cases, had returned to their preinfection weights. Their recovery to a state of generally good health is remarkable relative to the morbidity and mortality observed with the nonmedicated controls or dogs treated on infection days 1 and 2. Further, it is of interest that dogs showing clinical signs of canine hookworm parasitism can apparently be successfully treated as late as 1 or 2 days prior to that time when death would be imminent.

It is thought that the digest solutions would not adversely affect the somatic larvae although none were recovered from the thigh digest solutions for all of the dogs. No attempts were made to check other host tissues. Therefore, it is assumed that the use of "hookworm naive" dogs permitted extensive larval migration via the lungs and the subsequent development of the large numbers of hookworms which were recovered from the gastrointestinal tracts of these dogs.

Acknowledgments

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Fine Structure of the Larval Stage of *Paragordius varius* (Leidy, 1851) (Gordioidea: Paragordidae). II. The Postseptum

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ABSTRACT: The structure of the postseptal portion of the larval stage of *Paragordius varius* has been examined by means of electron microscopy. The postseptal body wall consists of a cuticle, an underlying hypodermis, and six longitudinal muscles arranged peripherally. Contained within the postseptal area are two glands and two groups of undifferentiated cells. The postseptal gland consists of several vacuole-filled cells whose contents apparently collect in a cuticularized duct. This duct traverses the septum and opens at the tip of the proboscial portion of the preseptum. The other gland, the pseudointestine, consists of four cells enclosing a large cavity. These cells secrete small granules into the lumen of the gland and coalesce into larger entities, the refringent granules. The lumen of the pseudointestine opens to the exterior via a canal (exit duct) onto the posterior ventral surface of the body.

In this study the electron microscope was employed to obtain a more accurate concept of the larval anatomy of *Paragordius varius*. The anatomy of the preseptum (anterior portion of the larva) and a brief historical review are presented in Zapotosky (1974).

Materials and Methods

Larval stages of *Paragordius varius* were collected by incubating egg strings collected from two ovipositing females. Egg strings with fully developed larvae were fixed in ice-cold, phosphate-buffered 1% osmium tetroxide (pH 7.2) for 4 hr. The specimens were dehydrated in a graded series of ethanol and then placed in a graded series of epoxy resins and 100% ethanol. Tissue sections were counterstained with uranyl acetate and lead citrate, then examined with the electron microscope. For a more detailed description of specimen treatment see Zapotosky (1974).

Observations

General features

The larval body of *Paragordius varius* consists of two major areas: a preseptum (an-

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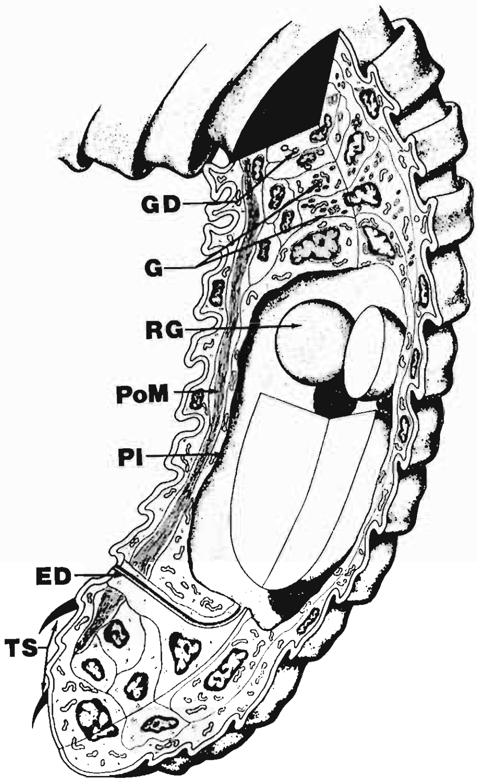

Figure 1. A three-dimensional diagram of the postseptum of the larva of *Paragordius varius*. A section has been removed to show internal structures.

terior) and a postseptum (posterior) divided externally by a constriction and internally by the septal complex. The postseptum (8–10 μ in diameter and 30–38 μ in length) is generally cylindrical, possessing a somewhat rounded and slightly enlarged posterior end (Fig. 4). Two pair of spines are located on the posterior ventral portion of the postseptum (Figs. 3–5). For an overall arrangement of internal structures see Figure 1.

