[Jpn. J. Parasitol., Vol. 39, No. 1, 1–6, February, 1990]

Ultrastructural Changes in the Esophagus, Intestine, and Excretory Organ of Larval *Anisakis* (Ascaroidea: Nematoda) after Incubation in Artificial Gastric Juice

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(Accepted for publication; November 7, 1989)

Abstract

The role of excretory and digestive organs in *Anisakis* larva was described based on their morphological changes after incubation in artificial gastric juice. The excretory organ consisted of a large cell with a main duct. The cytoplasm was filled up with many round granules of various sizes and electron densities. The wall of both intestine and ventriculus consisted of columnar cells which were partially occupied by dense granules. The granules in the ventriculus and intestinal cells were unchanged when *Anisakis* larva was incubated for 10 hrs. On the other hand, the ultrastructural change in the excretory organ was observed in larvae after incubation. Many granules in the excretory organ became less dense and fused together. The excretory organ seems to play an important role when the worm penetrates into human tissues.

Key words: **Ultrastructure, ventriculus, intestine, gastric juice, excretory organ,** *Anisakis*

Introduction

Ultrastructural characteristics of larval *Anisakis* have been described, in particular, from the taxonomical point by scanning electron microscopy (SEM) (Soleim, 1974; Aji *et al.*, 1982; Smith, 1983; Fujino *et al.*, 1984; Weerasooriya *et al.*, 1986; Fukuda *et al.*, 1988). The larva has a characteristic excretory organ, the renette cell. However, little is known about its histology and function (Ruitenberg and Loendersloot, 1971; Lee *et al.*, 1973).

Human anisakiasis has recently become a center of interest of nematode infection in Japan (Ishikura, 1978; Oshima, 1987). Most of the larval Anisakis penetrate into the wall of the human stomach, which is rich in acidic gastric juice. However, there is little information on functional and fine structural changes in the excretory organ and the gut cells when the worm has penetrated into human bodies.

In the present study we describe ultrastructural changes of the excretory organ, esophagus, ventriculus and intestine after incubation in artificial gastric juice to simulate the worm taken into a human stomach, and elucidate a role of many granules in the cells of these organs.

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Materials and Methods

Third stage larvae of *Anisakis simplex* (= *A.* type-I) were collected from internal organs of mackerels (*Scomber japonicus*). After washing with 0.85% NaCl-solution, the worms were incubated in artificial gastric juice (12N hydrochloric acid: 7ml, pepsin; 1g, H_2O: 1000 ml) at 37°C for 2, 4, 8, and 10 hrs respectively. Control larvae were treated in the same manner using 0.85% NaCl-solution instead of gastric juice.

The esophagus, ventriculus, and intestine were cut into several pieces of about 2 mm in length. Then the specimens were immediately pre-fixed for 2 hrs in Karnovsky's fixative (Karnovsky, 1965) in an icebox. After several rinses in cacodylate buffer, the specimens were post-fixed for 2 hrs with 2% osmium tetroxide (pH 7.4). The fixed materials were dehydrated in a graded series of ethanol and embedded in Epon (Oken

Syoji Co. Ltd., Tokyo). The materials were cut with a Reichert OmU2 ultramicrotome, double-stained with uranyl acetate and lead hydroxide, and examined under a Hitachi HS-8 electron microscope.

Results

Ultrastructure of normal excretory organ, ventriculus and intestine.

In the esophagus and ventriculus, the luminal surface of a tri-radiate lumen was covered with a thin cuticle approximately 80 nm thick (Fig. 1, arrow). The wall of the esophagus consisted of mainly well-developed muscle fibers arranged radially. The ventriculus lumen sometimes branched out at the periphery. The wall of the ventriculus consisted of columnar cells which were arranged radially in cross section. In these cells, most of the cytoplasm contained homogeneous granules of various sizes and electron-densities (Fig. 1). Many mitochondria, well-developed smooth endoplasmic reticulum, and a few filaments were scattered in close vicinity to the cuticle. A basement membrane, approximately 330 nm thick, covered the outer surface of the ventriculus. A cell membrane of the ventriculus showed notable infoldings (Fig. 2), and many mitochondria were distributed around these infoldings. A nucleus (Fig. 2, N) which was surrounded by an undulating nuclear membrane occupied a position near the cell membrane.

The intestine (Fig. 4) consisted of columnar cells and a tri-radiate lumen in cross-section. A lot of microvilli (Fig. 4, V) with several microtubules in the center were on the inside lining of the columnar cells. The columnar cell contained electron-dense granules with a maximum diameter of about 2.4 μm. In this cell, a nucleus was situated near the lumen. The cytoplasm contained a great number of glycogen particles (Fig. 3, 4, Gp) and many mitochondria (Fig. 4, M) which were located mainly at the periphery of the cell.

