Rhabditis pellio Schneider (Nematoda) From the Earthworm, *Aporrectodea trapezoides* Duges (Annelida)[1]

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Abstract: Studies were conducted on the behavior of the nematode, *Rhabditis pellio*, in the earthworm, *Aporrectodea trapezoides*, from southern California. Juvenile and adult nematodes were found in the bladders and tubules of the metanephridia of the host. Similar nematodes that entered the coelom were encapsulated and incorporated into multiple capsules ("brown bodies"). It was demonstrated that this host response is an effective defense reaction since dead and dying nematodes, as well as living forms, were found in the capsules. *Key words:* nematode, defense reaction.

Earthworms can serve as intermediate, sole, or transport hosts of nematodes. Representatives of the nematode orders Spirurida, Strongylida, and Ascaridida utilize earthworms as intermediate hosts, and members of the Drilonematoida occur as mature adults in the coelom of earthworms, whereas species of *Syngamus* utilize earthworms as transport hosts (5). Still another group of nematodes, members of the Rhabditidae, can utilize earthworms as temporary hosts. It is a representative of the Rhabditidae that is discussed here.

The earliest report of rhabditid nematodes from earthworms appears to be made by Dujardin (4), who described *Rhabditis terricola* and mentioned that it could be found in the body cavity of earthworms. Dujardin's description encompassed more than one species, however, and *R. terricola* is now considered strictly a soil-inhabiting species. Schneider (12) later described *R. pellio*, which is the first description of a rhabditid taken from earthworms. Bütschli (1) redescribed what he considered was Schneider's *R. pellio*. However, the male that Bütschli described had a leptoderan bursa, whereas Schneider described the male with a peloderan bursa. It was clear that there were two nematodes described as *R. pellio*, and within a 3-year span, three alternative names were published to replace the *R. pellio* of Bütschli. These were *R. leptodera* Hertwig (6), *R. johnsoni* Micoletzky (11), and *R. maupasi* Seurat in Maupas (9). The latter name had priority and is currently in use. Since than, 12 additional species of rhabditid nematodes have been described from living earthworms, most of these by Völk (14). Although many European authors have commented on the association between *R. pellio* and earthworms, few identified the hosts. Aside from Cuénot (2), who found *R. pellio* in *Allolobophora terrestris* Lav., only Völk (14) identified four oligochaetes that harbored this species. There appears to be no host records for *R. pellio* in North America, and apparently only Dougherty and Calhoun (3) established the presence of this nematode in the United States when they isolated it from unidentified earthworms in northern California. The present study describes the association between *Rhabditis pellio* Schneider and the earthworm, *Aporrectodea trapezoides* Duges, from southern California.

MATERIALS AND METHODS

Stages of *Rhabditis* (*Pellioditis*) *pellio* were obtained from specimens of *Aporrectodea trapezoides* collected in Riverside, California. Nematode colonies were established by placing metanephridia or portions of the body wall of *A. trapezoides* on plates of nutrient

