Formation of crystals within cells of two mermithid nematodes

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SUMMARY

Disc-like platelets of supposed proteinaceous nature composed of dense subunits approximately 8 nm in diameter arranged in a crystalline pattern were observed in the pseudocoelom of two mermithid nematodes, *Gastromermis* sp. and *Romanomermis culicivorax*. In addition morphologically similar subunits were observed in the nuclei and cytoplasm of hypodermal cord cells. It is proposed that the subunits are formed intracellularly and pass into the pseudocoelom where they aggregate into large platelets. It is possible that these crystals represent excretory products stored in a non-toxic state, so that they will not weaken the relatively small invertebrate host of these internal parasites.

RÉSUMÉ

Formation de cristaux à l'intérieur des cellules de deux Mermithides

Les auteurs ont observé dans le pseudocoelome de deux nématodes Mermithides, *Gastromermis* sp. et *Romanomermis culicivorax*, des petites plaques ayant l'apparence de disques protéiques et composées de sous-unités denses d'un diamètre approximatif de 8 nm présentant un arrangement cristallin.

Des éléments cristallins ont été aussi observés dans les noyaux et le cytoplasme des cellules des cordes hypodermiques. Les sous-unités de ces petites plaques se formeraient à l'intérieur des cellules, puis passeraient dans le pseudocoelome où elles s'agrégeraient en formations plus importantes. Ces cristaux pourraient représenter des produits d'excrétion stockés sous forme non toxique, donc non nocive pour les insectes hôtes relativement petits de ces parasites internes que sont ces Mermithides.

An earlier study (Poinar, Leutenegger & Thomas, 1970) described disc-like platelets of supposed proteinaceous nature from the pseudocoelom of free living post-parasitic juveniles of a mermithid nematode now considered in the genus *Gastromermis*. At that time, a survey showed that similar objects had been reported in various species of mermithid nematodes. Bugnion (1878) called them blood corpuscles in *Limnomermis aquatilis* (Duj.), Hagmeier (1912) referred to them as free-floating hyaline bodies in *Paramermis aquatilis* and other mermithids whereas Müller (1931) and Dollfus (1946) considered them as possible internal parasites.

The above mentioned study showed the discs to consist of a highly organized pattern of parallel, dense lines. However, the origin and formation of the discs were not investigated.

Materials and methods

The platelets were examined in two species of mermithid nematodes in the present study. One was the undescribed species of *Gastromermis* studied by Poinar, Leutenegger and Thomas (1970) which parasitizes midges of the genus *Cricotopus* in fastflowing mountain streams in California. The second species was *Romanomermis culicivorax* Ross & Smith, a parasite of mosquito larvae originally collected in Louisiana and mass-reared by Dr. J.J. Petersen.