Body wall

The surface of the postseptum is thrown into folds giving the body an annulate appearance. This annulation is superficial and involves only the cuticle and the hypodermis. The folds are irregular and in longitudinal sections the numbers of folds on each side of the postseptum do not correspond. The ultrastructure of the cuticle is the same as found for the postacanthal region of the preseptum (see Zapotosky, 1974). However, the hypodermis consists of a nucleate layer immediately underlying the cuticle (Fig. 8). No cell membranes were observed between hypodermal nuclei, although bounded internally and externally by these membranes. It is assumed to be of a syncytial nature.

A large pair of motile tail spines (3 μ long) are located laterally and posterior to a smaller (2 μ long) pair of ventrolateral spines (Figs. 1, 3–5). These spines are modifications of the cuticle and have a similar makeup as reported for the spines of the preseptum (see Zapotosky, 1974).

Muscles

The postseptal musculature consists of a set of longitudinal muscles (six peripheral cells), the mucles of the lateral tail spines, and fibrils found in association with the exit duct of the pseudointestine.

The postseptal parietal muscles originate on the septal complex and insert near the posterior

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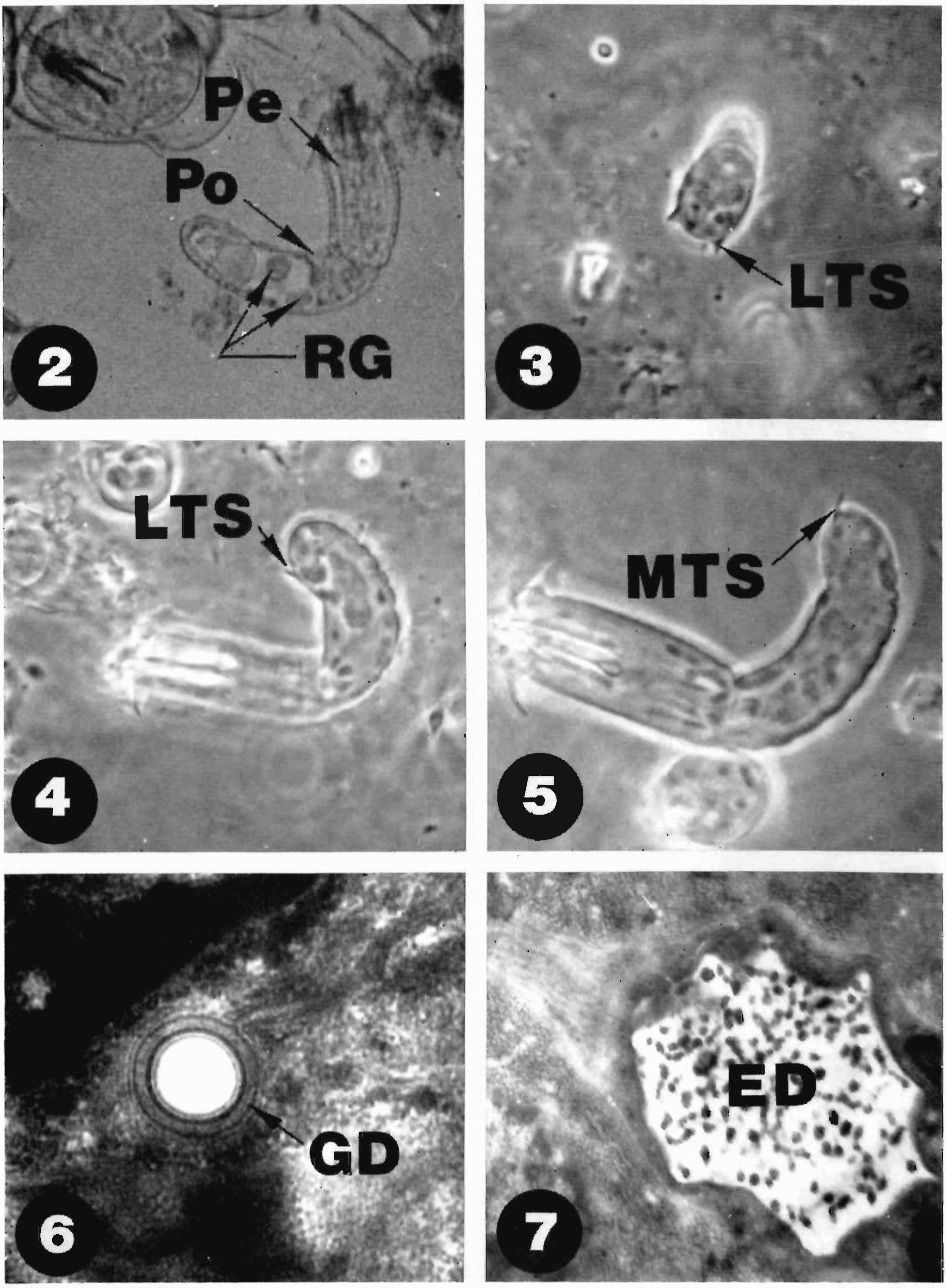
Figure 2. Light micrograph of a living larva escaping from egg membranes. 800 ×.
Figure 3. Phase contrast micrograph of a living larva, dorsal view. 800 ×.
Figure 4. Phase contrast micrograph of a living larva, lateral view. 800 ×.
Figure 5. Phase contrast micrograph of a living larva, lateral view. 800 ×.
Figure 6. Cross section through the gland duct. 67,300 ×.
Figure 7. Cross section through exit duct of the pseudointestine. 31,890 ×.