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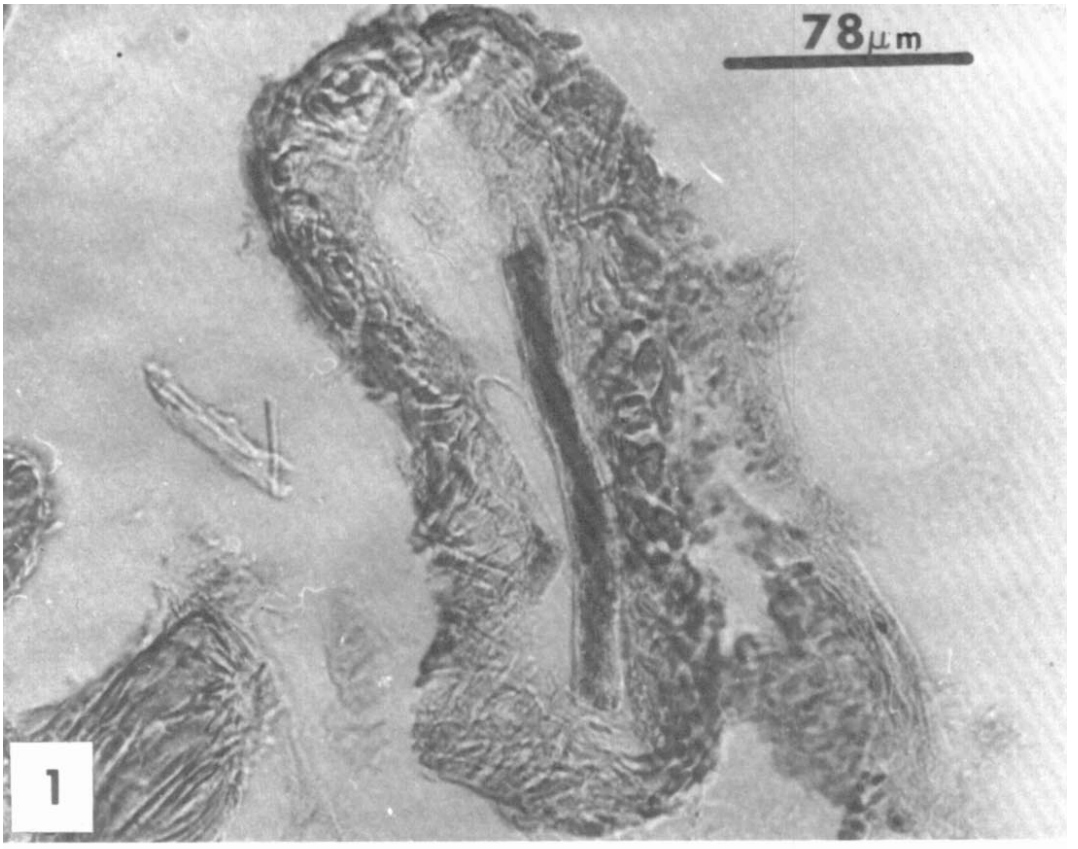
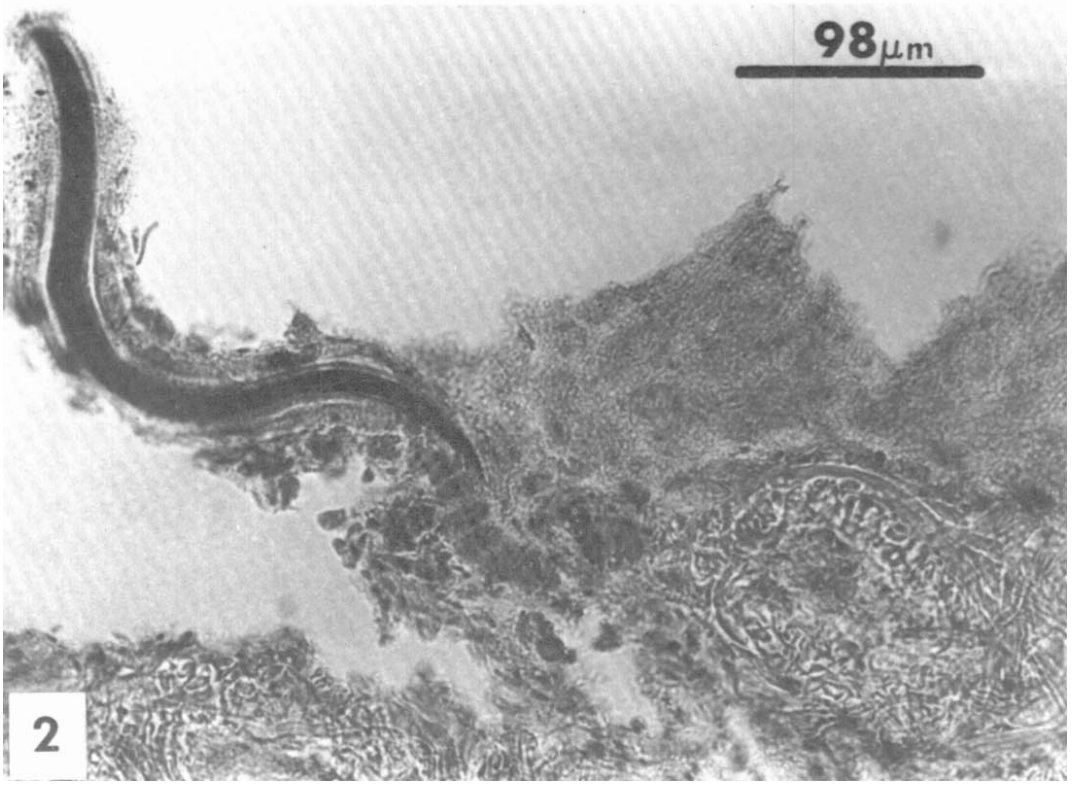
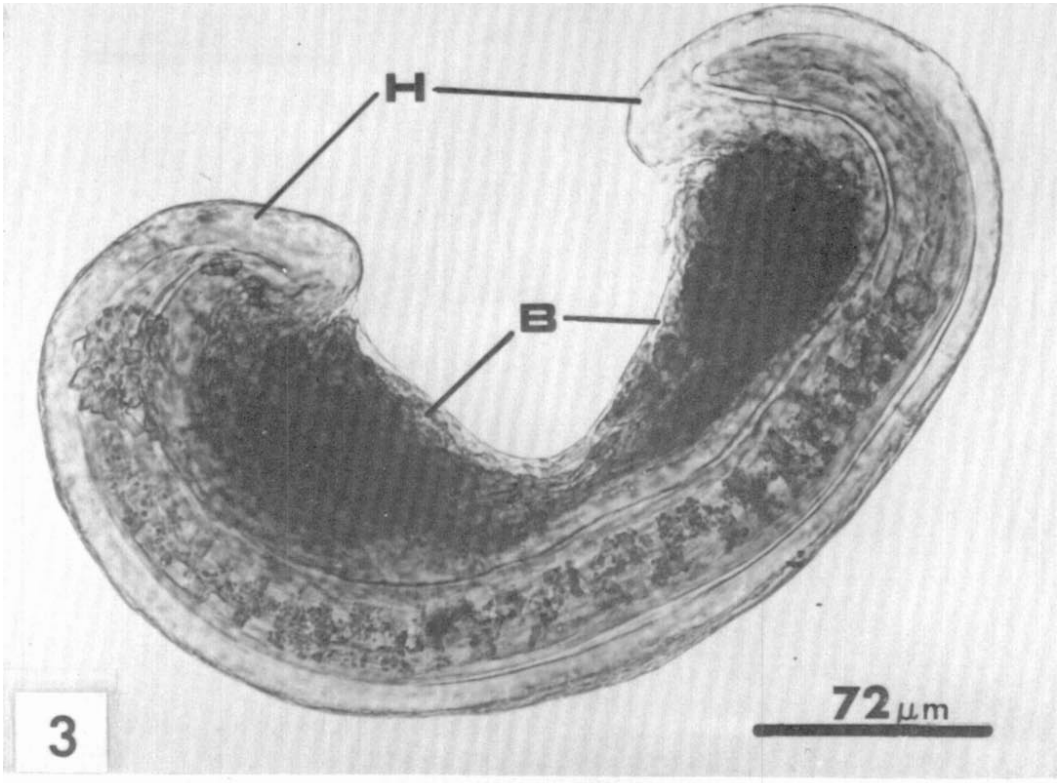