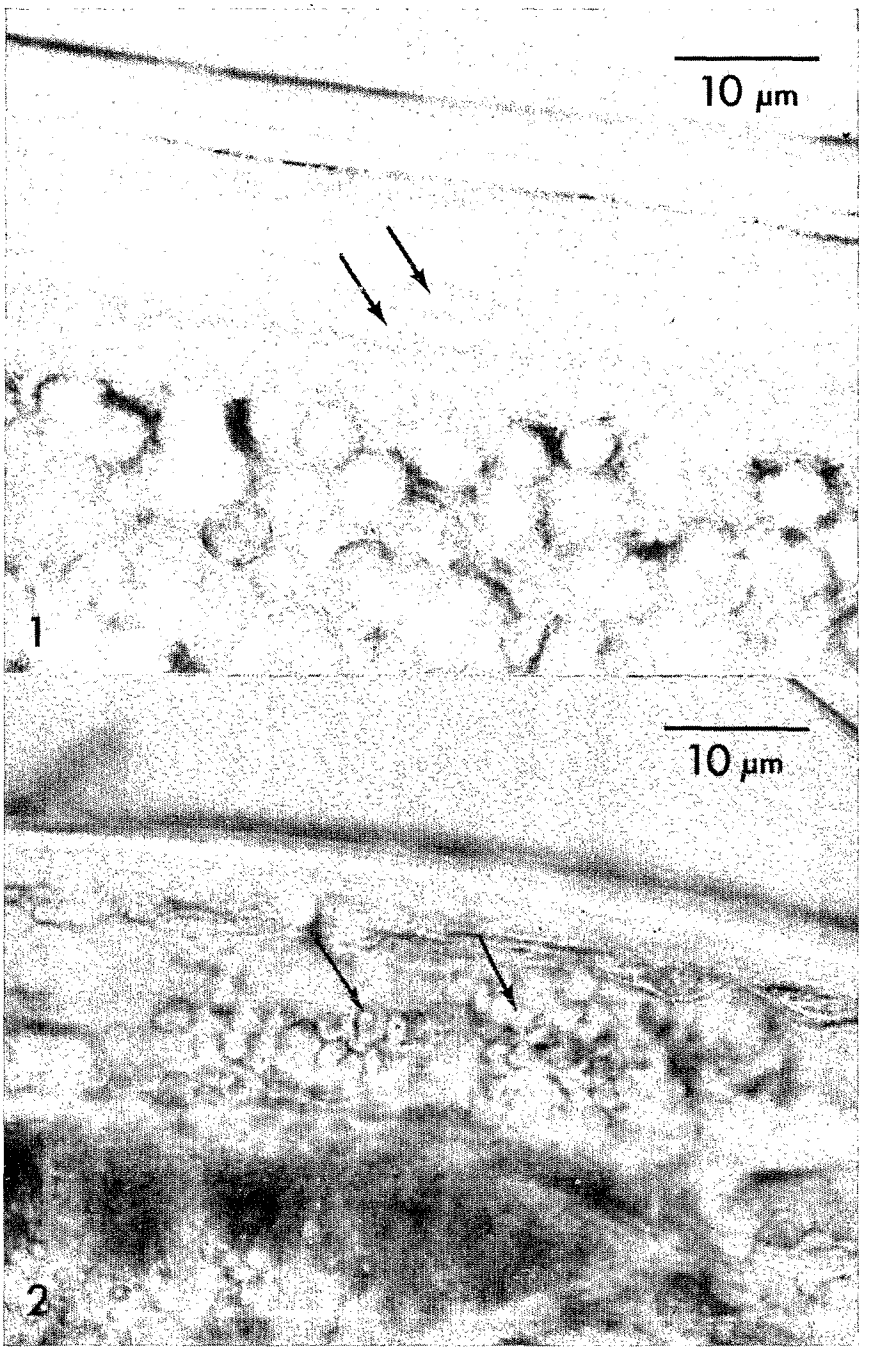

Fig. 1 (above) : Light microscope whole mount of *Gastromermis* sp. showing two adjacent protein platelets floating in pseudocoelom (arrows).

Fig. 2 (below) : Light microscope whole mount of *Romanomermis culicivorax* showing clusters of protein platelets accumulated in the pseudocoelom (arrows).

