The Structure and Function of the Scolex Glands of Three Caryophyllid Tapeworms

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ABSTRACT: The histology, histochemistry, and fine structure of the scolex glands are described for adults of Hunterella nodulosa, Glaridacris catostomi, and Glaridacris laruei. Frontal glands, found in H. nodulosa, consist of a mass of cells at the scolex apex with ducts leading to the syncytial tegument. Faserzellen, observed in G. catostomi and G. laruei, consist of a deeply staining mass of cells in the medullary parenchyma of the neck. Histochemical tests revealed the cytoplasm of the scolex glands of all three species to be rich in RNA and protein, but alkaline and acid phosphatase activity was not detected. Electron microscope observation showed these cells to have extensive endoplasmic reticulum, abundant ribosomes, Golgi, and numerous electron lucent vesicles in the cytoplasm. Vesicles originating from the frontal glands of H. nodulosa were observed releasing their contents to the surface; it appears that the frontal glands of this species secrete an adhesive substance which aids the parasite in attaching to its host. The function of the Faserzellen, on the other hand, could not be demonstrated for the adult worm. The role of these glands in determining site selection and causing intestinal pathology in the host is discussed.

Scolex glands in tapeworms have been shown to serve a variety of functions. In the Pseudophyllidea, for example, they are generally considered to be penetration glands (Williams, 1966; Bråten, 1968). A proteolytic function was also demonstrated for the cyclophyllideans Aploparaxis furcigera and Hymenolepis parvula by Slais (1961) and Taenia solium by Faroqi (1958). However, when Ohman-James (1973) was unable to demonstrate the presence of proteolytic enzymes in the scolex glands of Diphyllobothrium ditremum, she suggested that the secretory product of this species might serve as an adhesive instead. In contrast, cells in the rostellum of Hymenolepis diminuta were clearly shown to play a neurosecretory role; it was found that the release of secretory material from these cells triggered the strobilization of the worm (Davey and Breckenridge, 1967). There is also evidence that scolex glands are involved in initiating the strobilization of Echinococcus granulosus (Smyth, 1971).

In the Caryophyllidea two kinds of glands have been reported in the anterior end of the worm: frontal glands found near the scolex apex, and Faserzellen found in the medullary parenchyma of the neck. According to some authorities (Pintner, 1906; Sekutowicz, 1934; Wiśniewski, 1930) the two glands are homologous, but this point remains to be proven. The present study, part of a Ph.D. Thesis (Hayunga, 1977), was undertaken to examine the histology, histochemistry, and fine structure of these glands in Glaridacris catostomi Cooper, 1920, Glaridacris laruei (Lamont, 1921) Hunter, 1927, and Hunterella nodulosa Mackiewicz and McCrae, 1962, in order to determine their function and their role in causing intestinal pathology in the host.

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Materials and Methods

Host fish, *Catostomus commersoni* Lacépède, were collected by seine from several streams and ponds in the vicinity of Albany, New York. Fish were killed by a sharp blow to the head, dissected rapidly, and the intestinal helminths fixed within minutes after the fish were killed.

Worms were fixed for 16 hr in 10% neutralized formalin at 4°C, washed, and stored in 70% ethanol, then subsequently dehydrated and embedded in either a mixture of paraffin and beeswax (4:1) or in JB4 glycolmethacrylate plastic (Rud-dell, 1967). Paraffin sections were cut at 7 µm on an AO Spencer 820 microtome; plastic sections were cut at 1–3 µm on a Sorvall JB4 microtome using glass knives. JB4 plastic was obtained from Polysciences, Inc. Paraffin sections were stained as described in Clark (1973) and Humason (1972); protocols for staining plastic sections may be found in Hayunga (1977).

Localization of enzymatic activity was accomplished using naphthol substrates coupled with azo dyes, following the methods described by Burstone (1958) for alkaline phosphatase, by Barka and Anderson (1962) for acid phosphatase, and by Chayen et al. (1969) for leucyl-aminopeptidase and succinic dehydrogenase. Tissue was fixed in either 10% formalin or 100% acetone for 4 hr at 4°C and infiltrated with liquid Tissue-Tek® mounting medium for 16 hr at 4°C. Frozen sections 20 µm thick were cut on an AO Cryo-cut cryostat at −20°C. Controls consisted of sections processed without the appropriate substrate.

