[Jap. J. Parasit., Vol. 31, No. 3, 197-201, June, 1982]

Cytological Studies on the Bushy Cells in the Pig *Ascaris* (*Ascaris suum*)

II. Ultrastructure and Phagocytotic Function

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(Received for publication; March 29, 1982)

Key words: *Ascaris suum*, bushy cells, ultrastructure, phagocytosis

In the bushy cells of the pig *Ascaris*, the phagocytotic function was light- and electron microscopically reported to occur previously by a number of authors (Bolla *et al.*, 1972). These previous reports were based, on the whole, upon the accumulation or storage of foreign substances in the cytoplasm of the bushy cells of the ascaris. There have, however, not been any evidence for images of phagocytotic processes in such cells. In the present study, precise ultrastructural features of the phagocytotic processes in the cytoplasm of the bushy cells have been examined by means of transmission electron microscopy. According to the results obtained in the present work, it is suggested that the bushy cells show an activity of taking up iron particles.

Materials and Methods

Ten female pig *Ascaris* (*Ascaris suum*) were obtained from adult pigs sacrificed in the slaughter house of Nagoya City, Japan. These worms were kept alive in physiological Ringer solutions maintained at 37 C and 0.1 ml of 1% ferritin (Miles Laboratories, Inc. USA) solution was injected into their celiac cavity. The ferritin injected worms were furthermore kept alive in the Ringer solutions containing 1% ferritin at 37 C for about twenty minutes before their sacrifice for the removal of bushy cells and related tissues.

From the living worms, bushy cells with neighboring tissues were dissected out and immediately cut into cubes with a side less than 1 mm and fixed in chilled (4 C) 0.15 M phosphate buffered (pH 7.2) 2% osmium tetroxide or chilled (4 C) 0.15 M cacodylate buffered (pH 7.4) 2.5% glutaraldehyde for 1.5 to 4 hours. The osmium tetroxide fixed tissues were dehydrated in an ethanol series of ascending concentrations and embedded in Epon 812, as prescribed by Luft (1961). The glutaraldehyde fixed tissues were rinsed in cacodylate buffer (pH 7.4) for 2 to 4 hours, postfixed at room temperature for 2 hours in 0.15 M cacodylate buffered (pH 7.4) 1% osmium tetroxide, dehydrated in the graded ethanol series and embedded in Epon 812 as mentioned above. From the Epon embedded tissue blocks, thick sections with a thickness of approximately 1 μm were cut on an Ultramicrotome (JUM No. 5), stained with toluidine blue and used for trimming tissues containing bushy cells. From the trimmed tissue blocks, ultrathin sections with an interference colour of silver gold were cut on another Ultra-