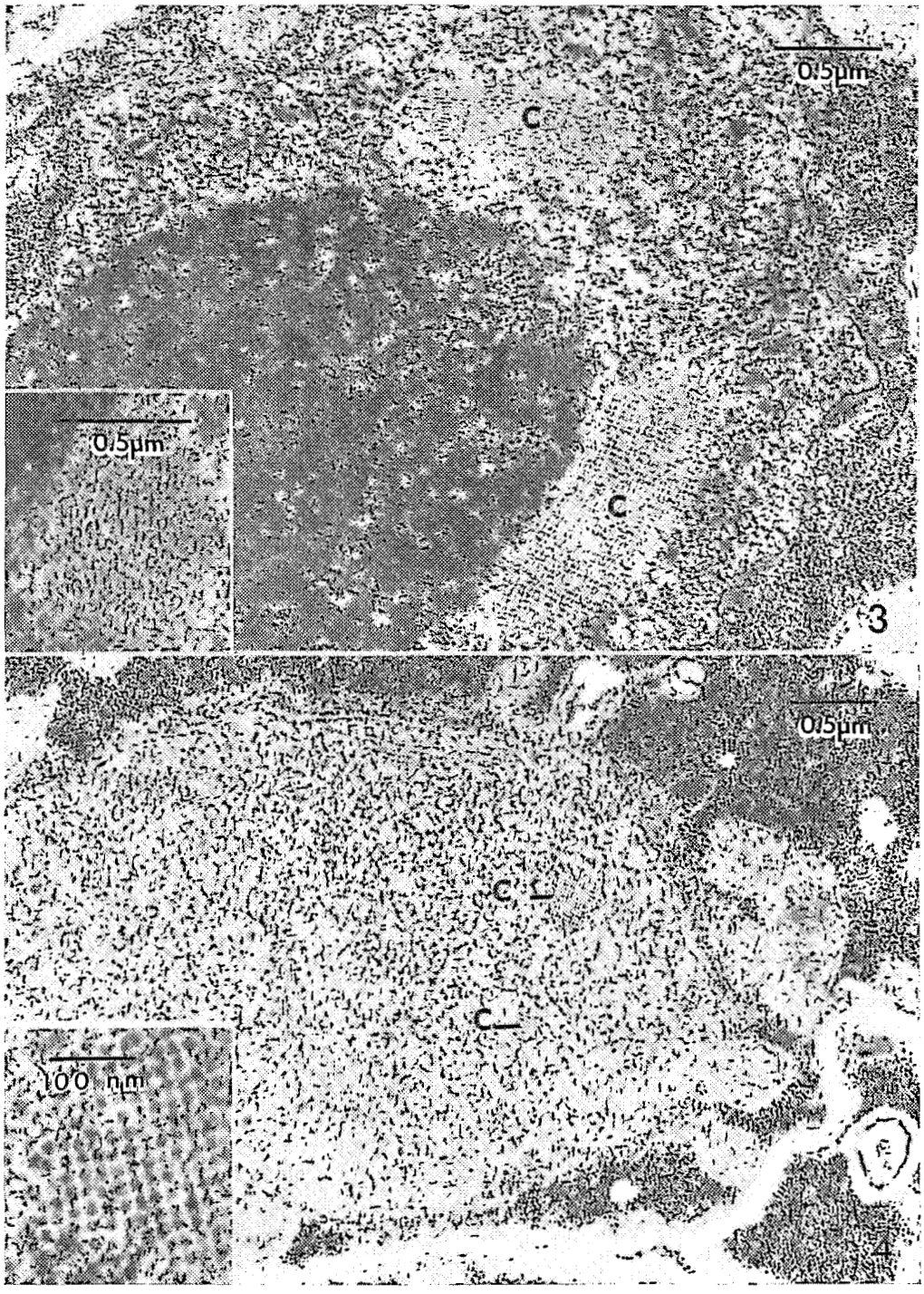

Fig. 3 (above) : Crystalline lattice (C) in the nucleus of a hypodermal cell of *Gastromermis* sp. Insert : higher magnification of crystalline subunits.

Fig. 4 (below) : Crystalline lattice (C) in the nucleus of a hypodermal cell of *Romanomermis culicivorax*. Insert : higher magnification of crystalline subunits.