Abbreviations: C, cuticle; ED, exit duct; EM, egg membrane; G, postseptal gland; GD, gland duct; GDC, gland duct cell; H, hypodermis; LTS, lateral tail spine; MTS, median tail spine; Par, partition containing exit duct; Pe, preseptum; PI, pseudointestine; PIC, pseudointestinal cell; Po, postseptum; PoM, postseptal muscle; Se, septum; TS, tail spine.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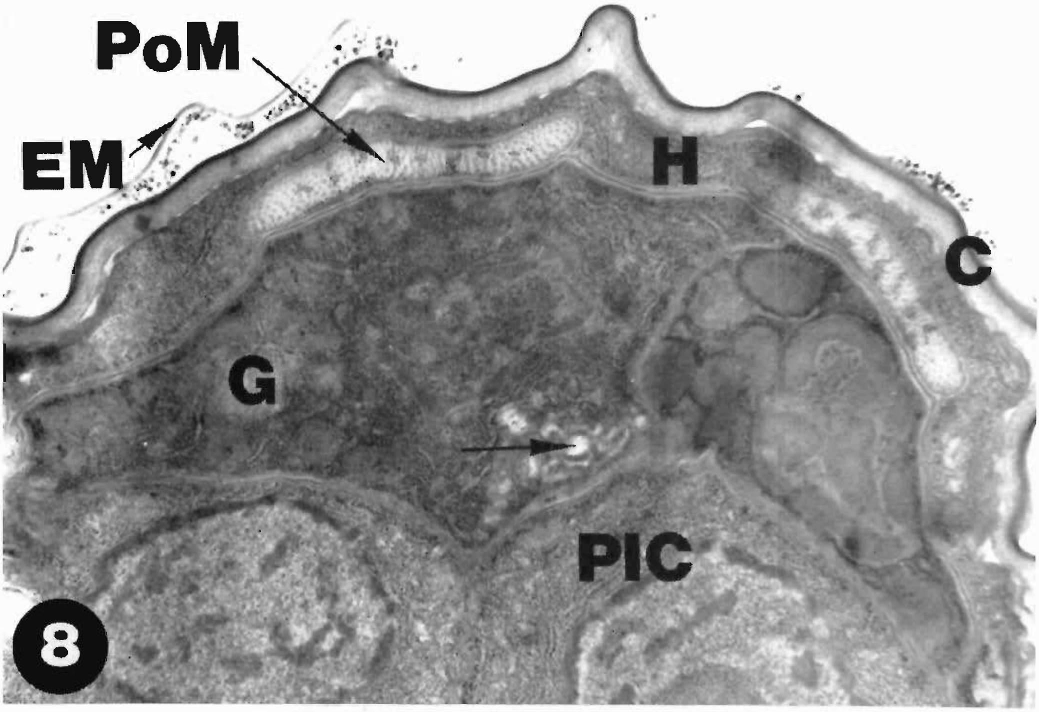
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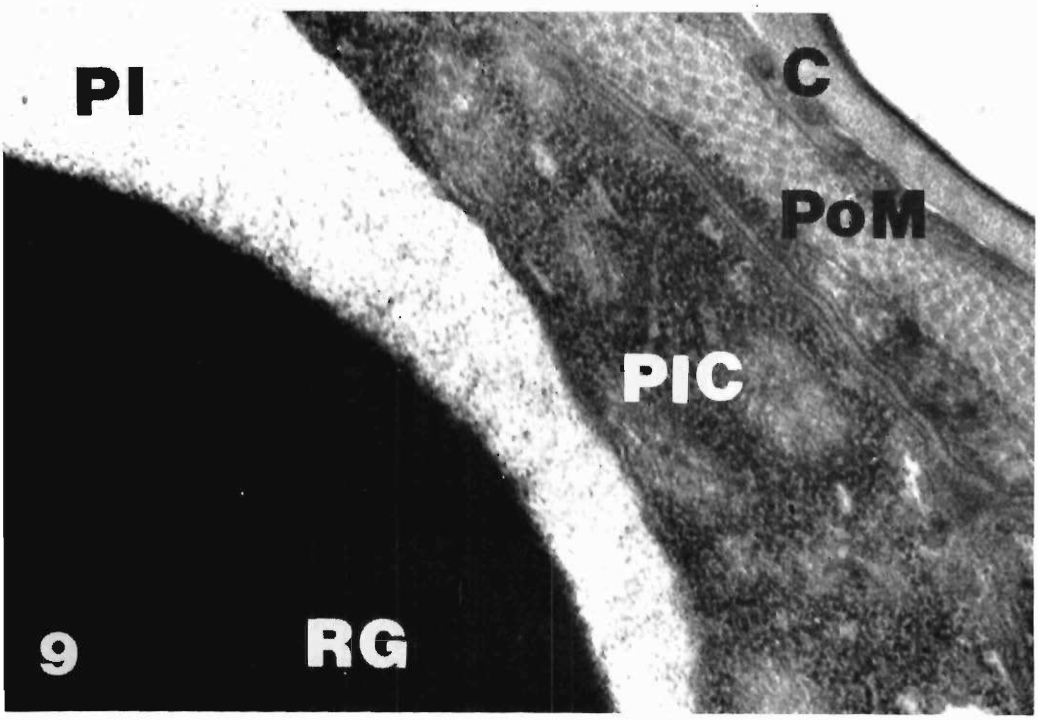
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extremity of the postseptum. The muscle tracts are oblong in cross section (0.3 by 2–2.5 μ) and contain fibrils 200–300 Å in diameter (Figs. 8, 9). The nuclei are located in a slightly swollen portion of the cell adjacent to the septum.