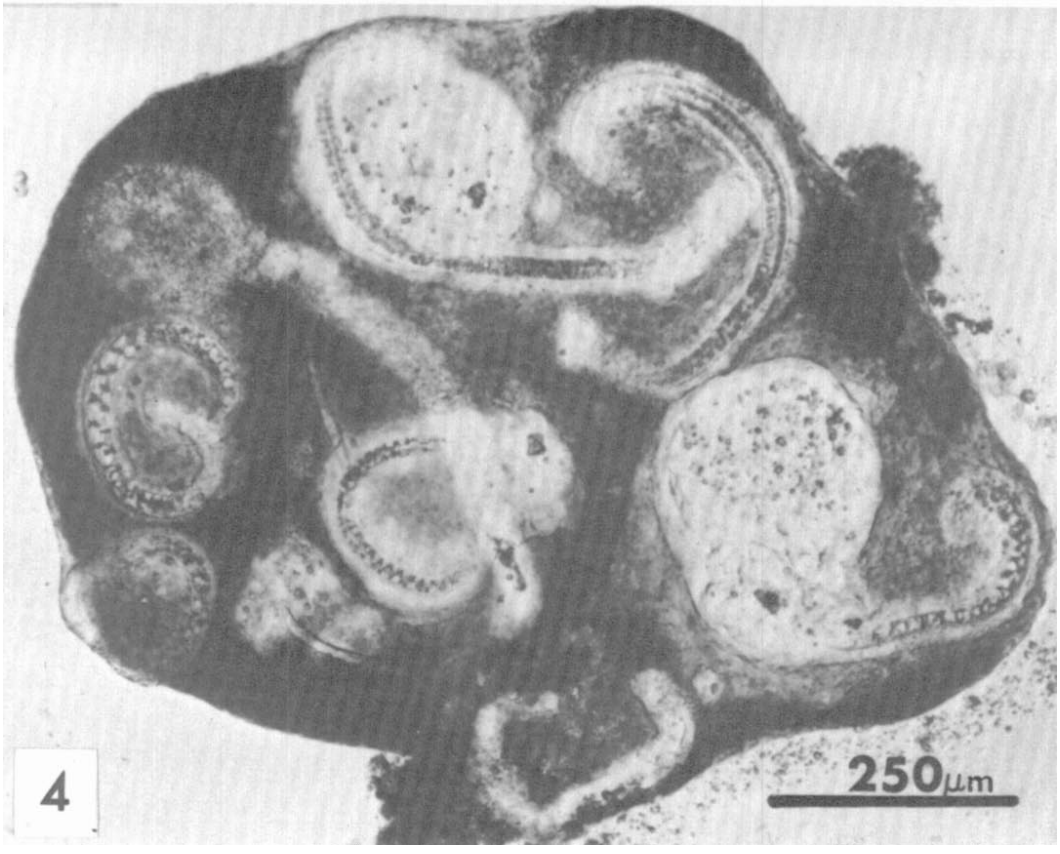
FIG. 1-2. The nematode, *Rhabditis pellio*, in the earthworm *Aporrectodea trapezoides*: 1) within a metanephridial bladder, and 2) in a metanephridial tubule.


