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Fine Structure of Endoderm of Embryo of *Ageniaspis fuscicollis* (Chalcidoidea, Hymenoptera)*

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Synopsis

Ageniaspis fuscicollis is a parasitic insect developing polyembryonically. The cells of the ectodermal and endodermal layers of the embryo absorb the food materials synthesized in trophamnion. In fine structure the cells of endoderm are similar to the trophamnion. During epidermis formation stage the endoderm-trophamnion contact disappears, and then the endodermal cells take over the function of trophamnion.

Introduction

Ageniaspis fuscicollis is a parasitic hymenopteran developing polyembryonically in caterpillars of *Yponomeuta malinella* (Lepidoptera). Caterpillars of *Yponomeuta* infested by *A. fuscicollis* contain long, branching cords filled with numerous parasite embryos whose number can reach 180. Marchal (1904) demonstrated that all embryos develop from one egg as a result of its divisions. He called this type of reproduction polyembryony, and the membrane surrounding the embryos and enabling them to absorb nutritive materials from the haemolymph — as trophamnion.

The egg of *A. fuscicollis* is devoid of yolk and its segmentation is total. Prior to the beginning of segmentation the ooplasm, constituting the proper embryo material, separates from the peripheral one, containing the chromatin of non-rejected polar bodies. The chromatin of the polar bodies changes into giant, lobe-shaped trophamnion nuc-

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leus — the paranucleus, which in the course of further development divides amitotically (Silvestri, 1908; Martin, 1914; Kościelska, 1963; Kościelski, 1981). According to Ivanova-Kasas (1961, 1972) the trophamnion is an embryonic membrane (serosa) whose formation has been shifted to early development stages. This type of membrane is characteristic of the polyembryonic Hymenoptera. As indicated by the ultrastructural studies on the polygerm of *A. fuscicollis*, the outer surface of the trophamnion is provided with microvilli and absorbs exogenous materials by meas of the micropinocytosis (Kościelski *et al.*, 1978). Multivesicular bodies resulting from the fusion of the micropinocytotic vesicles are a form of yolk precursor. Thus the micropinocytotic vitellogenesis usually taking place in the oogenesis, in the trophamnion same process is used as a means of accumulating food reserves for the developing embryos. It can be supposed that the disappearance of yolk and the change in the way of cleavage (from partial to total) following it, and the use of the food accumulating mechanism originating from that of the egg, are two important factors responsible for the origin of the polyembryony in Hymenoptera.

Further ultrastructural studies showed that the embryos of *A. fuscicollis* absorb food materials synthesized in the trophamnion, also by means of micropinocytosis. The trophamnion cytoplasmic membrane, contacting directly with the embryos, forms numerous folds which interlock with embryo ectodermal microvilli. In older embryos the trophamnion contacts also with the endoderm cells through a wedge-like process penetrating the dorsal groove of the embryos. In the terminal section of the wedge situated close to the endoderm, the cytoplasmic membrane folds are especially strongly developed. This direct contact enables also the endoderm cells to absorb micropinocytotically materials from the trophamnion (Kościelski and Kościelska, 1985).

The present paper deals with the fine structure of the endoderm of the embryo of *A. fuscicollis* at the dorsal groove stage.

Material and Methods

The polygerms of *A. fuscicollis* were dissected in physiological solution from caterpillars of *Y. malinella* and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 2 hr. Then the material was rinsed in the buffer and postfixed in 1% OsO_4 for 2 hr. Following the dehydration in ethanol and acetone the polygerms were embedded in Epon 812. Ultrathin sections were contrasted according to Reynolds (1963) and examined in the electron microscope Tesla BS 613. Semithin sections were stained with 1% methylene blue in 1% borax.

Results

The endoderm cells in *A. fuscicollis*, despite their position inside the embryo, contact with the trophamnion through the dorsal groove (Figs. 1, 2). The lumen of the groove penetrates, as narrow canals, between the endoderm cells. The cell surface contacting with the canal lumen is provided with microvilli. The lumen of the canals is filled with a granular material (Fig. 3). In the apical parts of the cells micropinocytotic