Postseptal gland and gland duct

The postseptal gland consists of several cells located between the gland duct cells and the anterior end of the pseudointestine. The bulk of the gland occupies a median position in the postseptum but also partially envelops the lateral margins of both the gland duct cells and the pseudointestine (Figs. 8, 10). The gland cells contain a moderate amount of endoplasmic reticula and numerous membrane-bound vesicles. The vesicles are of two types, or perhaps phases, a large irregular type vesicle with finely granular contents, the other with a relatively clear lumen and large granules (Fig. 10).

The gland duct and its enveloping cells originate on the anterior portion of the postseptal gland, traverse the septum, and open at the tip of the proboscis. There are at least three cells in association with the duct: one located at the posterior end of the preseptum and two tandem cells immediately posterior to the septum (Fig. 10). Light microscopic examinations indicate a coiled structure in these two areas and a balled, stringlike structure enveloping and somewhat posterior to the postseptal gland. In cross sections the duct appears cuticular, consisting of three circular lamellae. The lumen of the duct is of relatively constant diameter throughout its length (0.18–0.21 μ) (Fig. 6). The duct is enclosed by a unit membrane, thrown roughly into a u-shaped loop. The curved portion of the "u" surrounds the canal while the free ends are apparently continuous with the cell membrane of the enveloping cell. Although a direct connection between the gland cells and the lumen of the cuticular duct was not observed, all the gland cells examined did possess a reticular-like structure (Figs. 8, 10).