78µm
1


98µm
2


H
B
72 µm
3


250µm
4

agar. For histological studies, 12-mm sections of earthworms were embedded in 10% gelatin, frozen, and sectioned with an International Harris Cryostat model CT. Sections cut at 8, 16, and 24 μm in thickness were attached to microscope slides with Mayer's albumin solution and fixed for 30 min in Bouin's solution. Following fixation, the sections were washed in 50% and 70% ETOH for 5 min to remove the picric acid, then rehydrated to distilled water. The sections were then stained for 2 h in Giemsa solution, dehydrated in an alcoholic series to xylene, and mounted in Permount. The nematodes were stained deep blue in contrast to the lighter stained adjacent tissues. Observations on living nematodes in the hosts were made during dissections.

The "brown bodies" containing *R. pellio* were obtained by opening terminal segments of earthworms and squeezing out the contents of the coelom.

RESULTS

Rhabditis pellio occurred in the metanephridia and coelom of living *Aporrectodea trapezoides.* Those specimens in the metanephridia were mostly active juveniles. Some were still in the "dauer" or "infective" stage, whereas others were developing juveniles or, rarely, young adults. These nematodes were found in the bladders and tubules of the metanephridia (Fig. 1, 2).

Nematodes found in the coelom were mainly juveniles or young adults that were invariably in the process of being encapsulated and incorporated into "brown bodies." During the encapsulation process, individual nematodes were first covered with a thin homogeneous deposit, possibly representing an initial humoral response of the host. They were then covered with layers of blood cells containing brown pigment granules (Fig. 3). During this process, the growing capsules were passed to the posterior end of the host where they coalesced with similar capsules containing nematodes, protozoans, chaetae, and debris of unknown origin. These entities formed the so-called "brown bodies" (Fig. 4) that have been mentioned so frequently in the oligochaete literature.

Most of the nematodes were probably living when first attacked by the host's defenses (Fig. 5). However, in mature capsules, half of the nematodes were already dead and in various stages of degeneration when examined. At one stage, the nematode cadaver resembled a mass of small loosely attached particles (Fig. 6). Older specimens were completely decomposed, with only a faint outline remaining. When "brown bodies" containing living nematodes were placed on nutrient agar, only a few nematodes escaped, and cultures were rarely established.

