Studies in cysticercoid histology. I. Observations on the fully developed cysticercoid of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea)*

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In recent years the cestode *Hymenolepis diminuta* has become one of the laboratory animals frequently used in investigations of many aspects of tapeworm biology. It is therefore surprising that no studies are available on the microscopic structure of the cysticercoid. Moreover, observations on cysticercoid structure of other species are either fragmentary or deal primarily with gross morphology. The present report contains observations on the histology of fully developed, normal cysticercoids of *Hymenolepis diminuta*, and represents the first study of a series dealing with normal cysticercoid structure in different tapeworm species.

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**Materials and Methods**

Cysticercoids of *Hymenolepis diminuta* were grown in the confused flour beetle *Tribolium confusum* at 30°C, maintained on enriched flour. For studies on the fully developed cysticercoid, beetles were dissected in normal saline at least 10 days after infection. Five, six, or seven-day old cysticercoids were obtained for preliminary studies on the development of certain tissues. Live material was observed under the phase microscope for the presence of flame cells. For the preparation of sections, cysticercoids were fixed in different fixatives (Bouin's, Zenker's, acid alcohol, and formalin, etc.) depending on the stain to be used. Cysticercoids were embedded in paraffin and sections cut at 10 microns. Stains used were Mallory's aniline blue collagen stain as directed by Gridley (1953), Mallory's phosphotungstic acid hematoxylin, methyl-green pyronin, Langeron's alizarin red S, Mayer's hemalum, Feulgen, Bodian copper protargol as described by Lillie (1954), and Davenport's protargol method (Davenport *et al.*, 1939). Gomori's trichrome was used with Bouin's-fixed material as outlined by Lillie (1954), except that light green was substituted for fast green and the alum hematoxylin was omitted.

**Description**

The appearance and overall organization of the fully developed cysticercoid of *Hymenolepis diminuta* are illustrated in Figure 1 which represents a longitudinal section of this organism. The names employed to identify the different tissues or areas were selected for the purpose of description only and, with few exceptions, are not meant to suggest similarities in organization with adult tapeworms.

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The outermost layer or the external membrane (Fig. 1) is a thin and relatively delicate structure surrounding the whole cysticercoid. A surface view reveals thickened lines arranged in parallel fashion (perhaps an artifact) or an irregular network. In cross-section one observes that the membrane consists of two easily separable sheets, the outermost being very fragile and easily damaged or lifted above the second one beneath it. The space between these two layers reacts positively with fat stains (Voge, 1960). Beneath the external membrane, there are irregularly distributed acellular deposits which vary considerably in size and shape, and stain a uniform red with Mallory's aniline blue stain.

Next to the external membrane and in contact with its inner component is the peripheral layer which surrounds the body of the cysticercoid but does not extend into the tail. It consists of numerous hair-like processes arising from the surface of a single layer of cells (Figs. 1 and 2). These cells are flask or pear-shaped, or they may appear nearly rectangular depending on the section and perhaps the method of preparation. The cytoplasm of these cells, as well as the hair-like processes stain a deep blue with Mallory's aniline blue, while the small cell nuclei stain red. Among the hair-like processes there are scattered small granules which stain an intense red with Mallory's aniline blue but are not revealed with Mayer's hematoxylin or Gomori's trichrome. The latter two stains are not satisfactory for the demonstration of the peripheral layer.

Beneath the peripheral layer are situated relatively large cells which, in fully grown cysticercoids, stain weakly so that the cell borders are not always distinct. The nuclei are relatively large with well defined nuclear membranes and contain a small spherical mass of chromatina. These cells make up most of the intermediate layer (Fig. 1) and are present in the body of the cysticercoid but do not extend into the tail. The intermediate layer also contains a number of relatively small, deeply staining nuclei. Whether these represent remnants of degenerating cells or nuclei of other cell types could not be determined. Some of the nuclei are associated with elongated strands of cytoplasm and resemble the nuclei commonly observed in the tail parenchyma.

Beneath the intermediate layer is the fibrous layer. It is composed of a dense arrangement of fine fibers with interspersed elongated nuclei. Fibrous processes from this layer pass through the intermediate layer (Fig. 1) and connect with the cells of the peripheral layer (Fig. 3). Moreover, a dense network of fibers extends posteriorly towards but not into the tail. With Mallory's aniline blue and Mallory's phosphotungstic hematoxylin all fibers stain a brilliant blue or pink to red, while the nuclei appear red, or purple. Silver stains are useful in demonstrating the fibrous processes. All other stains used did not stain the fibers.

Beneath the fibrous layer is a narrow layer of very elongate cells which in Figure 1 is referred to as the lining of the cysticercoid cavity. The nuclei are definitely associated with strands of cytoplasm which do not stain as intensely as the fibers of the fibrous layer, but otherwise resemble them closely. Some of these cells are in contact with the narrow strip of dense tissue surrounding the scolex.