Pseudointestine

The pseudointestinal sac is a sausage-shaped structure composed of four cells. The cells enclose a large central cavity in which are contained two anterior granules and a large posterior mass. The lumen of this organ opens to the exterior via a ventromedial duct (Fig. 1).

The pseudointestine is thin-walled in its middle portion, while both the anterior and posterior ends are enlarged and contain nuclei. The two anterior cells are adjacent, forming a complete cap over the anterior end of the pseudointestine (Fig. 8). These cells are located over, and secrete, the anterior two refringent granules (Fig. 9). The posterior two cells of the pseudointestine are larger than the anterior cells and are found adjacent only in their ventral and posterior portion. Between the posterior cells there is a mesentery-like partition which contains the exit duct of the pseudointestine (Fig. 11). Both anterior and posterior cells have large open nuclei with relatively large amounts of ribosomes and rough endoplasmic reticula.

The lumen of the pseudointestine is round to oval in cross section (4.5–6 μ in diameter) and sausage-shaped in longitudinal sections (12–13 μ long). The refringent granules enclosed within the lumen are intensely osmiophilic and are composed of even smaller entities of about 33–45 Å in diameter. The refringent granules are apparently formed by coalescence of the small entities secreted by the cells (Fig. 9). The anterior two granules usually appear spherical or spherical with truncated adjacent sides (Fig. 2), while the shape of the large posterior mass (usually not readily seen in light microscopic studies) follows that of the enclosing sac.

The lumen of the sac opens onto the median ventral surface of the body by means of an L-shaped duct approximately 1–1.5 μ in diam-

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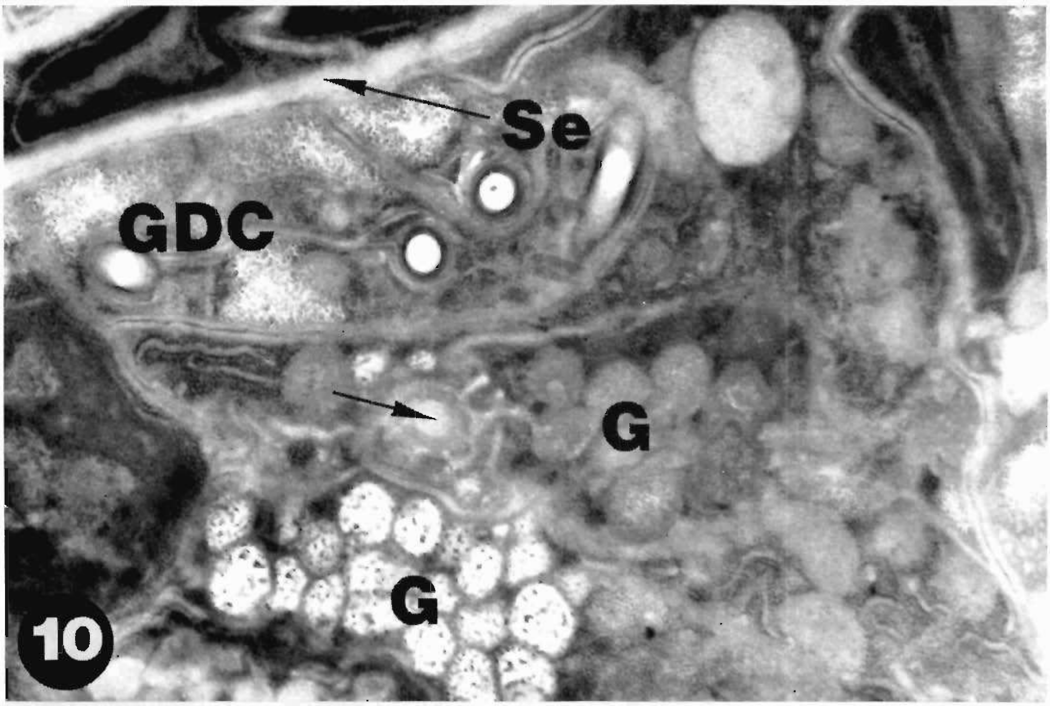
Figure 8. Cross section through the anterior cells of the pseudointestine; arrow indicates reticularlike structure. 23,300 ×.

