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Morphological Study of *Trichinella spiralis*: an Overall Picture of a Muscle Larva as Revealed by Longitudinal Sectioning

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Abstract

"Squashing and fixation" technique was introduced to make a longitudinal section of muscle larvae of *T. spiralis* and an overview of the larva was first described with hematoxylin-eosin (HE) staining profile. In brief, live muscle larvae were isolated by digestion with artificial gastric juice, squashed between two glass slides in the presence of the half strength Karnovsky fixative allowing the larvae to coil in one plane. These parasites actually lost their three-dimensional structure. After embedding in Acrytron E, the coiled parasites were cut in a longitudinal direction and stained with HE. Longitudinal sections through almost the entire length of the parasites were repeatedly obtained and successfully revealed a crystal-clear orientation which permitted the identification of each organ including the esophagus, the stichosome, the midgut, the hindgut and the genital primordium. And the occurrence of a unique banded structure, characterized by the alternative occurrence of eosinophilic and clear cells, was first described in the anterior portion of the stichosome.

Key words: *Trichinella spiralis*, muscle larva, histology

Morphological knowledge of a parasite is of critical importance in the comprehensive understanding of the species as of biology, life cycle and pathological effects on the host. Although some authors have described the morphology of *T. spiralis* (Beckett and Boothroyd, 1961; Bruce, 1966; Bruce, 1970a; Bruce, 1970b; Chitwood, 1930; Despommier, 1974; Despommier and Campbell, 1983; Richels, 1954; Thomas, 1965; Wright *et al.*, 1985), their descriptions are still fragmentary and consequently there is still a need for a complete description of this parasite's ultrastructure. One reason for the scarcity of morphological studies of *T. spiralis* may be due to the presence of the cuticle, which acts as a barrier and inhibits a penetration of chemical fixatives resulting in poor morphology. Another difficulty which one may encounter, even when the tissue is properly prepared, is the morphological orientation of the parasite. Because of its slender and coiled configuration, random sectioning provides a glance looking of the parasite's body, and consequently, identification of each organ is difficult especially for unexperienced workers. Thus, conventional random sectioning has not adequately met our current research needs which require precise morphological interpretation. Therefore, in an effort to overcome such inherent problems, the development of a new technique was necessary that allows a full picture of *T. spiralis* muscle-larvae at the light microscope level. In this paper we describe the technical details of our newly developed method for the histological preparation of *T. spiralis* larvae in muscles, with subsequent descriptions of HE staining profile and the spatial arrangement of each organ.