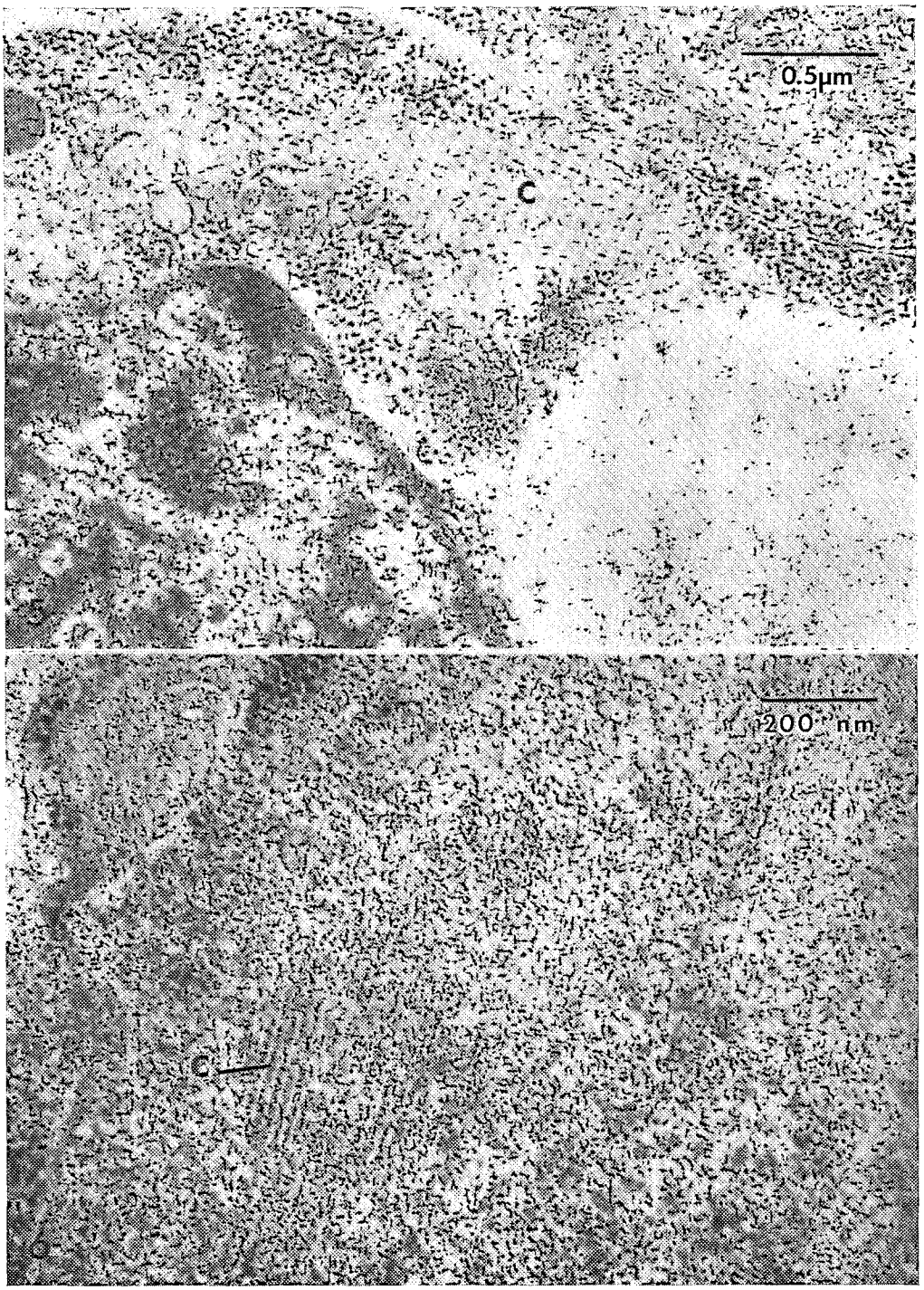

Fig. 5 (above): Crystalline lattice (C) in the cytoplasm of a hypodermal cell of *Gastromermis* sp.

Fig. 6 (below) : Crystalline lattice (C) in the cytoplasm of a hypodermal cell of *Romanomermis culicivorax*.