Figure 9. Cross section through a portion of the pseudointestine and the postseptal body wall. 50,800 ×.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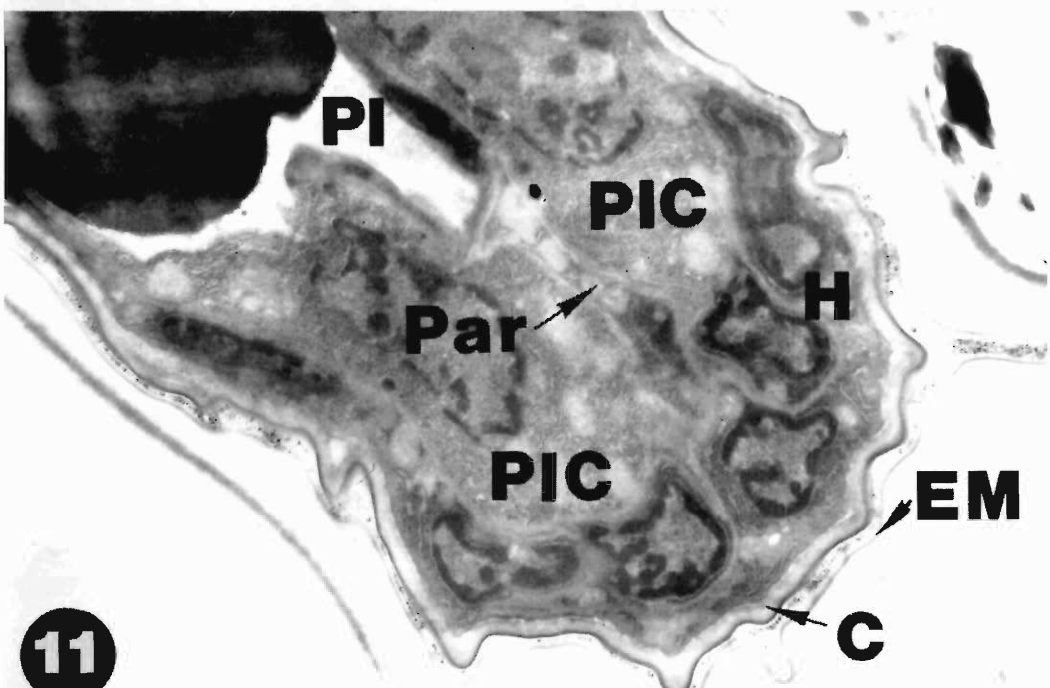
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eter. There is a short portion of the canal located medially and parallel to the longitudinal axis of the body (Fig. 11). This then turns perpendicular to the longitudinal axis about 6 μ from the end of the body and continues to the ventral surface (Fig. 1). The short leg of the duct apparently possesses fibrils (210–220 Å in diameter) attached to one of its sides (Fig. 7). The duct is lined by a cuticle which is structurally different from the external body wall cuticula or the lining of the gland duct. Located within this duct are granules or filaments in cross section. The duct and its fibrils are enclosed by a complex of cells (at least two or three) which forms the partition separating the two large posterior cells of the sac.

Undifferentiated cells

There are two groups of "undifferentiated" cells (i.e., showing no specialized organelles—such as fibrils, vesicles, etc.) found in the postseptum. One set of cells are located in the space between the posterior aspect of the pseudointestinal complex and the hypodermis. The other set of cells fill the space between the body wall and the postseptal gland and its duct.

Discussion

Montgomery (1904) and later Inoue (1958) correctly observed the presence of muscles beneath the postseptal hypodermis of *Paragordius varius* and *Chardodes japonicus*, respectively. They could not, however, determine the muscular arrangement or structure. Montgomery's observations were supported by both Dorier (1930) and Muhldorf (1914) when they induced the presence of a postseptal musculature from flexions and irregular torsions in the postseptum of *Gordius aquaticus*.