The excretory organ (Figs. 5, 6) consisted of a large cell with a main duct (Fig. 5, D). Some complex microducts (Figs. 5, 6, arrows) were ramified around the main duct. Many round granules (Figs. 5, 6, arrow-heads) filled up the whole cytoplasm. These granules varied in electron-density from dense to light, and dense granules were sometimes surrounded by less-dense areas. A basement membrane, approximately 70 nm thick, covered around the excretory organ. The cell membrane of this organ had many infoldings.

Excretory gland, ventriculus and intestine of worms incubated in artificial gastric juice.

The worms were still active even after incubation in the artificial gastric juice. When even in the worm that was incubated for 4 hrs, the morphological features of the ventriculus cells (Fig. 7) were similar to those of controls. Moreover, there was no material in the duct (Fig. 8, D). Electron-dense granules which were contained in the intestinal cells (Fig. 9) did not change even after 4 hrs of incubation. The intestinal cells were filled up with many glycogen particles (Fig. 10). These α-glycogen particles changed into hollow ones after 10 hrs of incubation. In the lumen of the intestine (Fig. 9, L), there was neither a change in the microvilli (Fig. 9, V) on the luminal surface, nor food debris in the lumen

Fig. 1. Cross-section (same to Fig. 12) of the ventriculus of a control worm. Ventriculus cells contain many dense granules. Arrow shows the cuticle on the luminal surface. Arrow-heads show granules. Bar indicates 1 μm. L: Triradiate Lumen Mu: Muscle fiber

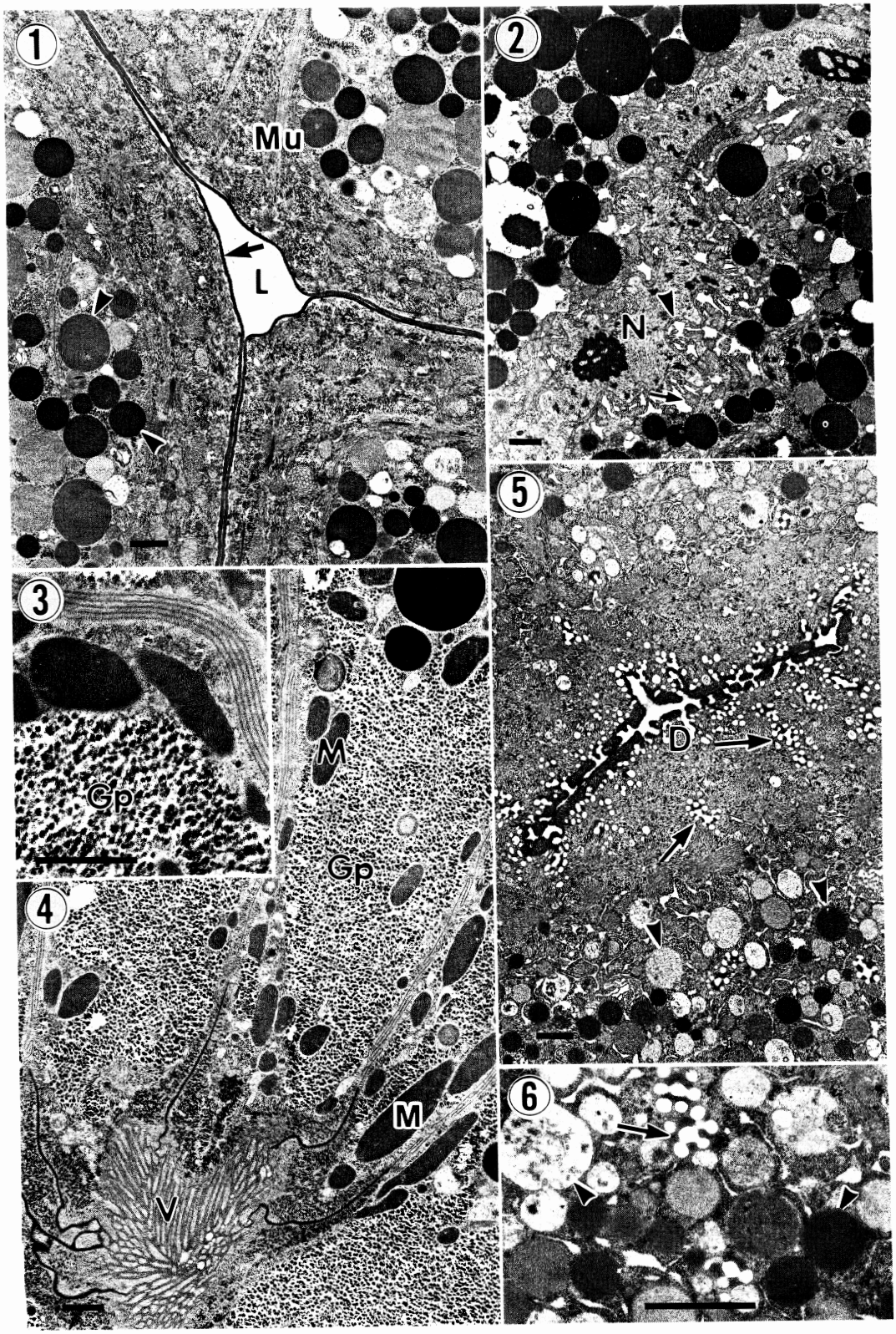
Fig. 2. The ventriculus cell of a control worm. Arrow shows cells membrane. Arrow-head shows nuclear membrane. Bar indicates 1 μm. N: Nucleus

