; Sg: Secretory granule. Bar = 50 μm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lipid droplets in the process of being excreted (Fig. 11). The number of excreted lipid droplets appeared to be regionally different and varied from cell to cell. Round, swollen lipid droplets appeared to emerge from within the epithelium. The luminal surface of these lipid droplets was partly covered with fragments of the plasma membrane that contained the lamellar cytoplasmic projections. Smaller round swellings which appeared near the lipid droplets were probably secretory granules. Some lipid droplets had already been excreted and left holes on the epithelial surface. Lipid excretion was also observed in LM and TEM (Figs. 12 and 13). In these micrographs, large round lipid droplets that were surrounded by a well-developed endoplasmic reticulum lay at the apical end of the gastrodermal cell (Fig. 12). Portions of the cytoplasm and plasma membrane that covered the apical end of the lipid droplets were thin and partly broken as observed by SEM. A few, short cytoplasmic projections were seen over the apical gastrodermis. Lipid droplets with some cytoplasmic elements were excreted into the lumen and regurgitated through the esophagus (Fig. 13).

Cytochemistry

AcPase activity was different in each cell (Fig. 10). Cells which contained numerous secretory granules had reaction deposits in the endoplasmic reticulum. Most of the secretory granules were negative in activity. Granular reaction deposits were also localized on the surface and along the inner component of the plasma membrane of the cytoplasmic projections. Adjacent cells which contained fewer secretory granules demonstrated weaker enzyme activity. No reaction occurred in control.

Numerous lipid droplets in the gastrodermis and gut lumen were demonstrated as neutral lipids by staining with Oil Red O or Sudan Black B (Fig. 14). The number of lipid droplets produced and excreted into the lumen varied in different regions of the gastrodermis as well as among individual cells.

Tracer studies

A single interrupted layer of cationized ferritin particles covered the surface of cytoplasmic projections and in the plasma membrane between the bases of the projections. Ferritin particles were also incorporated into membrane-bound vesicles within the gastrodermal cells (Fig. 8 inset). Dense amorphous material in the vesicles was also labeled with ferritin particles.

Discussion

This study demonstrated that the gastrodermis of *Paragonimus ohirai* and *P. westermani* is composed of a single cell type which exhibits a variety of forms as has been described in *Fasciola hepatica* (Gresson and Threadgold, 1959; Robinson and Threadgold, 1975). No distinctive morphological differences were found between *P. ohirai* and *P. westermani*. Robinson and Threadgold (1975) stated that the gastrodermal cytoplasm has an ultrastructure which reflects its metabolic activity. They classified the gastrodermal cells of *F. hepatica* into three types. These include secretory and absorptive types, which are mainly found in the lateral diverticula, and a third type which is concerned with the movement of food in the main caeca. Secretory

Fig. 7. TEM of an apical area of the gastrodermis of *P. ohirai* in the early or intermediate secretory phase. The cells are tall and filled with secretory granules and extensive rough endoplasmic reticulum. Cp: Cytoplasmic projection; Er: Endoplasmic reticulum; L: Lumen; Sg: Secretory granule. Bar = 3 μm

Fig. 8. TEM of an apical area of the gastrodermis of *P. westermani*. The cells are in the late secretory or early absorptive phase. Lysosomal bodies are present. Arrows indicate an invaginated area in the apical plasma membrane which is in the process of forming phagocytic vacuoles (arrowheads). Two types of secretory granules (Sgl and Sg2) are present. Bar = 1 μm. Inset: A vacuole containing cationized ferritin (arrowheads). Cp: Cytoplasmic projection; Er: Endoplasmic reticulum; L: Lumen; Ly: Lysosomal bodies; Mi: Mitochondrion. Bar = 0.3 μm