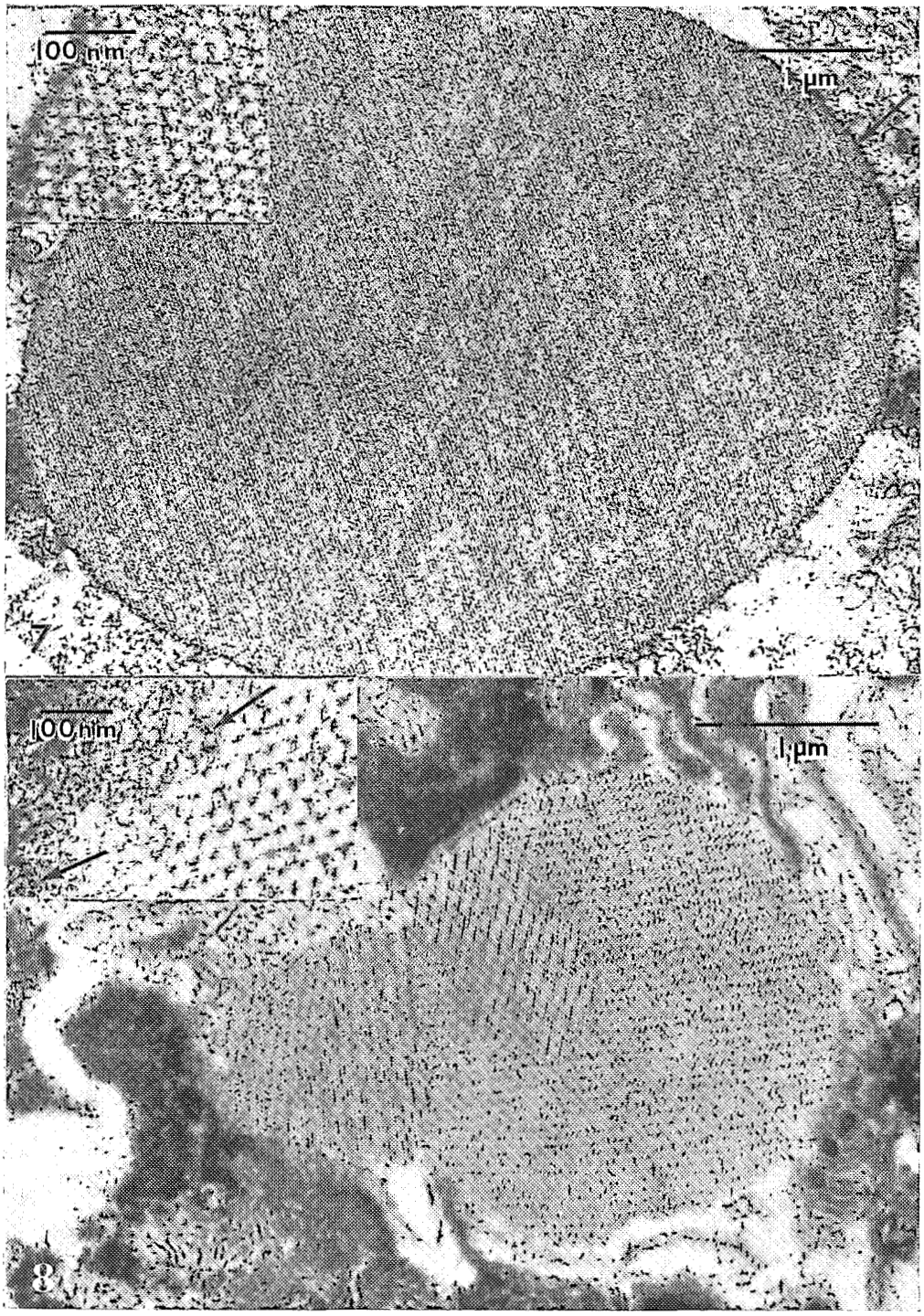

Fig. 7 (above) : Protein platelet in the pseudocoelom of *Gastromermis* sp. Arrow indicates limiting « membrane ». Note electron dense outer boundary. Insert : portion of a protein platelet in *Gastromermis* sp. showing structure similar to a crystalline lattice.