Specimens were fixed for electron microscopy with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer for 16 hr at 4°C, washed in buffer, then postfixed with 1% osmium tetroxide in cacodylate buffer for 90 min. Specimens were dehydrated with ethanol followed by propylene oxide, then embedded in Epon 812. Ultrathin sections were stained with a 2% solution of uranyl acetate in 50% methanol for 30 min at 60°C, and counterstained with saturated aqueous lead citrate for 2 min at room temperature. Sections were examined using the AEI EM6B transmission electron microscope at an accelerating voltage of 60 kV.

All observations reported are of adult worms; larval tapeworms could not be found in the field and attempts to raise procercoids in the laboratory were unsuccessful.

Observations

*Hunterella nodulosa*

Longitudinal sections of *H. nodulosa* reveal a conspicuous mass of cells near the scolex apex. These cells have an affinity for neutral red and aldehyde fuchsin, and appear to be homologous to the frontal glands described by Will (1893), Mrázek (1901), and Wiśniewski (1930) for other caryophyllid species. Faserzellen (neck cells), described below, were not found in *H. nodulosa*.

The frontal glands of *H. nodulosa* are very large cells with numerous cytoplasmic processes that anastomose to form an extensive syncytial network. Most of their cytoplasm stains purple with hematoxylin and eosin, but there are also large aggregations of eosinophilic granules in these cells (Fig. 1). The nucleus is basophilic and contains numerous chromatin granules and a prominent nucleolus. The frontal glands occur quite close to the surface near the scolex apex (Fig. 3) and cytoplasmic processes from the cells nearest to the surface can be traced...
Figures 1–5. 1. Frontal gland cell of *H. nodulosa* showing an aggregation of eosinophilic granules (arrow). Note also the large nucleus (n) and prominent nucleolus. Epon section, toluidine blue. 2. Cross section through the neck of *G. laruei* showing prominent Faserzellen (FZ) in the medullary parenchyma. Plastic section, toluidine blue. 3. Section through the cortical parenchyma of *H. nodulosa* near the scolex apex. Note the proximity of the frontal glands (FG) to the surface; tegumental cells are not found in this part of the worm. Plastic section, hematoxylin and eosin. 4. Faserzellen of *G. laruei*. This cell is characterized by its prominent nucleolus and by the mottled appearance of its cytoplasm. Plastic section, hematoxylin and eosin. 5. Mid-sagittal section of *G. laruei*. Note Faserzellen (FZ) in the medullary parenchyma. Paraffin section, trichrome stain.
Table 1. Histochemical localization of various substances in the cytoplasm of the scolex glands.

<table>
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<th>G. laruei</th>
<th>G. catostomi</th>
<th>H. nodulosa</th>
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<td>Alcian blue</td>
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<td>PAS</td>
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<td>Best carmine</td>
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<td>Pyronin</td>
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<td>Neutral red</td>
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<td>Aldehyde fuchsin</td>
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<td>Millon’s</td>
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<td>Bromophenol blue</td>
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<td>Oil Red O</td>
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<td>Leucyl aminopeptidase</td>
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<tr>
<td>Succinic dehydrogenase</td>
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+ positive reaction; ++ stronger reaction; +++ very strong reaction; ++++ extremely strong reaction; – negative reaction; ± inconclusive results; 0 no data.

directly to the syncytial tegument. In terms of their staining properties the frontal glands most closely resemble the tegumental cells. (Tegmental cells are not found at the scolex apex.) Frontal glands stain positively with pyronin, Millon’s reagent and bromophenol blue, indicating that they are active in protein synthesis (Table 1). The cells are PAS positive and there are small deposits of glycogen (as shown by PAS control and Best carmine) in the intercellular spaces. Attempts to localize alkaline phosphatase in these cells were inconclusive. However, acid phosphatase activity (which is present in the tegumental cells and in most of the syncytial tegument) was absent from both the scolex glands and from the tegument immediately above the glands. This suggests that the syncytium covering the body is different, chemically, from that covering the scolex apex, and that this difference is due to the nature of the secretory products synthesized by the cells immediately beneath the syncytium.