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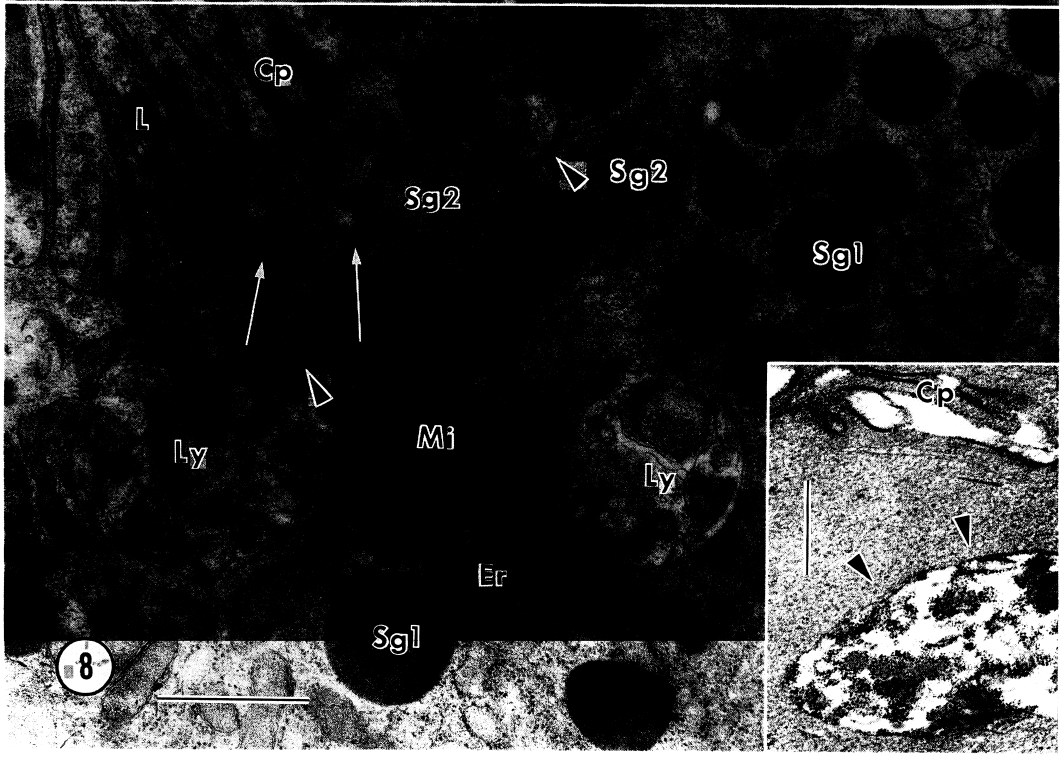
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and absorptive cells were subdivided into early, intermediate and late forms based on their morphology and cellular features.

In the *Paragonimus* species examined, cross sections of the gastrodermis showed regional and local as well as cellular morphological differences. We found that the morphology of the gastrodermis varied considerably and depended on the amount of food in the gut lumen and a peristaltic movement of the gut. Localized groups of cells were in the same morphological and probably physiological phases. At the anterior most end of the gut, uniformly tall groups of gastrodermal cells that lacked secretory granules may have been in the absorptive phase, or may have been functionally differentiated as in the esophagus and caeca of *Schistosoma mansoni* (Ernst, 1975). Regardless, the morphological and cellular features of each phase of the *Paragonimus* were similar to those of *F. hepatica* (Robinson and Threadgold, 1975). Cells in the early secretory phase were pyramidal with few secretory granules and numerous cytoplasmic projections along the luminal edge. Cells in the intermediate secretory phase were columnar, tall and were filled with secretory granules that were scattered throughout the cytoplasm. These cells were unlike the corresponding cell type in *F. hepatica,* in which granules are distributed mainly in the apical cytoplasm. The secretory granules we observed were distended cisternae of the rough endoplasmic reticulum. Cells in the late secretory phase were also tall and included linear membranous structures and some secretory granules. Cells in the absorptive phase, as in *F. hepatica,* were characterized by membranous structures and lysosomal bodies that contained the endoplasmic reticulum, mitochondria and unidentifiable material.