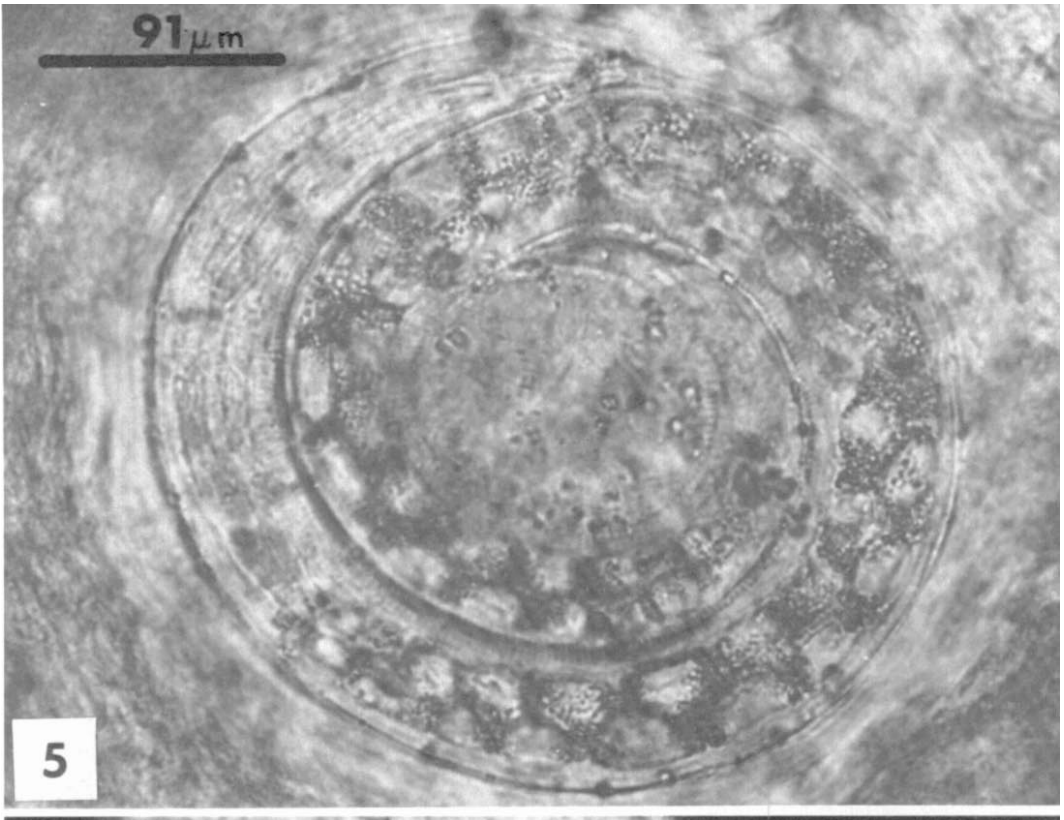
All of the 20 specimens of *A. trapezoides* which were collected contained large numbers of *R. pellio*, yet no sign of inflammation in the host was noted.

DISCUSSION

The life history of *Rhabditis pellio* was briefly discussed by Cuénot (2), who suggested the nematodes entered the host through the nephridiopores. Our findings confirm his belief and indicate that entry is probably made by dauer juveniles since this stage is encountered in the metanephridia. These dauer stages develop into feeding juveniles that move through the bladder and tubules and occasionally enter the coelom through the nephrostome. Although young adults were also observed in living hosts, no reproducing adults, eggs, or young juveniles were encountered. If any development occurs, it probably does so in the metanephridia since the nematodes appear to elicit an immediate host response upon entering the coelom. This is based on the fact that free nematodes were never recovered from the coelom. Nematodes also were never found in the body wall musculature as reported by Lankester (8).

Maturation of *R. pellio* probably occurs in two ways. The nematodes can escape to the outside through the nephridiophores and establish themselves by feeding on bacteria in the soil. Or they can remain in the host until it dies and then feed on the decaying carcass. Earthworms that were killed and placed on agar plates contained masses of reproducing *R. pellio* after several days. *R. pellio* can be maintained continuously on agar cultures

FIG. 3-4 3) The nematode, *Rhabditis pellio*, soon after entering the coelom of the earthworm, *Aporrectodea trapezoides.* H = homogeneous deposit. B = host blood cells. 4) A multiple-capsule or "brown body" of *A. trapezoides* containing various stages of *R. pellio.*