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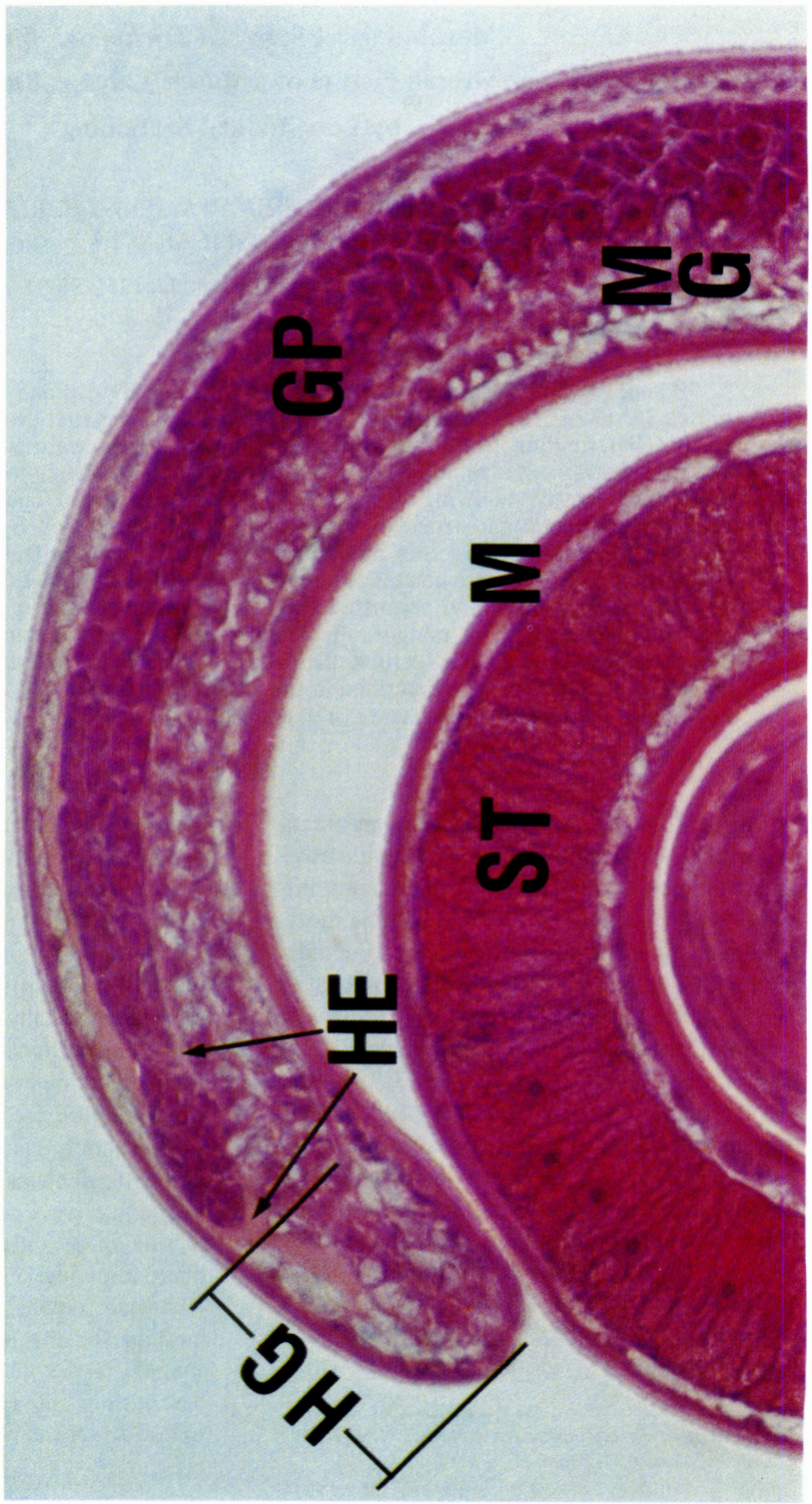