The postseptal gland of this study corresponds, although denoted differently, to a structure similar to that reported by earlier authors. Inoue (1958) and Montgomery (1904) referred to the structure as, simply, gland, while Dorier (1930, 1932, 1935) dubbed it "appareil glandulaire." Following the lead of Vejdovsky, Muhldorf (1914) called it the "braune druse" or brown gland. In 1884, Vejdovsky (Muhldorf, 1914) described a brown organ in the anterior end of the parasitic stage of a gordioid, supposedly developing from this larval gland. In light microscopic examinations, the larval postseptal gland of *P. varius* was colorless. Indeed, the parasitic stage of *P. varius* did not possess any brown structure, except the remains of the preseptum.

Both Montgomery (1904) and Dorier (1930) described the larval postseptal gland of *P. varius* and *G. aquaticus* as a cellular mass with eight large nuclei. Additionally, Dorier (1930) noted longitudinal lobes and transverse grooves in the gland of *G. aquaticus*. In a similar study Muhldorf (1914) correlated changes in the gland with its state of development. The final form of the gland was greatly elongate with its flattened or pointed end extending deep into the body cavity. The light and electron microscopic examinations of this study showed many nuclei in the area between the septum and the anterior end of the pseudointestine. However, the electron photomicrographs indicated the gland to consist of two or three uninucleate cells possessing a vacuole-packed cytoplasm.

In *P. varius*, Montgomery (1904) described a duct that arose near the base of the postseptal gland, extending through the gland on its course through the septum. Inoue (1958) noted a similar arrangement for *C. japonensis*. However, Dorier (1930, 1932, 1935), in his studies on *Parachordodes gemmatus*, *Parachordodes alpestris*, *Parachordodes violaceus*, and *G. aquaticus*, reported a proximal ball-like terminus that also ran across the septum to the tip of the proboscis. Zapotosky (1974) noted that when the proboscis of the preseptum was retracted the duct appeared coiled at the pro-

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Figure 10. Longitudinal section through the anterior portion of the postseptum, arrow indicates reticularlike structure. 30,000 ×.

Figure 11. Longitudinal section through the posterior portion of the postseptum; star indicates the proximal portion of the exit duct. 12,500 ×.

boscial base. Additionally, this study indicates both a ball-like terminus and some coiling of the duct posterior to the septum. Also it is shown here that three or four nucleated cells envelope the gland duct throughout its course. The reticular structure shown within the cells may represent a collection system (ball-like terminus) for the glandular secretions.

The function of the gland could not be determined by this study. Still, by virtue of the gland's cytology (large amounts of ribosomes and membrane-bound granular vesicles), it does not appear to be an excretory structure as hypothesized by Muhldorf (1914). Its relationship to the proboscis seems to indicate a function associated with the perforating apparatus of the preseptum (see Zapotosky, 1974).

Dorier (1930) correctly described the "intestinal sac" or pseudointestine as an elongated tube which occupies a large part of the postseptum. As he observed, the walls of this tube are thin except at the nucleated anterior and posterior end. The posterior end of the "intestine" of *G. aquaticus* was reported by Muhldorf (1914) to connect to the posterior ventral wall by a solid cuticular style or closed blastopore. Montgomery (1904) recorded the same condition in *P. varius* noting additionally the presence of two to four nuclei apposed to the surface of the stalk. However, both Dorier (1935) and Inoue (1958) observed that the larvae of *P. gemmatus* and *C. japonensis* possessed a small open canal to the ventral surface. As shown here, *P. varius* has a dorsoventral partition between the two posterior cells. This nucleated partition contains a definite canal which opens on the ventral surface. In addition, fibrils were found in association with this exit duct, suggesting a possible sphinctor or opening device for the canal.