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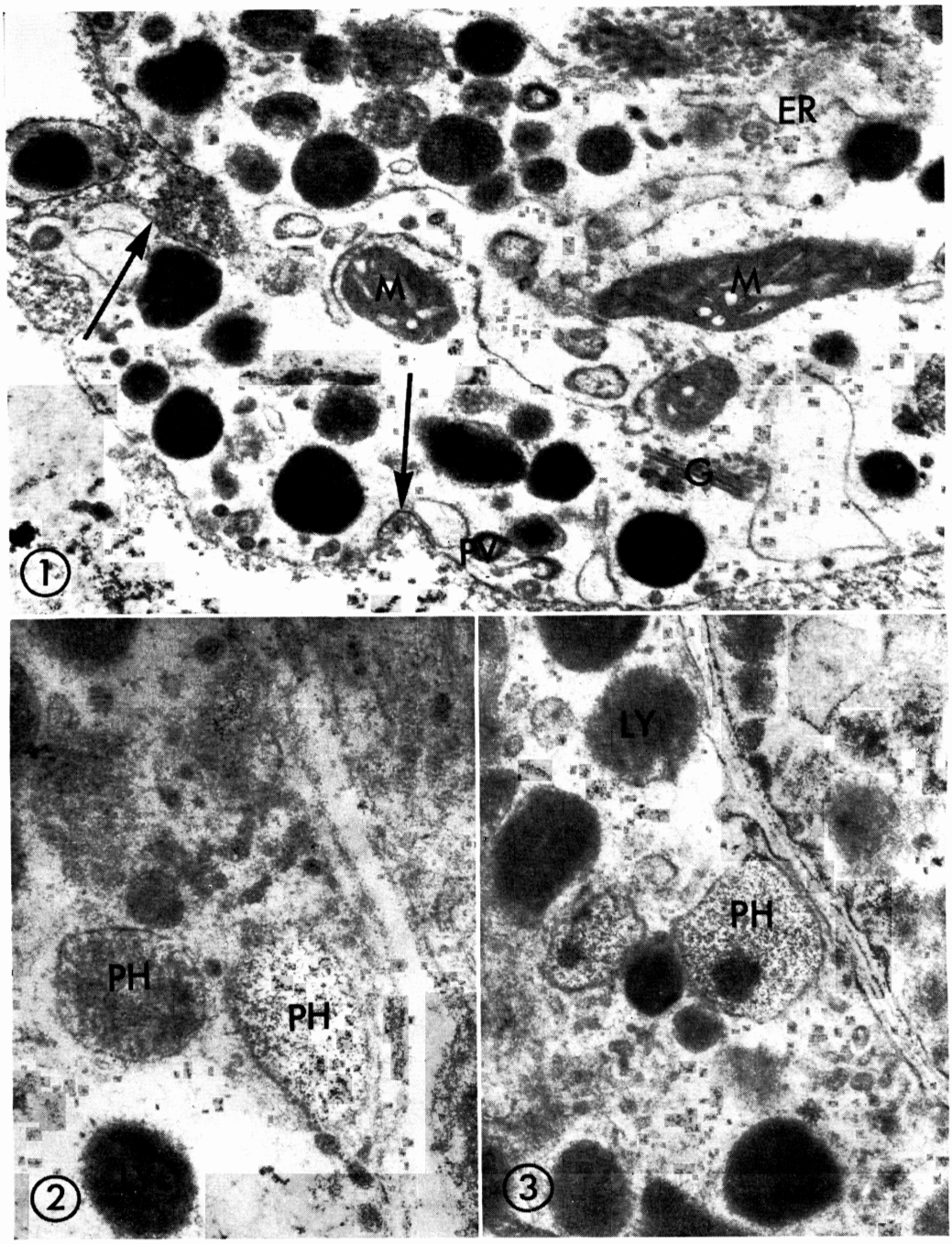

Explanation of Figures

Figs. 1–3 Parts of the peripheral cytoplasm of bushy cells in a pig *Ascaris*. Uranyl acetate-lead citrate stained.

Fig. 1 Ferritin injected. Both phagocytotic and pinocytotic invaginations (arrows) are noted. Mitochondria (M), Golgi complex (G), endoplasmic reticulum (ER), vesicles (PV). (×26,000).

Fig. 2 Ferritin injected. Phagosomes are obvious. Phagosomes (PH), lysosome (LY). (×33,000).

Fig. 3 Ferritin injected. Phagosomes (PH), lysosomes (LY). (×33,000).

microtome (Porter-Blum MT-1 type, Ivan Sorvall Co. Ltd. USA), mounted on copper grids and stained doubly with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Then, the stained sections were examined in a transmission electron microscope (Hitachi HU-11D or HS-4). Pictures were taken at original magnifications ranging from 2,000 to 10,000 times and photographically enlarged as desired.

Results

When the bushy cells of the pig ascaris were exposed to celiac fluids into which ferritin particles were experimentally induced, the plasma membrane and its subjacent cytoplasm were found to show a sequence of images suggestive of the phagocytotic and pinocytotic activities of the cells. In Fig. 1, clusters of ferritin particles were shown to be incorporated in an invagination of the cytoplasmic membrane. In the cytoplasm right beneath the plasma membrane, vesicles and vacuoles were visualized which contain ferritin particles possibly introduced by phagocytotic or pinocytotic activities of the cells (Fig. 2). In the peripheral cytoplasm of occasional bushy cells exposed to ferritin particles, some of ferritin particle-containing vacuoles were found to involve debris of electron opaque materials which were comparable in features to lysosomal dense bodies (Fig. 3).