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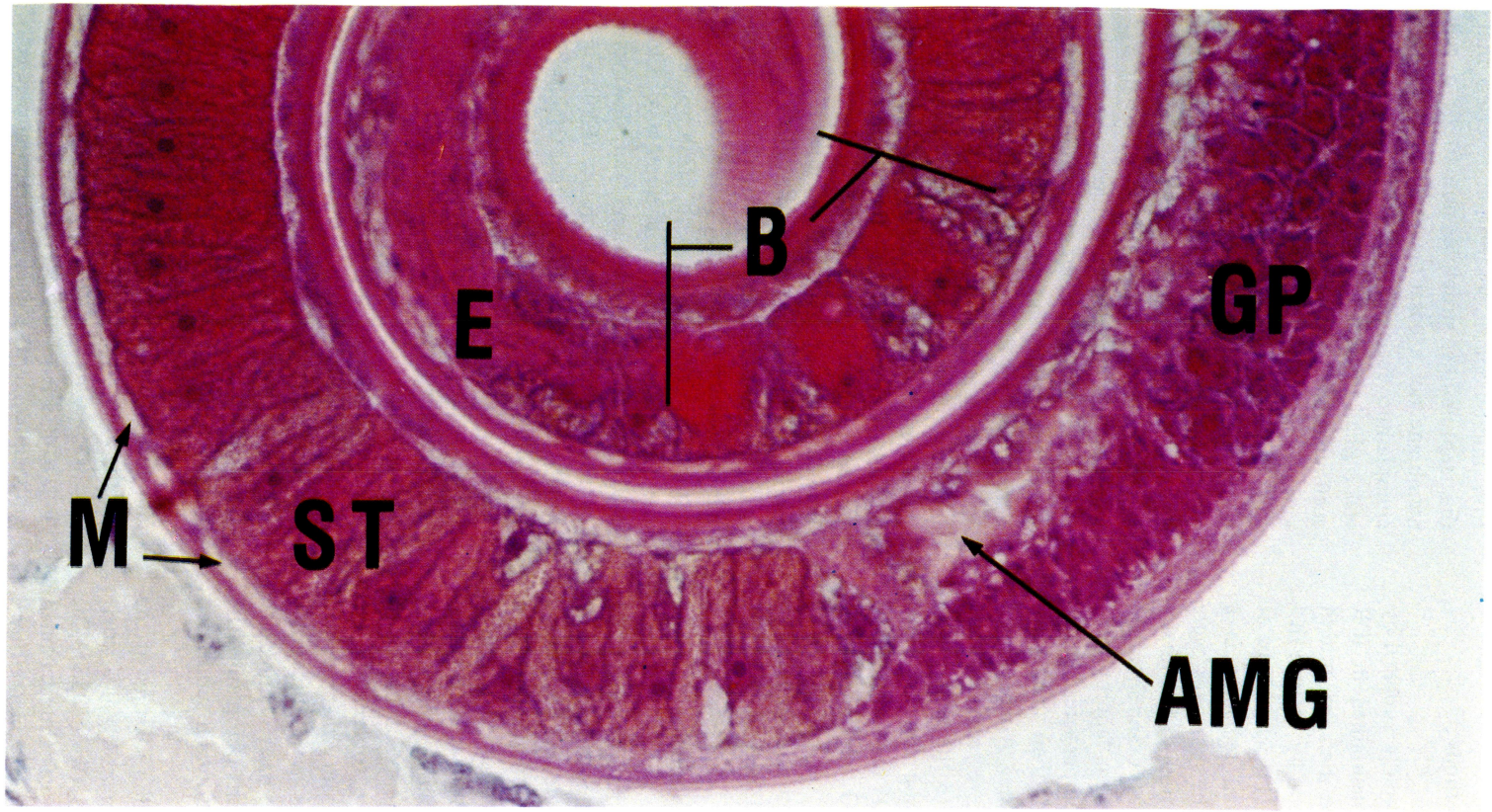

General longitudinal view of *T. spiralis* muscle larvae stained with hematoxylin and eosin. Abbreviations: esophagus (E), the banded structure (B), the stichosome (ST), the ampullar portion of the midgut (AMG), the midgut (MG), the hindgut (HG), the genital primordium (GP), the hemolymph (HE), the muscle (M).

Materials and Methods

T. spiralis (polish strain kindly supplied by Prof. T. Yamaguchi) was maintained in ICR mice. Muscle larvae of more than one month post-infection were isolated by pepsin-HCl degestion (Despommier, 1971), when kept at 4°C they ceased motion and remained in a corkscrew configuration. The parasites were then suspended in the half strength Karnovsky fixative (Karnovsky, 1965) at 4°C and squashed between two glass slides with "appropriate" pressure. All parts of squashed parasites became coiled in the same plane losing their characteristic stereoscopic configuration. After enough fixation time, two slides were separated, and the parasites, still attached to the slides, were dehydrated through ascending concentrations of alcohol and embedded in Acrytron E (MITSUBISHI RAYON CO., LTD., Tokyo Japan). Semi-thin sections of 1–2 μm were cut parallel to the coiled plane and stained with HE according to the standard method.

Observations and Discussion

The worm presented a cylindrical shape and tapering at both anterior and posterior portions. The fore-end was very delicate and beyond the limit of resolution for light microscope. At a more distal region and anterior to a typical stichosome, a banded structure was visualized (B in Fig.), which was morphologically characterized by alternative occurrence of 2 kinds of cells, one with eosinophilic cytoplasm and the other with "clear" cytoplasm when stained with HE. The former cells were discernible from the stichocytes on the basis of their size (about 12 μm in length) in the cytoplasm. The latter cells had the clear cytoplasm subdivided by a septum like structure.