Two or more large granules develop within the pseudointestinal sac of most gordioid larvae. Muhldorf (1914) described such globules in *G. aquaticus* and speculated that they were the remains of mesenchyme cells absorbed by the "intestine." Similar granules were noted in *C. japonensis* by Inoue (1958), in *P. gemmatus* by Dorier (1935), in both *P. alpestris* and *P. violaceus*, also by Dorier (1932), *G. robustus* by May (1919), and *P. varius* by Montgomery (1904). Montgomery (1904) believed these to be granules of metabolic waste. He suggested the "intestine" served as a waste reservoir until the next stage was reached. The cells of the pseudointestine contain large amounts of ribosomes and rough endoplasmic reticulae. Thus, these cells are apparently active in the production and secretion of proteinaceous materials into the lumen of the pseudointestine. Dorier (1935) noted that the larvae of *P. gemmatus* while encysting emits from the orifice of the pseudointestinal sac a viscous substance. While the secretion of the cyst was accomplished, he saw the two inclusions (granules of the pseudointestinal sac) diminish progressively. Inoue (1958) notes a similar condition in *C. japonensis*. These observations and the cytology indicates that the pseudointestine is in reality a gland used in larval encystment.

Dorier (1930) and Muhldorf (1914) referred to the mesenchymatous or rest mesenchyme cells which appear as large numbers of nuclei between the posterior extremity of the intestine and its posterior end. Similar cells are noted in the larvae of *P. gemmatus*, *C. japonensis*, and *P. varius* (Dorier, 1935; Inoue, 1958; Montgomery, 1904). In this study of *P. varius* many of the nuclei in the tail region can be accounted for, i.e., there are numerous hypodermal nuclei, two cells in association with the ventral tail spines, muscle cells to the lateral tail spines, and various nuclei in association with the exit canal of the pseudointestine. Still, there are several cells which do not have a readily apparent role, or association with another structure. Further developmental studies are necessary to determine the role of these cells.

No excretory organs or nervous system were found; however, a nerve primordium consisting of a double ventral row of large ectodermal cells has been reported for the larvae of *G. aquaticus* by Dorier (1930) and Muhldorf (1914), *G. robustus* by May (1919), and *P. varius* by Montgomery (1904). These cells were not seen here; still a complex of several cells with no structural peculiarities were observed on the ventro-anterior side of the pseudointestine. These cells did not appear to be in a double row and structurally resembled the "rest mesenchyme" cell noted earlier.

Muhldorf (1914) also reported a body cavity which develops from the blastocoel of *G. aquaticus*. In light and phase microscopic examination, a cavity was observed in both the preseptum (when the perforating apparatus was extruded) and in the postseptum (in permanent mounts only) of *P. varius*. Electron photomicrographs of larvae with the proboscis invaginated showed no cavity in either the pre- or postseptum.

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Metazoan Parasites of *Fundulus heteroclitus* (Linnaeus, 1766) from Insular Newfoundland[1]

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ABSTRACT: Five hundred and fifty-seven *Fundulus heteroclitus* (Linnaeus, 1766), from four locations on the west coast of insular Newfoundland, were examined for metazoan parasites, using conventional parasitological techniques. Six genera of parasites were recovered (two Monogenea, two Digenea, one Cestoda, one Acanthocephala). It was found that the parasite burden (*Gyrodactylus prolongis*, *G. stephanus*, *Urocleidus angularis*, *Homalometron pallidum*, and *Neoechinorhynchus rutili*) of *F. heteroclitus* was not homogeneous for sample area, sex of host (Monogenea), and length of host. Significant differences in the preferred site of attachment of the three species of Monogenea were noted. Seasonal variations in incidence and intensity were noted for some parasite species. Seasonal cycles were related to seasonal variations in selected environmental factors, and possible changes in host diet and physiology.

The mummichog, *Fundulus heteroclitus* (Linnaeus, 1766), a cyprinodont fish, is widespread in western Atlantic coastal and brackish waters (Leim and Scott, 1966; Scott and Crossman, 1973). It is a popular laboratory animal and is the host for a number of metazoan parasites (Dillon, 1966; Hoffman, 1967). To date only two papers (Gowanloch, 1927; Fantham and Porter, 1948) concerned with the parasites of members of the genus *Fundulus* from the Atlantic provinces of Canada have been published. *F. heteroclitus* is found at several localities in southwestern Newfoundland (Scott and Crossman, 1964).

In May 1973 a study was initiated to determine the occurrence, distribution, and seasonal dynamics, in relation to selected environmental parameters, of the metazoan parasites of *F. heteroclitus* in Newfoundland.

[1] This paper consists largely of material submitted by the senior author in partial fulfillment of the requirements for the degree of M.Sc., Memorial University.