Ultrastructural observations show that the frontal glands transport their secretory products along the cytoplasmic processes in much the same manner as do the tegumental cells (see Hayunga and Mackiewicz, 1975, for comparison). Membrane bound pores passing through the tegument (as reported by Kwa, 1972; Öhman-James, 1973; Arne and Threadgold, 1976) were not observed in H. nodulosa; the secretion of the frontal glands of this species appears to be intrategumental. Electron lucent vesicles, found in the cytoplasm of the frontal glands (Fig. 7), can be followed along the cytoplasmic processes and into the syncytium, where they fuse with the surface membrane and release their contents (Fig. 6). Aggregations of these electron lucent vesicles correspond to the eosinophilic deposits seen with the light microscope; large electron lucent vesicles are not found in other parts of the tegument but only near the scolex apex. The cytoplasm of the frontal glands is rich in ribosomes and endoplasmic reticulum.

Previous investigations (Mackiewicz and McCrae, 1962; Mackiewicz et al., 1972; Hayunga and Mackiewicz, 1975) have reported that the scolex of H. nodulosa is separated from host tissue by a thin layer of amorphous eosinophilic material, the so-called “eosinophilic matrix.” The present study indicates that
Figures 6–8. 6. Tegument of *H. nodulosa* near the scolex apex. Note the large electron lucent vesicles in the syncytium and the granular material being released at the surface (arrow). ×17,000. 7. Frontal gland of *H. nodulosa* showing numerous electron lucent vesicles in the cytoplasm (arrow); nucleus (n). Aggregations of these vesicles appear to correspond to the eosinophilic deposits seen in Figure 1. ×7,000. 8. Faserzellen of *G. catostomi* showing the fine structure of the intercellular deposits (arrow) and arrangement of ribosomes in the cytoplasm. ×17,000.
the eosinophilic matrix is a secretory product of the frontal glands. Further observations on this matrix and the fine structure of the parasite-host interface are described elsewhere (Hayunga, 1977, 1979).

Glaridacris laruei

In G. laruei there are no frontal glands but there is a conspicuous mass of cells, the Faserzellen, found in the medullary parenchyma of the neck anterior to the vitellaria. The Faserzellen appear as individual cells when viewed in cross section (Fig. 2), but longitudinal sections reveal that they are arranged as a syncytial network (Fig. 5). In addition, isolated Faserzellen cells can be found in the medullary parenchyma among the first three or four vitelline follicles. The cytoplasm of these cells is characterized by numerous dark basophilic strands, and the nucleus by a large nucleolus (Fig. 4). The Faserzellen have an affinity for toluidine blue, fast green, neutral red, pyronin, and aldehyde fuchsin (Table 1). They stain positively for protein with Millon’s reagent and bromophenol blue, and give a slightly positive PAS reaction, but no glycogen deposits could be demonstrated in either the cytoplasm or the intercellular spaces. Electron microscope examination shows the Faserzellen of G. laruei to be almost identical in appearance to the frontal glands of H. nodulosa; their cytoplasm is rich in ribosomes and endoplasmic reticulum and contains numerous aggregations of electron lucent vesicles.

Glaridacris catostomi

Frontal glands are also lacking in G. catostomi. In this species, the Faserzellen are confined to the neck region anterior to the vitellaria. The syncytial cytoplasm appears mottled with hematoxylin and eosin staining; each nucleus is basophilic and has a prominent nucleolus. The Faserzellen are rich in RNA and protein, as demonstrated by methyl green-pyronin and bromophenol blue, and positive staining with neutral red and aldehyde fuchsin suggests secretory activity (Table 1). The spaces between the cells contain numerous glycogen deposits. (Such intercellular deposits of glycogen are found throughout the body of G. catostomi, primarily in the medullary parenchyma.)

Electron microscope examination reveals the Faserzellen of this species to be rich in ribosomes, endoplasmic reticulum, and Golgi. The ribosomes are found surrounding areas of electron lucent cytoplasm, but no aggregations of vesicles are found in these cells (Fig. 8). In the spaces between the Faserzellen there are numerous inclusions comprised of concentric dark and light bands (arrow, Fig. 8). These deposits bear a striking resemblance to the calcareous corpuscles (which are larger) of other species; the chemical composition of the deposits was not ascertained and their function remains obscure.