Fig. 8 (below) : Protein platelet in the pseudocoelom of *Romanomermis* culicivorax. Insert shows structure similar to a crystalline lattice. Arrows indicate subunits in the pseudocoelom.

For ultrastructural investigations, adults and postparasitic juveniles of both species and parasitic stages of *R. culicivorax* were prefixed for 2 h in a 4 % solution of glutaraldehyde, buffered with 0.1 M phosphate buffer, then fixed for 1 h in 1 % osmium tetroxide. After dehydration in an alcohol series and embedding in Araldite, sections cut with a Porter-Blum MT-2 microtome were stained with uranyl acetate and lead citrate and examined with an RCA F3 and a Philips 300 electron microscope.

Results

In both species of living nematodes, platelets could be seen floating freely in the pseudocoelom or aggregated in clusters between the trophosome and muscle layer (Figs 1 and 2). In *Gastromermis* sp., the platelets were flat circular discs and had an average diameter of 23.2 µm (14.6-30.5) and an average width of 5.5 µm (3.7-7.3). They were hyaline and not refringent under bright field. They possessed an indistinct limiting « membrane » (Fig. 7).

In *R. culicivorax* the platelets were smaller, averaging 2 µm by 2.5 µm in diameter. The structure of the platelets varied from oval (Fig. 2) to lobulate (Fig. 8). They did not possess a limiting membrane or interface structure variation (insert Fig. 8).

High magnification showed that the platelets were composed of dense subunits approximately 8 nm in diameter. The subunits were arranged in a cystalline pattern with an average center to center spacing of 23 nm (inserts Fig. 7, 8). In *Gastromermis* sp. the subunits were generally aligned within one or two planes, whereas in *R. culicivorax* the subunits were more frequently aligned in many planes giving the platelet a heterogeneous appearance (Figs. 7, 8).

In both nematodes, small units of crystalline lattice similar to those in the platelets of the pseudocoelom occurred within the nuclei of the hypodermal cord cells. In *Gastromermis* sp. these units often occurred adjacent to extensive electron-dense nucleoli (Fig. 3). In *R. culicivorax*, they were present in clear nuclei with reduced chromatin and no apparent nucleoli (Fig. 4). In other hypodermal cells of both nematodes, crystalline structures similar to those in the platelets occurred in the cytoplasm of the cells (Figs. 5, 6).

When cut in cross section, the subunits of the crystalline arrays found in the nucleus, cytoplasm and pseudocoelom were round with a dense outer wall and less dense center which gave them a hollow appearance (inserts, Figs 4, 7, 8). Cross bridges interconnecting the units could be seen (insert, Fig. 8). Sections cut in older planes showed the subunits as dense parallel bands (Figs 3, 4, 5, 6). The angles between the subunit array varied according to the plane of section. The insert of Fig. 8 shows units similar to those of the platelet at the boundary of the pseudocoelom (arrows).

Discussion

The morphological subunits composing the platelets of supposed proteinaceous nature found in the pseudocoelom of *Gastromermis* sp. and *R. culicivorax* are similar in structure to those crystals found in the nucleus and cytoplasm of the hypodermal cord cells. Similar subunits were found at the edge of the crystalline lattice in the hypodermal cell cytoplasm and at the boundaries of the platelets in the pseudocoelom. This suggests, but does not imply, that the crystalline subunits are assembled within the cell and secreted into the pseudocoelom where they aggregate into platelets.

The substructure of the platelets from the two nematode species is remarkably similar, being composed of subunits of the same size and spacing and may be of similar composition.

Mermithid nematodes in general do not possess a complete intestine with an external opening, nor do they possess a functional excretory system as do most other nematodes. Although the exact chemical nature of these platelets is not yet known they may possibly represent waste material stored in a non-toxic form. This would ensure that normal cellular metabolites would not be released in liquid state into the nematode and in turn be carried into the insect where they could interfere with normal development of both host and parasite.

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