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Toxocara canis (Nematoda, Ascarididae): ultrastructure of the rachis and the ovarian wall

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Abstract. The oogonia and oocytes in the ovaries of *Toxocara canis* are joined to a cytoplasmic process called the rachis. The rachis is a muchbranched cytoplasmic mass without cell components in the germinal zone. At the end of the germinal zone and in the growth zone the cytoplasmic mass is formed into a central axial cylinder, containing small dense granules, lipid drops and glycogen. Throughout the growth zone shell granules similar to those present in the oocytes are also present in the rachis. Anterior to the opening of the ovaries into the oviduct the rachis disappears. The ovarian wall is composed of epithelial cells, adjoining the basal lamina. They are characterized by the presence of large numbers of mitochondria, especially in the germinal zone. The epithelial cells in the growth zone also contain rough endoplasmic reticulum, ribosomes and bundles of microfibrils. A dense tubular material occurs between the basal membrane of the epithelial cells and the basal lamina as well as in the wall intercellular spaces in the ovarian growth zone. Multivesicular labyrinth-like formations can also be observed in the epithelial intercellular spaces in the central portion of the *T. canis* ovary.

Oogonia as well as oocytes are attached to the rachis and lie close to the ovarian wall during oogenesis in ascarids. The first part of the ultrastructural studies on the reproductive system of *Toxocara canis* was devoted to the ultrastructure of the oogonia and oocytes (Bruňanská 1993a). In the present investigation, attention is directed to the ultrastructure of the rachis and epithelial cells comprising the ovary.

MATERIALS AND METHODS

Adult female *Toxocara canis*, isolated during a dissection of dog digestive tract, were washed in saline solution (Baldwin and Moyle 1947). The whole worms, as well as their isolated reproductive organs, were fixed in 3.5% glutaradehyde in 10 mM cacodylic buffer at room temperature for 3 hr. They continued to be washed in 100 mM cacodylic buffer at 4° C overnight. The washed, fixed worms were divided into 10 equally long portions and postfixed in 2% cacodylic-buffered OsO_4 at room temperature for 2 hr. The reproductive tract, divided into ovaries, oviducts and uteri, was postfixed in the same way. The material was dehydrated through a graded series of alcohols, saturated by propylenoxid-Durcupan and embedded in Durcupan ACM. Semi-thick sections (1 μm) were stained in 1% methylene blue and 1% sodium tetraborate for about 15s, rinsed in distilled water, viewed and photographed with an OPTON optic microscope. Ultrathin sections were cut on an ultramicrotome Tesla BS 490 and LKB Ultratome III, contrasted by 7% uranylacetate and lead citrate and viewed under a Tesla BS 500 electron microscope.

RESULTS

Light microscopy

Oogonia of *Toxocara canis* are arrayed around the muchbranched cytoplasmic mass, or the rachis, in the germinal zone of the ovaries (Fig. 1). Cytoplasmic ramifications of the rachis appear in the shape of the letter H in the cross semi-thick sections from the germinal zone. During very early stages of oogenesis the rachis assumes a cylindrical shape. The rachis disappears near the ovarian-oviduct junction.

Electron microscopy

The rachis contains neither cell organelles nor cytoplasmic inclusions in the germinal zone and it is surrounded by cytoplasmic membrane with numerous invaginations (Fig. 2). Its continuity with oogonia is secured by numbers of cytoplasmic bridges of various width, occasionally containing also microfilaments. The walls of the widest bridges are depicted in Fig. 2.

The cytoplasmic bridges connecting the rachis with the oocytes in the growth zone also show the presence of microfilaments (Fig. 3). Microfilaments are oriented parallel or vertical to the long axis of the oocytes. At the beginning of the ovarian growth zone the rachis contains mostly lipid drops and small dense granules. In further portions of the ovaries it contains inclusions identical with those found in the adjoining oocytes: lipid drops, dark dense granules, some small shell granules and glycogen (Fig. 4). The lipid drops and dense granules increase in size as do those in the adjoining oocytes. At the points where oocytes with large shell granules occur these large shell granules can be observed in the rachis (Fig. 5). The rachis contains no cell organelles in the growth zone.