91 µm
5

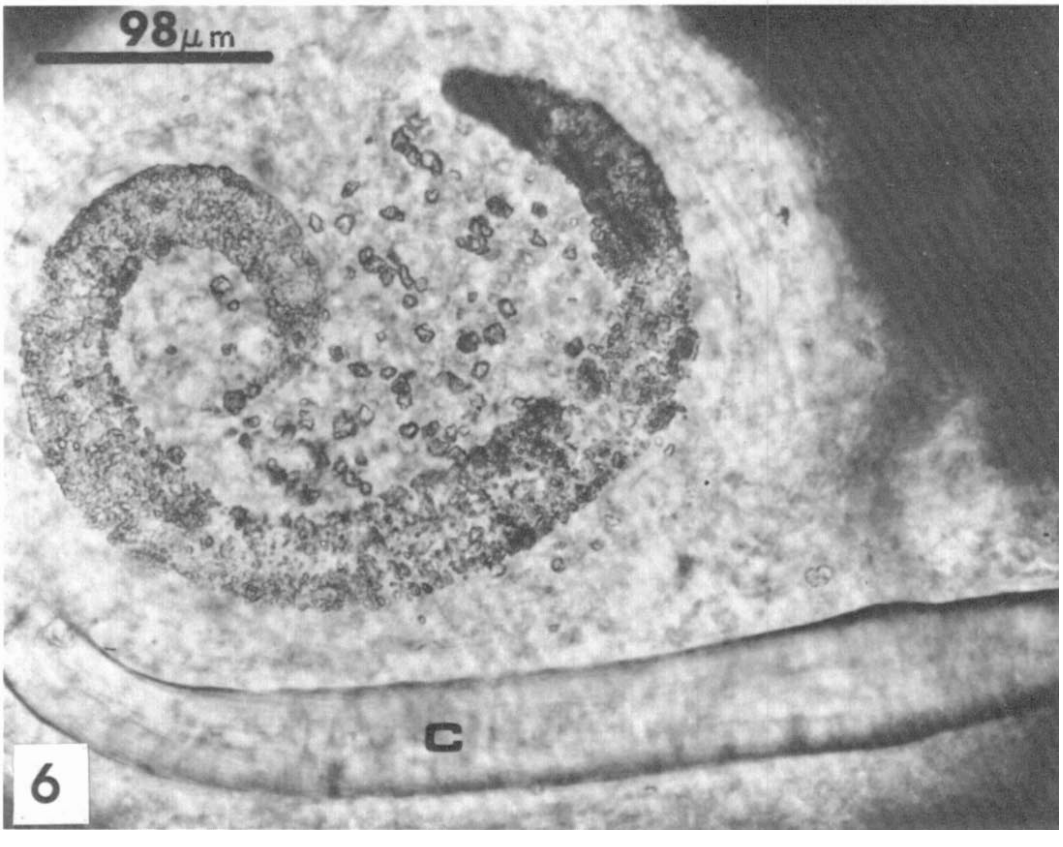

98 µm
C
6

FIG. 5-6. 5) A living nematode, *Rhabditis pellio*, coiled up in a multiple-capsule or "brown body" in the earthworm *Aporrectodea trapezoides*. 6) A disintegrating specimen of *R. pellio* in a multiple-capsule or "brown body" of *A. trapezoides*. C = chaeta.

seeded with various bacteria. Thus, it is clear that earthworms are not an essential stage in the nematodes' development. The association may have initiated as a means of protection for the nematode, especially when the habitat became dry. The nematodes then became able to undergo partial development in this new habitat, and were assured of an ample food supply when the host died.

The term "brown bodies" that represents spherical masses of host cells surrounding parasites and other debris was apparently coined by Johnson (7) who described them surrounding juveniles of *Rhabditis maupasi* Seurat in *Lumbricus terrestris* L. Metchnikoff (10), one of the first to observe nematodes in these structures, called them phagocytic masses, whereas Cuénot (2) described them as phagocytic nodules, and Tu (13) named them "small spheres" (Bälchen).

Brown bodies are actually single or multiple host capsules surrounding foreign debris or organisms. The host reaction begins as a homogeneous deposit on the surface of the nematode or foreign body. Metchnikoff (10) considered this deposit as a supplementary cuticle secreted by the nematode, whereas Cuénot (2) described it as a secretion from the host's blood cells. Our observations indicate that it may be a deposit from the noncellular portion of the hemolymph, thus representing a humoral response. Host bood cells follow this initial deposit, and become flattened and appressed to one another.

That this process is a successful host defense reaction is obvious by the number of dead and dying nematodes in the capsules. Another interesting aspect of this reaction is that it also occurs against the host's chaetae that fall into the coelom. Conversely, some nematode parasites of earthworms apparently do not elicit a host defense reaction; e.g. members of the nematode-parasitic Drilonematoidea which occur free in the coelom. This may indicate an adaptation on the side of the parasite and show a long-standing, host-parasite relationship.

Although *R. pellio* enters earthworms, it does not possess the resources to reproduce in a living host. Only when the host dies and conditions revert to those found in the soil, can the life cycle be completed. Also, it is clear that *A. trapezoides* is able to encapsulate those nematodes that enter the coelom, and that this encapsulation process is an effective defense reaction.

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