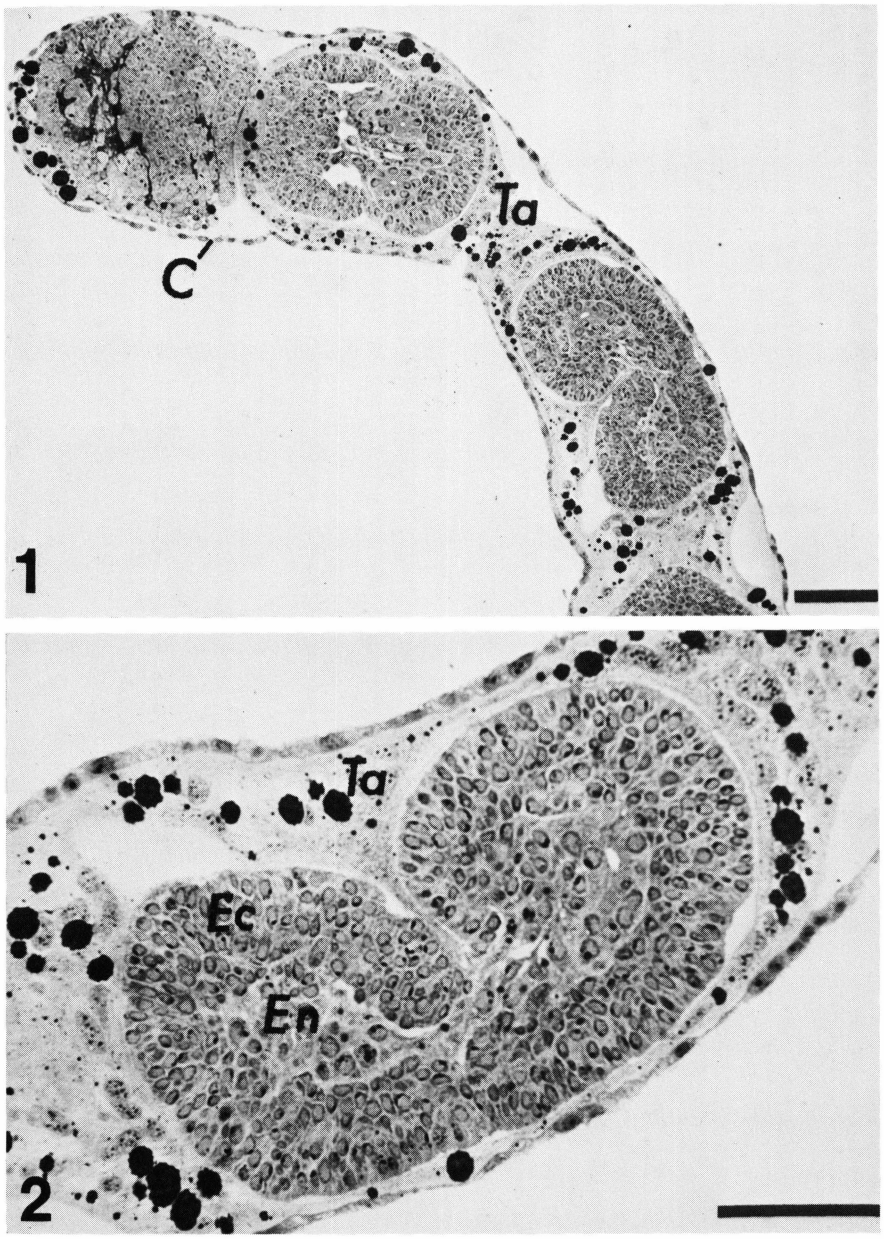
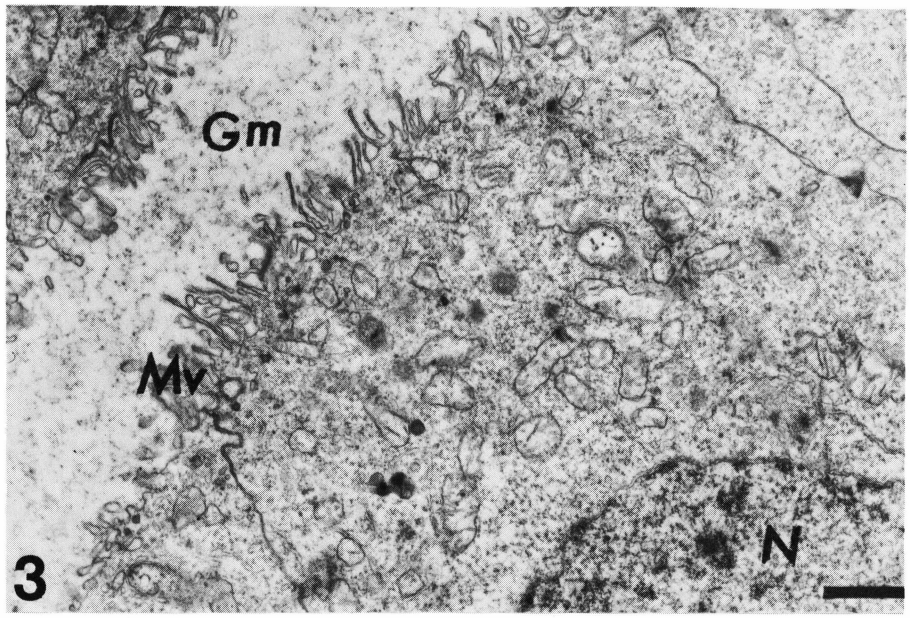