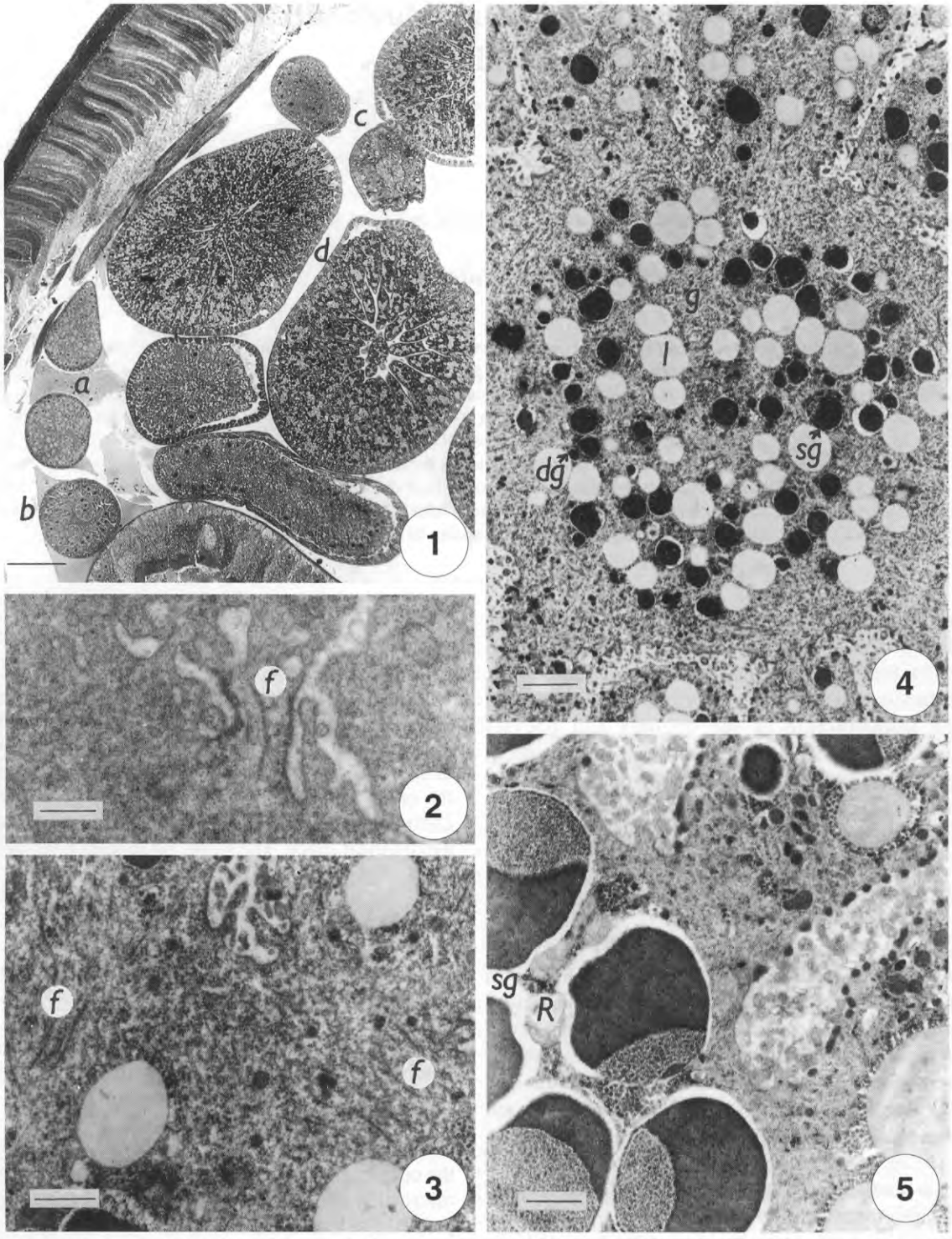

Fig. 1. Various planes of sections through the germinal and growth zones of *Toxocara canis* ovaries. The rachis is a muchbranched cytoplasmic mass (a) formed into a letter H (b) in the germinal zone. The rachis assumes a typical cylindrical shape at the end of the germinal zone (c) and in the growth zone (d) it is centrally disposed. A semi-thick section, stained with methylene blue. Bar = 15 μm. **Fig. 2.** Cytoplasmic bridges, connecting the rachis R with oogonia in the germinal zone, have pronounced electrondense walls and contain microfilaments (f). The rachis matrix contains no cytoplasmic organelles in this portion of the ovaries. Bar = 0.6 μm. **Fig. 3.** The rachis surface is characterized by numerous invaginations. At the linking points of the cytoplasmic bridges variously oriented microfibrils (f) occur inside the rachis. Bar = 1 μm. **Fig. 4.** The rachis connecting young oocytes contains lipid drops (l), dark dense granules (dg), occasional small shell granules (sg) and glycogen (g). Bar = 2 μm. **Fig. 5.** Large shell granules (sg), present in growing oocytes, can also be observed in the rachis (R). Bar = 0.7 μm.

The dense material, described from the surface of oocytes (Bruňanská 1993a) is not now observed on the surface of the cytoplasmic bridges.

The connections between the oocytes and the rachis diminish (Fig. 6) and the last portions of the ovaries show no presence of a rachis.

The ovarian wall in *T. canis* is composed of epithelial cells adjoining the basal lamina. It is 3–4.5 μm and 8 μm wide in the germinal and the growth zone, respectively. The characteristic feature of the epithelial cells in the ovarian wall is the presence of large numbers of mitochondria especially