The stichosome (ST in Fig.) is one of the most prominent organs composed of a single row of over 50 stichocytes (Wu, 1955). The junction of two stichocytes was almost planar, giving an appearance of being segmented by a septum-like structure (Thomas, 1965). Electron microscopical observation revealed that this septum-like structure not to be a cell membrane nor a basal membrane, but a rough endoplasmic reticulum-rich cytoplasm which was pushed towards the cell periphery by large accumulation of granules and glycogen (Yoshikawa *et al.*, 1987). The stichocyte presented a round nucleus with a prominent nucleolus, which stained dark violet, and its cytoplasm exhibited more or less granular appearance. The HE staining pattern of each stichocytes varied depending on the type of stichocyte granules and the amount of glycogen in the cytoplasm. More detailed analyse are currently under investigation in our laboratory by direct comparison of two adjacent sections by light and electron microscopy.

About midway along the parasite the esophagus entered the midgut (MG in Fig.), which extends through almost the whole of the posterior region of the parasite. At the junction between the esophagus and the midgut was the ampullar portion of the midgut (AMG in Fig.) accommodating amorphous material. HE staining of the midgut epithelial cells showed bright cytoplasms. The midgut with brush border on the cell surface (Beckett and Boothroyd, 1961; Bruce, 1966) narrowed and ran downward parallel to the genital primordium (GP in Fig.), finally ending in the short hindgut (HG in Fig.) with epithelial cells of thick cuticular lining which differed slightly from the external cuticle (Bruce, 1966). Although the midgut ran straight, the lumen of the midgut ran in a zigzaged manner.

A subcutaneous muscular system lies below the cytoplasm which is a thin extension from the cord cells (Beckett and Boothroyd, 1961). The inner half of each muscle cell cytoplasm is occupied by glycogen that usually occur in clumps (Beckett and Boothroyd, 1961; Takahashi *et al.*, 1986). The presence of this abundant glycogen provided an appearance of bright line underneath the cuticle in HE staining, which line ran from the anterior tip to the posterior tip of the worm being periodically interrupted by the nucleus of the muscle cells. Lying parallel to the midgut was the genital

primordium (GP in Fig.) which was composed of numerous small cells with clear nucleus, prominent nucleolus which stained violet, and a rather structureless cytoplasm which stained light violet. The genital primordium is surrounded by a single layer of basal membrane. The midgut is also covered by the same basal membrane (Takahashi *et al.*, 1987a). At the anterior portion of the genital primordium, the two basal membranes were in close apposition, while, at the posterior portion of the genital primordium, there was a widened space filled with hemolymph (HE in Fig.).

Cytoplasm of a great many cell types of the muscle larvae, including the cord, the muscles, the stichocytes, the epithelial cells of the gastrointestinal tract, contained aggregated glycogen in varying degrees (Beckett and Boothroyd, 1961; Takahashi *et al.*, 1986). When glycogen occurred in a large quantity, the glycogen rich cells gave a "clear" appearance by HE staining (Kim *et al.*, 1987).

In morphologically oriented study of *T. spiralis* larvae, the importance of a general longitudinal sectional view should be emphasized where the appearance of each organ varies according to position along the length of the worm and the angle by which it is cut. As typically shown in Figure, our *T. spiralis* larvae sections through the entire length of the worm revealed the crystal-clear identification and the spatial arrangements of each organ. A semi-thin section of Acrytron E-embedded specimens, thinner than a conventional paraffin section, provided higher microscopic resolutions. Because of hydrophilic nature of the Acrytron E, HE staining is feasible. These advantages promote the limitless use of our technique of "squashing and fixation" for histologically and cytochemically profilling *T. spiralis* at the light microscope level. The present contribution is the first outcome of the application of this technique, which offers the opportunity to elucidate the overall structure of *T. spiralis* larvae, and a unique banded structure was first identified in the anterior area to the stichosome. Other results obtained from the use of this technique including histological sex characteristics of *T. spiralis* larvae (Takahashi *et al.*, 198 b), histochemical properties of the stichosome granules, and localization of the antigenic substances are to be published.

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