Fig. 1. Fragment of polyembryonic cord of *Ageniaspis fuscicollis*. Embryos at dorsal groove stage lie in trophamnion (Ta). On surface of trophamnion cyst of host cells (C). Numerous lipid drops visible in trophamnion. Semithin section. Scale: 50 μm.

Fig. 2. Embryo at dorsal groove stage. Trophamnion cytoplasm (Ta) penetrates embryo dorsal groove as a wedge: ectoderm (Ec), endoderm (En). In endoderm numerous lipid drops. Semithin section. Scale: 50 μm.



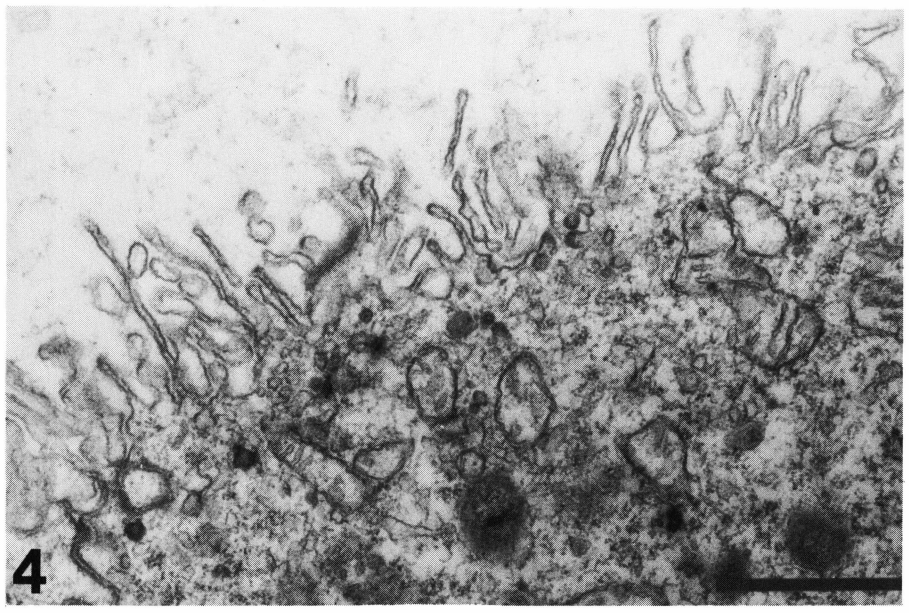


Fig. 3. Electron micrograph showing polarized endoderm cells contacting with canal lumen: microvilli (Mc), nucleus (N). In canal lumen granular material (Gm). Scale: 1 μm.

Fig. 4. In polarized endoderm cell numerous pinocytotic vesicles and dense bodies visible. Scale: 1 μm.

vesicles are visible as well as multivesicular bodies and dense bodies. In the cytoplasm there are numerous, elongate mitochondria of a typical structure (Fig. 4).

The remaining endoderm cells do not form a compact arrangement; between them there are space filled, like the lumen of the canals, with a granular material (Fig. 5). Probably they are continuous with the canals. The cytoplasmic membranes of these cells do not form microvilli, thus the cells are not polarized. Very rarely direct connections between the cells are observed, as cytoplasm bridges (Fig. 5). The cytoplasm, rich in mitochondria and free polyribosomes, contains numerous parallel cysterns of granular cytoplasmic reticulum. Sporadically, multivesicular bodies are observed. Lipid inclusions of various size, sometimes occupying a considerable part of the cytoplasm are a characteristic feature of the endoderm cells (Fig. 5).