Discussion

According to Wiśniewski (1930), the frontal glands of Archigetes were presumed to be penetration glands, and thus homologous to similar glands found in

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3 This term has been handed down from the early descriptive literature, much of which is in German. The word “Faserzellenstränge,” which means “bundle of fibrous cells,” was introduced by Will (1893) to describe these conspicuous cells in the neck. Mrázek (1901) used the shorter word “Faserzellen” (“fibrous cells”), while Wiśniewski (1930) preferred the term “Halszellen” (“neck cells”).
pseudophyllid larvae. On the other hand, Hunter (1930) and Szidat (1937) suggested that the frontal glands of caryophyllids might aid in attachment. This latter view is supported by the observation of Mackiewicz (1972) that frontal glands appear more numerous in species with poorly developed attachment organs. Furthermore, caryophyllid tapeworms generally do not penetrate the gut, and there is no solid evidence that the frontal glands secrete proteolytic enzymes.

In *H. nodulosa* no evidence of proteolytic activity could be detected for the frontal glands. They were found, instead, to secrete a mucoprotein that forms the “eosinophilic matrix” found between parasite and host. This secretion appears to function primarily as an adhesive. Electron microscope examination shows that tegumental microtriches are attached to the matrix, and that the matrix closely adheres to host tissue (see Hayunga, 1979). It was originally suggested that, in the absence of any holdfast organs on the scolex of *H. nodulosa*, attachment was maintained by the hooklike microtriches (Mackiewicz and McCrae, 1962; Hayunga and Mackiewicz, 1975). It now appears that for this species attachment is accomplished by the microtriches in conjunction with the adhesive secretions of the frontal glands.

If the frontal glands of *H. nodulosa* are not penetration glands, then how does one account for the severe damage caused by this species, or for the observation that frontal glands appear most numerous in species that cause considerable damage to host tissue, while little pathology is caused by species lacking these glands? Hayunga (1977, 1979) suggested that the secretion of the frontal glands, although primarily an adhesive, also acts as a strong irritant, and that the nodule is formed as part of the inflammatory response of the fish. Thus, the frontal glands would be indirectly responsible for the pathology associated with this species. Clearly, more work is needed both in following the pathogenesis of the nodule caused by *H. nodulosa*, and in examining the scolex glands of other pathogenic species such as *Monobothrium ulmeri* and *Djombangia penetrans*.

The function of the Faserzellen remains obscure. Pintner (1906) and Wiśniewski (1930) both considered the Faserzellen to be homologous to the frontal glands, while Mrázek (1901) believed them to be the vestigial remains of a digestive system once present in the original protocestode. The positive staining of the Faserzellen with aldehyde fuchsin suggests a neurosecretory role, because scolex neurosecretory cells that triggered the strobilization of *H. diminuta* were shown to have an affinity for that dye (Davey and Breckenridge, 1967). However, if the Faserzellen are neurosecretory cells, they clearly must serve some other function because caryophyllid tapeworms do not undergo strobilization. A final possibility is suggested by the work of Sekutowicz (1934), who reported that Faserzellen found in adults of *C. laticeps* were the vestigial remains of frontal glands found in the procercoid stage. Histological studies of proceroids of *Glaridacris* and experimental studies of developing caryophyllids would be of great value in elucidating the function of these cells.

Although there is no direct correlation between the presence of scolex glands and the preferred site of attachment, there is evidence that scolex morphology can play a major role in determining site selection (Mackiewicz et al., 1972; Hayunga, 1977). Undoubtedly, both scolex glands and external scolex morphology evolved concurrently for the caryophyllids. It now appears that two successful strategies have been taken by these worms. In *H. nodulosa* we see an
extreme case where the scolex is poorly developed and attachment is maintained by the adhesive secretion of the frontal glands, while in G. catostomi and G. laruei the attachment organs are well developed and there is no need for adhesive glands. Further study is needed to determine whether intermediate strategies are also followed, and to what degree scolex glands and scolex morphology influence competition between species.

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