Fig. 3. The intestinal cell of a control worm. Bar indicates 30 nm. Gp: Glycogen particles

Fig. 4. The intestine of a control worm. Bar indicates 1 μm. M: Mitochondria Gp: Glycogen Particle V: Microvilli

Fig. 5. The excretory organ of a control worm. Main duct runs through the center of the cell. Arrow shows some complex microducts. Arrow-heads show many granules. Bar indicates 1 μm. D: Main duct

Fig. 6. Higher magnification of the excretory organ of a control worm. There are many granules varying in size and density (Arrow-heads). Bar indicates 30 nm.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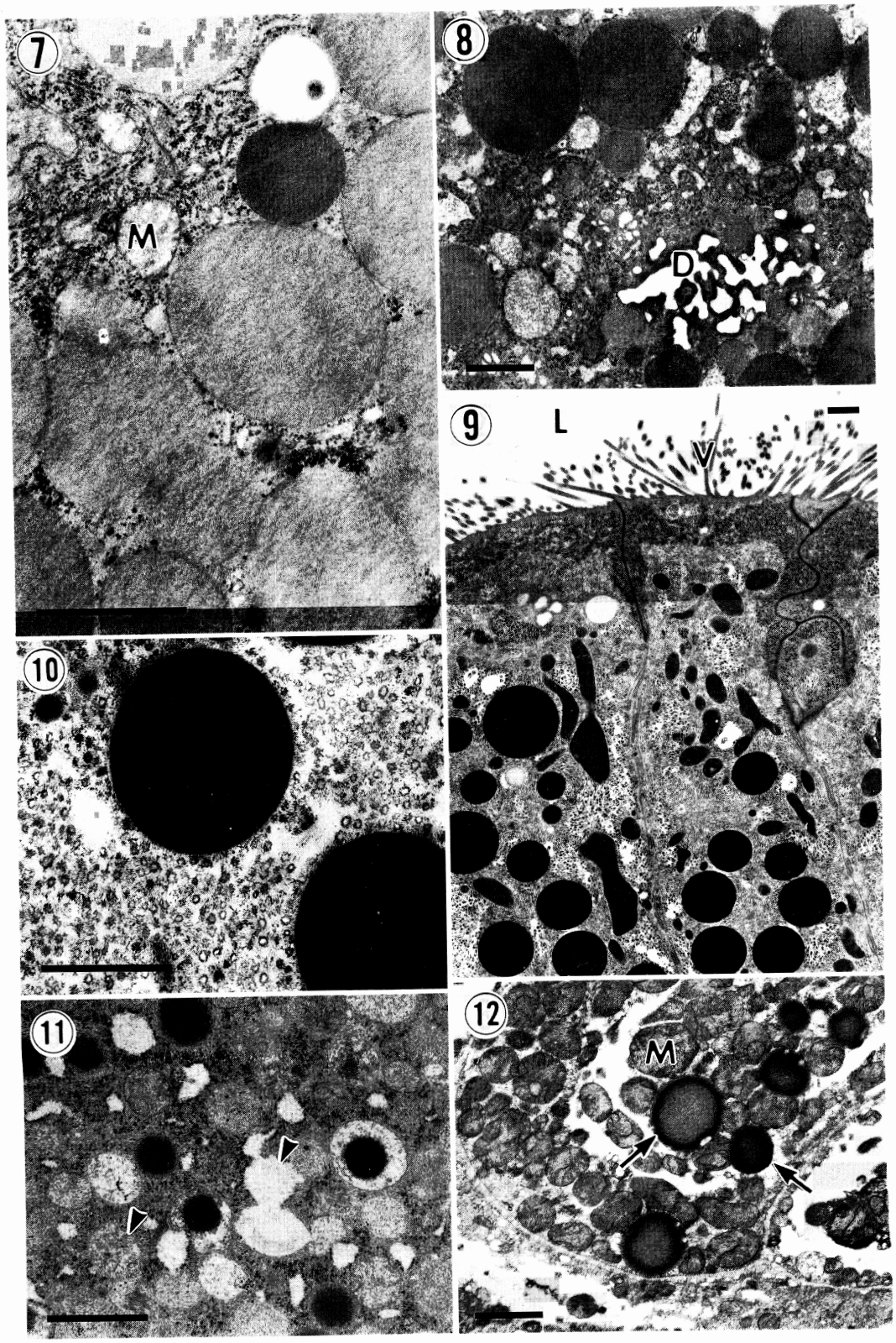
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M

(Fig. 9, L).