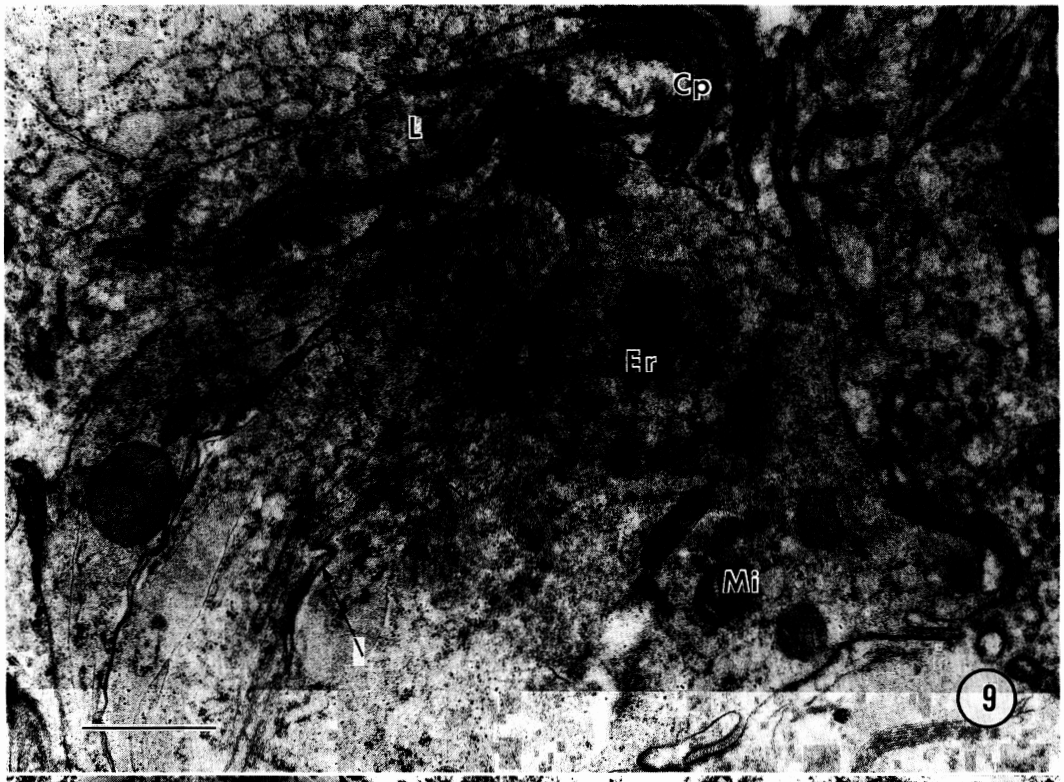
In cells of the absorptive phase, phagocytic activity was observed at the apical end of the cell. The apical plasma membrane between the bases of the cytoplasmic projections was partly invaginated into the cytoplasm to form phagocytic vesicles. The test with cationized ferritin demonstrated the incorporation of the particles into membrane-bound vesicles in the cytoplasm. These observations agree with phagocytosis in the gastrodermis of *Haematoloechus medioplexus* (Dike, 1969). Ernst (1975) did not observe the phagocytosis of a variety of high molecular weight tracers such as ferritin, peroxidase, thorotrast and latex beads in his studies of the gastrodermis of *Schistosoma mansoni.* Thorsell and Björkman (1965) also failed to demonstrate uptake of ferritin in *F. hepatica.*

Digestion of food in digenetic trematodes is predominantly extracellular which is completed intracellularly (Halton, 1967). Ultrastructural studies have not yet provided conclusive evidence for an endogenous source of hydrolytic enzymes in the gastrodermal cells (Erasmus, 1977). We observed two types of secretory granules with different densities in gastrodermal cells of *Paragonimus* species that were associated with cells in the secretory phase. The relative proportions of these two types vary by cell and region. It is not clear from our observations whether these granules are a part of different processes, are part of the same secretory process or are functionally different.