in the germinal zone (Fig. 7). The nucleus occupies a major portion of the cell in the germinal zone, while in the growth zone it tends to be localized in the apical portion of the cell (Fig. 8). The nucleus is lined with a double nuclear membrane and it contains a pronounced granular nucleous.

A dense material occurs between the basement membrane of epithelial cells and the basal lamina (Fig. 9). This dense material is also found in the wall intercellular spaces in the ovarian growth zone, where multivesicular labyrinth-like formations of unknown function can also be observed. A detailed study revealed the tubular character of this dense material (Fig. 10).

In addition to the conspicuous round and biscuit-shaped mitochondria, the epithelial cells in the growth zone of *T. canis* ovaries also contain rough endoplasmic reticulum, ribosomes and bundles of microfibrils (Figs. 9, 11).

DISCUSSION

The rachis appears associated with oogonia in the germinal zone of the ovaries in many nematodes (McLaren 1973, Lee 1975, Gutteková and Bruňanská 1988). The structure of the rachis in the ovaries of *Toxocara canis* resembles the structure described in ascarids (Foor 1967, Bogoyavlensky et al. 1982). It is a muchbranched cytoplasmic network in the germinal zone of the ovaries. While Bogoyavlensky et al. (1982) writes about the rachis as having its characteristic cylindrical shape only in the growth zone, in *T. canis* we can observe a cylindrically shaped rachis during a very early stages of oogenesis, at the end of the ovarian germinal zone, as in *Syngamus trachea* (Bruňanská 1991).

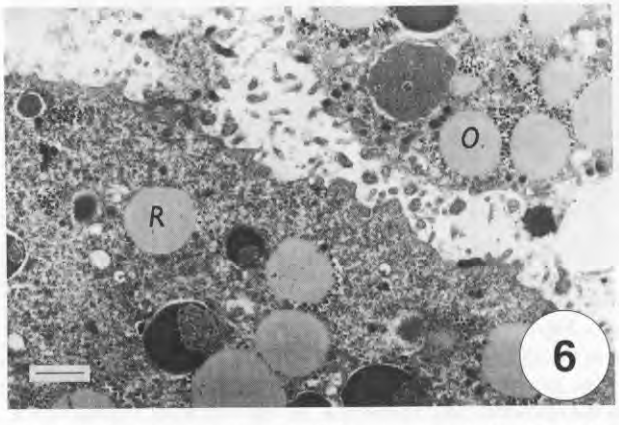

Fig. 6. In the posterior portions of the ovarian growth zone oocytes (O) separate from the rachis (R). Bar = 1.1 μm.