The excretory organ showed notable changes gradually according to incubation periods. When the worm was incubated for 4 hrs, many granules in the cell of the excretory organ (Fig. 11) showed a decline in their electron density. Their membranes disappeared, and the granules were fused together (Fig. 11, arrows). However, there was no change in the duct. In the muscle cells of the body wall, electron-dense bodies (Fig. 12, arrows) were observed after 10 hrs of incubation. Muscle filaments, however, did not show any change.

Discussion

Since Mueller (1927) reported that the excretory system of *Anisakis simplex* consisted of "a long ribbon-like uninucleate gland cell", such an excretory system has been referred to an Anisakis type. In this gland cell, he observed granules (0.5–0.8 μm) which stained orange with Mallory's double connective tissue stain. Ruitenberg and Loendersloot (1971) reported that the lumen of the excretory organ of Anisakis sp. larva was sometimes filled with basophilic granular substance having notable activities of phosphatases, oxidative enzymes and esterases. And they suggested that this organ had probably a function of secreting enzyme besides the function of excretion. Furthermore they stated that these enzymes might be involved in the penetration of larva into the host tissue. Lee *et al.* (1973) observed many secretory granules in the cytoplasm of the excretory organ of larval *Anisakis* by TEM. Lee (1970) estimated that the excretory organ may conduct osmoregulation and excretion, as if shown in the other nematode, *Nippostrongylus brasiliensis*.

Oshima *et al.* (1967) described the structure of the ventriculus and the intestinal wall of *Anisakis* larva from a taxonomical point of view by light microscopy. However, ultrastructural characteristics of these organs have not been reported.

In the present electron-microscopic study, the change in electron density was observed on the granules found in the excretory organ of anisakid larva after incubation for 4 hrs or more in artificial gastric juice. Since this organ is connected to an excretory pore located near a boring tooth on the anterior extremity of the worm (Fukuda *et al.*, 1988), the granules may be related to the formation of histolytic enzymes necessary for the worm to penetrate into human tissues.

The electron-dense granules were also observed in the ventriculus and the intestinal cell. These granules did not show any notable change even in the larvae that were incubated for 10 hrs in the artificial gastric juice. Therefore, these granules seem not to play a role when larva penetrates into human tissues. These are probably stored as a nutrient for their activity during larval stage, because food materials were not found in the ventriculus and the intestinal lumens.

Besides the electron-dense granules, the cells of the intestinal wall were filled up with many glycogen particles. These particles changed in electron density after incubation for 10 hrs in the artificial gastric juice. It seems that these particles were stocked as an energy source and were consumed when the larva moved actively in artificial gastric juice. Many mitochondria were distributed through the muscle cells. A few electron-dense bodies appeared near the mitochondria in the larva incubated for 10 hrs.

In conclusion, many granules in the digestive

Fig. 7. The ventriculus cell of a worm incubated in artificial gastric juice for 4 hrs. Bar indicates 1 μm. M: Mitochondria
Fig. 8. The ventriculus cell of a worm incubated in artificial gastric juice for 10 hrs. Bar indicates 1 μm. D: Duct
Fig. 9. The intestinal wall of a worm incubated in artificial gastric juice for 4 hrs. Bar indicates 1 μm. L: Lumen V: Microvilli
Fig. 10. The intestinal cell of a worm incubated in artificial gastric juice for 10 hrs. Glycogen particles are less electron-dense in each center. Bar indicates 1 μm.
Fig. 11. The excretory organ of a worm incubated in artificial gastric juice for 4 hrs. Arrow-heads show granules which are fused together. Bar indicates 1 μm.
Fig. 12. The body muscle cell of a worm incubated in artificial gastric juice for 10 hrs. Arrows show dense bodies. Bar indicates 1 μm. M: Mitochondria

organs (i.e. ventriculus and intestine) do not seem to have any role, but electron-dense granules in the excretory organ are possible histolytic enzymes, and the excretory organ seems to play an important role when the worm penetrates into human tissues.

Acknowledgements

The authors wish to thank Prof. Yoichi Ishii of Kyushu University for his critical reading of the manuscript.

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