Cytochemical studies of *P. westermani* demonstrated AcPase reaction product that was mainly associated with the endoplasmic reticulum and the inner component of the plasma

Fig. 9. TEM of an apical area of the gastrodermis of *P. westermani* which is in the absorptive phase. Few secretory granules are present. Membranous material (arrows) and poorly-developed endoplasmic reticulum are characteristic. Cytoplasmic projections are prominent. Cp: Cytoplasmic projection; Er: Endoplasmic reticulum; L: Lumen; Mi: Mitochondrion. Bar = 1 μm

Fig. 10. TEM of an apical area of the gastrodermis of *P. westermani,* after the reaction for AcPase. Enzymatic activity varies among the cells. Dense reaction product is associated with the endoplasmic reticulum in cell A, but not with the numerous internal secretory granules. By contrast, adjacent cells B and C have little reaction product in the cytoplasm and in cytoplasmic projections and also have fewer secretory granules. Er: Endoplasmic reticulum; L: Lumen; Sg: Secretory granules. Bar = 1 μm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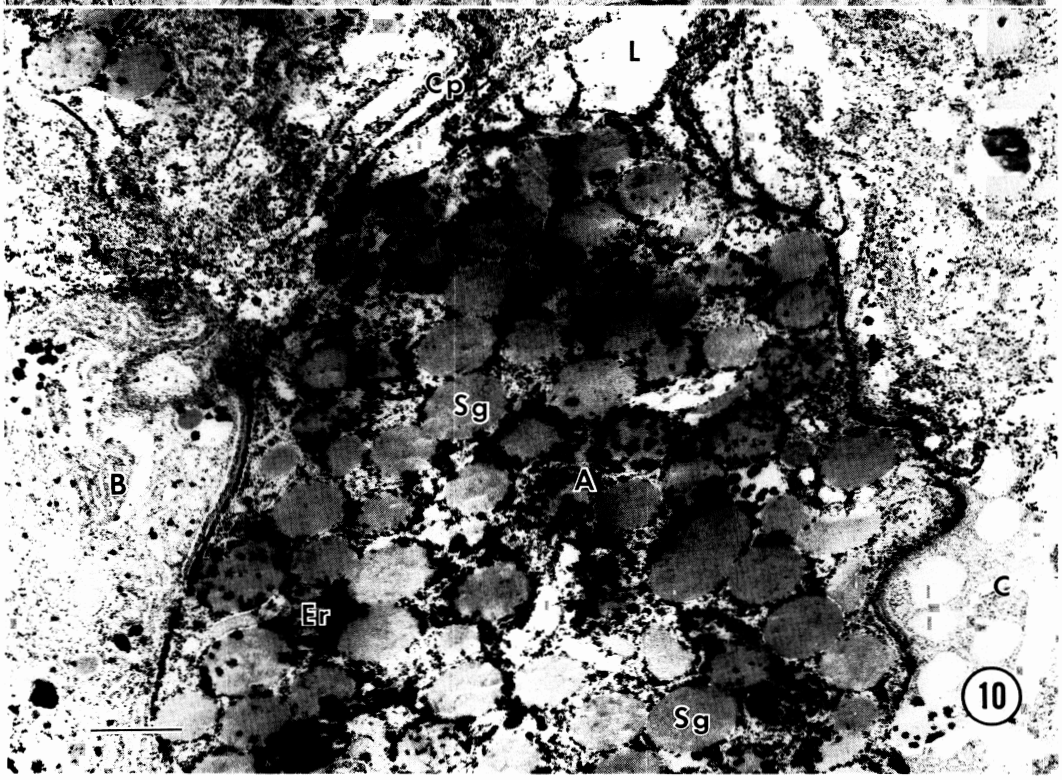
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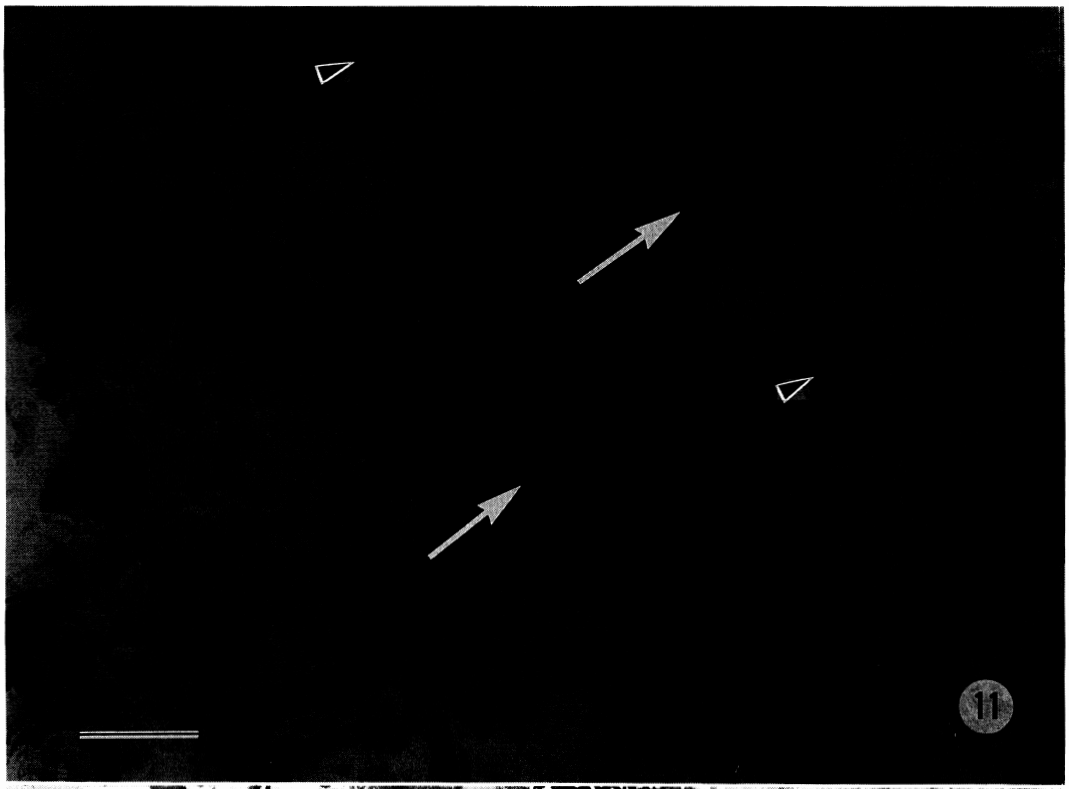