According to Foor (1967), the rachis from *Ascaris* in the growth zone of the ovaries contains lipid drops, dense granules, ribosomes and microtubular formations, but no refringent (shell) granules. Our results show, however, the absence of cell organelles such as ribosomes, but the presence of shell granules in the rachis connecting growing oocytes in *T. canis*. This finding is interesting and supports the opinion that the rachis facilitates the distribution of the cytoplasmic inclusions and substances passing into the gonads from the pseudocoelomic fluid and from the intestine (Foor 1983, Bruňanská 1991). It is believed that the function of the rachis is related to the degree of maturity of the oocytes (Bruňanská 1989). The absence of malate dehydrogenase in the rachis of *Ascaris* supports the hypothesis that the rachis has a transporting role in most nematodes (Bruňanská 1993b). The absence of ribosomes and mitochondria in rachis of *Toxocara* suggests that its synthetic activity is minimal.

The rachis probably provides physical support for the oocytes (Foor 1967) as suggested by the thickening of the bridge wall in *T. canis* ovaries, similar to that in *Ascaris lumbricoides* ovaries (Foor 1967) and by the presence of microfilaments in the bridge cytoplasm.

The direct transport of nutrients from the intestine into the oocytes could occur via the ovarian wall. The labyrinth-like formations described in this paper could be associated with this transport. The ovarian wall in *T. canis* also contains numerous mitochondria similar to those from other nematodes (MacKinnon 1987) and it is probably very active in respiration. Even though earlier authors considered the epithelial cells and the rachis to be the sites of yolk synthesis in *Ascaris* ovaries, later electronmicroscopic studies did not support this opinion since organelle systems associated with rapid synthetic activity were absent in both parts of the reproductive tract (Foor 1972). The ultrastructure of the ovarian wall in *T. canis* and *S. trachea* (Bruňanská 1992), however, indicates a synthetic activity of the ovarian epithelial cells. Other authors (Kochhar 1960, McLaren 1973) also support the opinion that the epithelial cells in the nematode reproductive organs share in production of the material that may be utilized by the oocytes.

The presence of the dense material in the intercellular spaces of the epithelial cells and in the labyrinth-like formations is of much interest. Electron-dense formations of different lengths were observed between the basal part of the plasma membrane and the basal lamina in *Dictyocaulus viviparus* as well (Gutteková and Bruňanská 1990). The dense material is believed to be utilized in the oocyte cytoplasm (or on the oocytes surface) for the building of dense and heterogenous (shell) granules, or contributing to the oolemma (Foor 1972, Bruňanská and Gutteková 1989, Bruňanská

1993a). The precursors could be resorbed and transported by the epithelial cells from the worm's pseudocoelomic fluid. The pseudocoelomic fluid has been shown to represent a rich source of metabolites for active protein synthesis that are necessary for the complete development of the oocytes (Lee and Smith 1965, Viglierchio and Gortz 1972).

The original micromorphological data on the *T. canis* ovaries presented in this and recent papers (Bruňanská 1993a), will allow comparison of the ultrastructural aspects of oogenesis in the related genera *Toxocara* and *Ascaris*, the worldwide agents of helminthic diseases. These findings are also the starting point for electronmicroscopic studies on the further development of oocytes in *T. canis*.