Discussion

In the bushy cells of *Ascaris,* the phagocytotic functions have been light microscopically demonstrated previously by numerous authors. In 1900, Nassonov injected India ink, dyes and bacteria (*Bacillus anthracis*) into the celiac cavity of the nematoda. He observed that these foreign solid particles were taken up by terminal swellings of the stellate (bushy) cells. In line with this report, Metalnikoff (1923) subsequently injected other bacteria such as tubercular bacilli, *Sarcina lutea* and micrococci from *Gulleric mellonella* into the celiac cavity of *Parascaris equorum* and likewise found that these microorganisms were taken up by the same cells. In the bushy cells of *Ascaris lumbricoides,* furthermore, phagocytotic activities were noted by Dobashi (1933) who injected a series of dyes (neutral red, trypaflavin, and safranin) into the body of the worm. Similar activities of the bushy cells were reported to exist in *Ascaris* worms by Murase (1943), who injected India ink and dyes into the worm bodies. In agreement with the results of all these previous studies, the present author (Ishikawa, 1960) revealed that, in the pig *Ascaris,* the bushy cells exhibit an activity of taking up India ink. In contrast with these results indicative of phogocytotic activities of the bushy cells in *Ascaris* worms, Müller (1929) failed to find any evidence of phagocytotic activities in any parts of the cytoplasm of the cells and could only detect agglomerations of injected materials on the cell surfaces. In 1961, Fukuda incubated living bushy cells from *Ascaris lumbricoides* in the Ringer solutions containing India ink at 37 C for 15 minutes and electron microscopically observed the fate of India ink particles in the cells. According to his observations, India ink particles with a diameter of less than 1 μm were first scattered in the peripheral areas of the cytoplasm and secondarily transported towards areas containing bundles of filamentous figures where they were seen to be accumulated together. In keeping with these results obtained by Fukuda (1961), Bolla *et al.* (1972) could visualize electron dense bodies in the peripheral areas of the cytoplasm of bushy cells from *Ascaris* worms. These dense bodies ranged in diameter from 0.5 to 1.0 μm, were spherical, ovoid

or irregular in shape and occasionally contained both myelin figures and vacuoles which suggest their lysosomal natures. In 1976, the present author (Ishikawa 1976) could show images of pinocytosis and the presence of lysosomal dense bodies in the cytoplasm of the bushy cells from pig *Ascaris*. Despite these previous studies by means of methods of both light and electron microscopy, however, it seems that the phagocytotic activities of the bushy cells from ascaris worms have been subjected to confusion, since any concrete evidence for the actual activities of phagocytosis has never been presented up till now, to the best of my knowledge. The present experimental study by means of methods of transmission electron microscopy has been performed, with a view to draw a conclusion as to the actual phagocytotic activity of the bushy cells. The results obtained in the present work can be regarded as substantiating the validity of actual phagocytosis in the bushy cells of pig *Ascaris*.

Summary

Pig *Ascaris* worms injected ferritin were kept alive in ferritin containing Ringer solutions. The cytoplasm of the bushy cells has been observed by an electron microscope. Evidence has been presented which indicates that ferritin particles are ingested into the cytoplasm by a process of phagocytosis or pinocytosis. These ferritin particles appear to be incorporated into dense bodies which are either phagosomes or digestive vacuoles of lysosomal nature.

Acknowledgments

The author expresses his sincere thanks to Associate Professor, Kazuyori Yamada for the constant support and encouragement during the course of the present study.

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ブタ回虫花房状細胞の細胞学的研究
II　超微構造と食作用

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ブタ回虫花房状細胞が食作用を備えることを実験電子顕微鏡学的研究によって証明した．すなわち，フェリチン含有リンゲル液中に生活させたブタ回虫の花房状細胞の細胞膜にはフェリチン顆粒の食作用による取込みまたは飲作用を示す像が認められ，さらにその近接細胞質にはリソゾーム性のファゴゾームや消化空胞が存在する．