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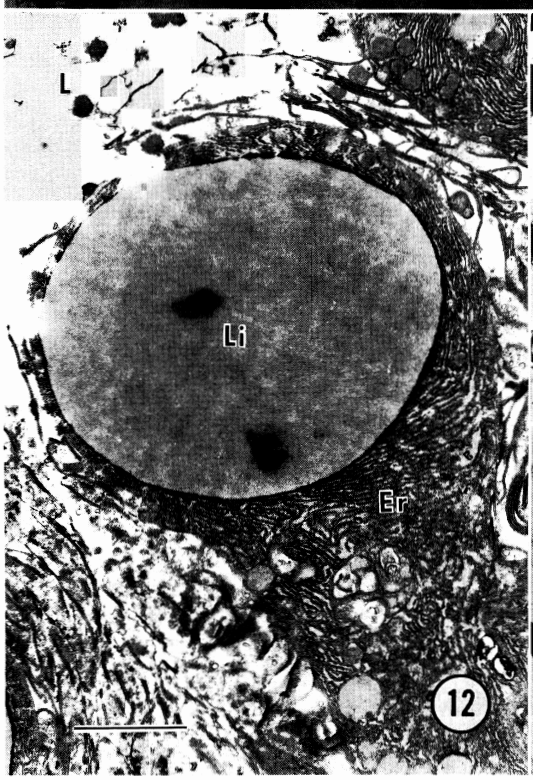
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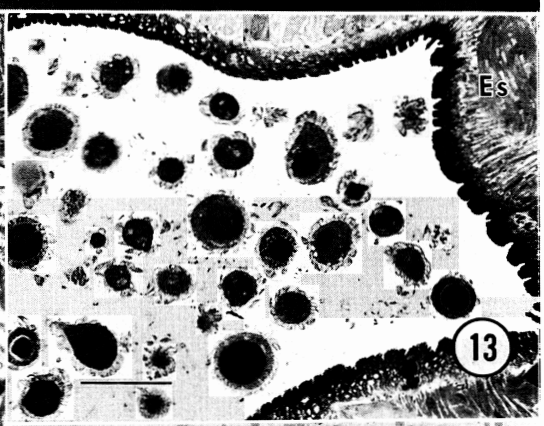
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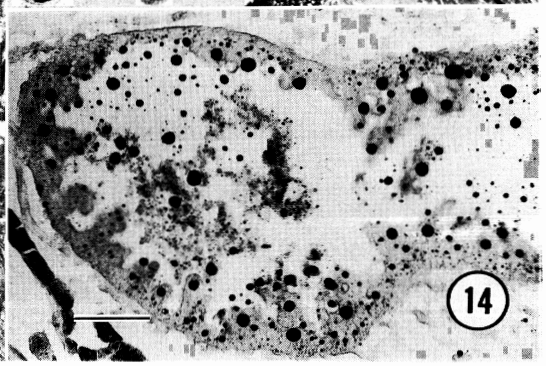
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membrane of cytoplasmic projections. Similar findings have been described in *H. medioplexus* (Dike, 1969). Although Dike (1969) demonstrated AcPase activity in numerous membrane bounded bodies in *P. kellikotti,* most of granules in our study did not have AcPase activity. Variations occurred in the intensity of the enzyme activity in each cell, and the reaction products appeared to be denser in cells that were in the secretory phase than those in the absorptive phase. These variations may reflect different physiological conditions in secretory and absorptive cells. Similar differences in enzyme activity were observed in the cells of *F. hepatica* (Fujino *et al.*, 1983). These findings suggest that AcPase is involved in the secretion and/or digestion of foodstuffs (Threadgold, 1968; Lumsden, 1975). It is still not clear, however, whether secretory granules have hydrolytic activity.