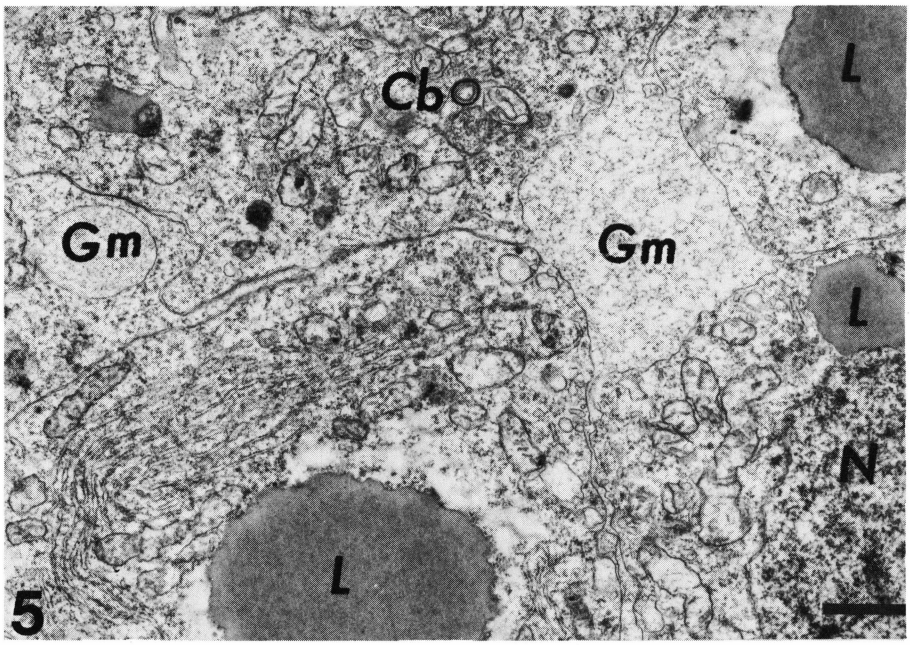


Fig. 5. Electron micrograph showing endoderm cells far from dorsal groove; in cytoplasm cysterns of granular endoplasmic reticulum, cytoplasm bridge (Cb) and lipid inclusions of various size visible, nucleus (N). Between endoderm cells spaces filled with granular material (Gm). Scale: 1 μm.

Discussion

The endoderm in *A. fuscicollis* results from the multipolar migration of the cells into the blastula (Kościelska, 1977). In the further course of its development the embryo shape changes from spherical to ellyptical, and at the moment of the dorsal groove appearance changes to kidney-like. The cells, that entered the inside of the embryo and contact with the trophamnion through the dorsal groove, show the presence of phospholipids. The phospholipids are the main energy source for the living

processes during the polyembryonic development (Ivanova-Kasas, 1961; Kościelska, 1962, 1963). The lipid-rich cells in the course of the development form the mid gut (Kościelska, 1977). There are no electron microscope studies on insect endoderm. The existing data pertain to the definite mid gut epithelium (Smith, 1968; Krzysztofowicz *et al.*, 1973).

Some structures contained in the endoderm cells of *A. fuscicollis* are similar to those of the trophamnion: micropinocytotic vesicles, multivesicular bodies, dense bodies and lipid inclusions. Like in the trophamnion, in the endoderm cells an intense protein synthesis takes place which is evidenced by the strongly developed granular endoplasmic reticulum. The endoderm cells start absorbing and accumulating the reserve materials as soon as they enter in contact with the trophamnion through the dorsal groove. In the monoembryonic development the endoderm differentiating into the mid gut encloses the egg yolk in its lumen. In *A. fuscicollis* the endoderm-yolk contact is retained as a close contact with the "external yolk " which — according to Marchal (1904) — is the trophamnion. The system of the canals distributes the materials coming from the trophamnion to endoderm cells situated farther from the dorsal groove. After the disappearance of the dorsal groove the further development is possible due to the endoderm in which the reserve materials, mainly lipids, are accumulated. Numerous lipid inclusions are present also in the mid gut cells. After having used the reserve materials the mid gut gets in connection with the fore and hind guts. Then the embryo at its larval stage can feed on its own account (Kościelska, 1977).

The electron microscope studies on the polyembryony in *A. fuscicollis* showed, that the development of the parasitic Hymenoptera is not only due to the trophic membrane — trophamnion — adaptations to the absorption of nutritive materials from the host haemolymph, but also due to the adaptations of the embryo cells to the micropinocytotic uptake of the materials synthesized in the trophamnion. This adaptation is shown, at early development stages, by both the ecto- and endoderm cells. At more advanced development stages the endoderm cells take over the trophamnion function synthesizing materials necessary for further development of the embryos.

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