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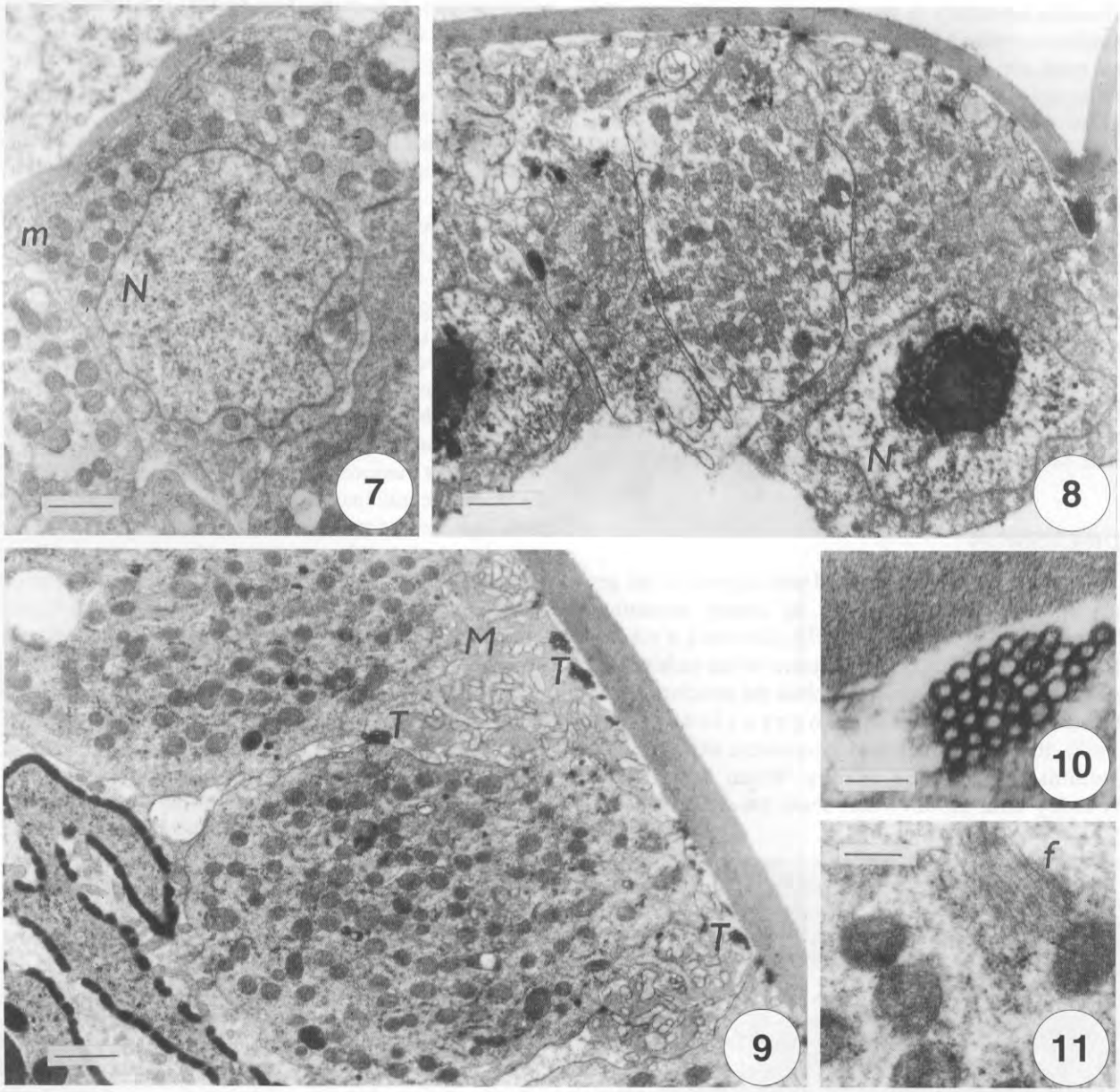

Fig. 7. The epithelial cells in the wall of the ovarian germinal zone contain a large nucleus (N), occupying most of the cell vollume. The cell cytoplasm contains numerous round mitochondria (m). Bar = 0.8 μm. **Fig. 8**. At the beginning of the ovarian growth zone the shape of epithelial cells changes to cylindrical. Their nuclei (N) are localized in the apical portion of the cell. Bar = 0.9 μm. **Fig. 9.** The intercellular spaces of the epithelial cells in the central portion of the ovarian growth zone contain multivesicular labyrinth-like formations (M). Dense material (T) occurs in them and in the spaces between the epithelial basal membrane and the wall basal lamina. Bar = 1 μm. **Fig. 10.** Dense material found in the ovarian wall has a tubular structure. Bar = 0.2 μm. **Fig. 11.** Bundles of microfilaments (f) and round mitochondria in the wall of the ovarian growth zone. Bar = 0.35 μm

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