The excretion of lipids by the gastrodermis has been reported in only a few digenetic trematodes, although neutral fat has been localized in the excretory system of most trematodes (Harris and Cheng, 1973). Large numbers of neutral fat droplets were demonstrated histochemically in the gastrodermis of *Leucochloridiomorpha constantiae* metacercariae (Harris and Cheng, 1973). They noted that the amount of lipid within the gut depended on the season when metacercariae were collected and also changed when the medium used for *in vitro* cultivation was altered. In *Paragonimus* species, large amount of neutral lipids were excreted by gastrodermal cells that were in the late secretory phase. The cytoplasm of cells in this stage contained well-developed endoplasmic reticulum that surrounded the lipid droplets. The excretion of lipid droplets falls into Kurosumi's (1961) category of 'apocrine' secretion. As he stated, "the secretion contains more or less the debris of cytoplasm and plasma membrane besides the secretory substance." Observations by SEM, TEM and LM showed that lipid droplets excreted into the lumen or which were in the process of being excreted from gastrodermal cells, were surrounded by cytoplasm and cytoplasmic projections. The function of excreted lipids in some trematodes has been discussed by a number of studies (Fried and Roberts, 1972; Fried and Jacobs, 1980; Fried *et al.*, 1980; Fried and Robinson, 1981). These authors stated that sterol esters and free sterols of neutral lipids function as chemo-attractants that induce pairing and aggregation of the trematodes. In *Paragonimus* species, adult worms are usually paired within the lung cysts, and lipids excreted from the gut may also facilitate this process.

Acknowledgements

The authors thank Dr. B. Fried of Lafayette College and Dr. C. T. Atkinson of Case Western Reserve University for reviewing the manuscript.

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Fig. 11. SEM of the gastrodermal surface of *P. ohirai.* The surface is covered by numerous triangular lamellar cytoplasmic projections. Arrowheads indicate lipid droplets that are about to be excreted. Arrows mark empty holes, from which lipid droplets have already been released. Bar = 0.3 μm

Fig. 12. TEM of an apical area of the gastrodermis of *P. westermani.* A large lipid droplet is being excreted from the epithelium. Note the well-developed endoplasmic reticulum. Er: Endoplasmic reticulum; L: Lumen; Li: Lipid droplet. Bar = 3 μm

Fig. 13. Cross section through the esophagus of *P. ohirai,* which has been stained with Toluidine Blue O. Lipid droplets with some cytoplasmic elements are about to be excreted from the gut and regurgitated through the esophagus. Es: Esophagus. Bar = 30 μm

Fig. 14. Cross section of the gut of *P. ohirai,* stained with Oil Red O. Numerous lipid droplets are excreted from the gastrodermis. Bar = 100 μm

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