The scolex is bordered by the cuticle which also lines the tissue immediately surrounding it (Fig. 1). The scolex consists of densely packed cells as evidenced by the presence of a large number of nuclei. The suckers are fully developed and their musculature distinct. In living material one observes
Figure 1. Free-hand drawing of longitudinal section of *Hymenolepis diminuta* cysticercoid showing relationships of different tissues in the fully developed organisms; ad: acellular deposits, cc: cysticercoid cavity, cp: cells of peripheral layer, cs: cuticle of scolex, em: external membrane, fl: fibrous layer, fp: fibrous processes of fibrous layer, hp: hair-like processes of peripheral layer, ic: intermediate cell layer, ic: lining of cysticercoid cavity, tp: tail parenchyma.
flame cells situated close to the outer margins of the suckers. Flame cells were not seen in any area other than the scolex. Excretory ducts could not be traced either in living material or in sections.

The cysticercoid tail consists of a fine irregular network of cells which are referred to as the tail parenchyma (Fig. 1). The nuclei differ in size and structure, one type being relatively large, the other smaller and staining more heavily. The same types of nuclei were observed in the intermediate layer of the body of the cysticercoid. The parenchyma is seen to extend beyond the body-tail junction into the posterior portion of the cysticercoid body.

Preliminary observations on developmental stages of *H. diminuta* cysticercoids have shown an organization considerably different from that of fully developed cysticercoids. The hair-like processes of the peripheral layer are not visible until the 8th day of development. They increase in extent and length thereafter. The cells from which they originate, however, are discernible before that time. Some of the fibrous connections between these cells and the fibrous layer are already established by seven days development but the fibrous layer proper is much less extensive than it is in older cysticercoids. Furthermore, the staining reaction with Mallory's aniline blue in the fibrous layer varies from blue to purple, indicating perhaps the presence of transitional cell or tissue stages. Individual fibers are not sharply delineated as they are in older cysticercoids. The cell borders of the intermediate layer are sharply defined and their cytoplasm stains intensely. Some of these cells have long and narrow processes which extend to the area just beneath the external membrane. These processes are not apparent in older cysticercoids.

**DISCUSSION**

The apparent complexity of organization of the fully developed cysticercoid of *Hymenolepis diminuta* is perhaps surprising when one considers the ultimate fate of all tissues other than those within the cavity. As far as known, only the scolex and the tissue immediately surrounding it will participate in the formation of the adult tapeworm. All other tissues are apparently discarded upon entry into the final host. Hence it may be assumed that the tissues outside the cysticercoid cavity function in some manner in the intermediate host and are perhaps essential for the maintenance and protection of the fully developed cysticercoid. With respect to passage into the final host it could be argued that protection from the host's digestive enzymes should not necessitate such extensive histologic differentiation.

While a discussion of the possible function of the different tissue layers would be premature, a consideration of the affinities and relationships of the cysticercoid tissues with those of the adult tapeworm will be presented. As already mentioned, the names assigned to the various layers surrounding the cysticercoid cavity (Figs. 1-3) were purposely general ones. From observation of the fully grown cysticercoid it is not certain that these tissues can be discussed in terms of what is known about the histology of adult tapeworms. The external cysticercoid membrane and the seemingly highly specialized peripheral layer superficially bear no resemblance to the cuticle of the scolex within the cysticercoid cavity. The large cells of the intermediate layer do not resemble any of the cells described in adult tapeworms. However, in developmental stages of the cysticercoid these cells resemble the so-called epithelial cells situated beneath the cuticle (see Wardle and McLeod, 1952, p. 14) or the subciliated cells (Hyman 1951, p. 319). The fibrous layer of...
the cysticercoid has a staining reaction quite similar to that of collagen fibrils in mammalian tissue but does not resemble any of the tissues described in adult tapeworms. Only the tail parenchyma of the cysticercoid resembles the description given of parenchyma in adults, although the cysticercoid tail does not contain any of the large cells described for adult parenchyma.

Preliminary observations indicate the presence of distinct differences in the histology of different species of hymenolepidid cysticercoids. The hair-like processes in the peripheral layer of *H. diminuta*, for example, are absent in both *H. nana* and *H. citelli*. For a better understanding of cysticercoid structure, further study of fully grown cysticercoids of different cestode species, as well as cysticercoid developmental stages must be undertaken. Knowledge of the progressive differentiation of cysticercoid tissue is essential for interpretation of the histologic structure of fully grown forms and for comparison with tissues of adult tapeworms. Investigations of some of these problems, including additional studies on fully developed cysticercoids, are in progress.

**LITERATURE CITED**


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**Figure 2.** Camera lucida drawing of peripheral layer showing the cells (cp), the hair-like processes (hp), and the external membrane of the cysticercoid (Mallory’s aniline blue stain).

**Figure 3.** Camera lucida drawing of fibrous layer (fl) and the fibrous processes (fp) which are connected with the cells of the peripheral layer (cp); appearance of the latter varies considerably depending on orientation of section and on method of preparation (protargol). Cells of intermediate layer are not shown.