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The Morphological Findings of *Trichinella spiralis* as Revealed by PAS and AZAN Stainings

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Abstract

The experiments described in the present report extend and amplify our previous morphological descriptions of *Trichinella spiralis* muscle larvae (Jpn. J. Parasitol. 36, 361–366, 1987) using PAS and AZAN staining approaches.

Longitudinal sections of *Trichinella spiralis* larvae in the muscle were prepared by means of a "squashing and fixation" technique in combination with PAS and AZAN staining procedures. The staining pattern thus obtained provided a comprehensive description of each organ studied. Structures identified as responsible for PAS positive staining included eosinophilic cells of the banded structure, stichocyte granules, esophagus-occupying substance, midgut-occupying substance and hemolymph. The strongest PAS positive staining was due to glycogen aggregates, which were distributed in a wide variety of cells such as the bright cells of the banded structure, stichocytes, muscle cells, cord, female genital primordial cells and epithelial cells of the alimentary tract. These glycogen aggregates stained very light blue by AZAN. A conspicuous blue staining by AZAN was observed in one third of the lower stichosome where α-stichocytes have been localized. The rest of the worm body stained yellow, red or orange by AZAN.

Key words: *Trichinella spiralis*, PAS, AZAN, histology

Introduction

Morphological investigations of *Trichinella spiralis* muscle larvae are confronted with particular problems because of this parasite's slender and coiled configuration. Conventional random sectioning allows only a glance of the body structure and, consequently, identification of each organ is difficult especially for unexperienced workers. The development in our laboratory of a "squashing and fixation" technique to make a longitudinal section of *T. spiralis* muscle larvae allows a crystal-clear orientation of each organ and, therefore, a detail description of the larvae as we have previously demonstrated by hematoxylin-eosin (HE) staining profiles (Takahashi, 1987b).

The use of this convenient technique along with PAS and AZAN staining approaches enabled the distinct characterization of organs such as the esophagus, the banded structure, the stichosome, the midgut, the hindgut and genital primordium. Our results further showed a conspicuous blue staining in the stichosome indicating that AZAN may be used in the detection of stichocyte α-granules, which have been reported to be antigenic in the early stages of trichinosis (Despommier and Müller, 1976; Silberstein and Despommier, 1984).

Materials and Methods

Trichinella spiralis (polish strain kindly supplied by Professor T. Yamaguchi) was maintained in ICR mice. Muscle larvae of more than 5 month post-infection were isolated by pepsin-HCl digestion (Despommier, 1974). The move-

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ment of larvae was ceased by storage at 4°C. The parasites were then suspended in half strength Karnovsky fixative (Karnovsky, 1965) at 4°C and squashed between two glass slides using a steady pressure. Squashed parasites coiled in one plane losing their characteristic corkscrew configuration. After enough fixation time, the two slides were separated and the parasites, still attached to the slides, were dehydrated with an ascending concentration of alcohol and embedded in Acrytron E (Mitsubishi Rayon Co., Ltd., Tokyo, Japan). Semi-thin sections of 1–2 μm were cut perpendicularly to the coiled plane and stained with PAS and AZAN according to the standard methods.

Results and Discussion

The Stichosome

By definition, the stichosome of *T. spiralis* is an organ composed of a single row of 50 to 55 discoid stichocytes occupying nearly one-half of the anterior region of the larvae (Chitwood, 1930; Richels, 1955; Wu, 1955; Villela, 1970). The cytoplasm of the stichocyte usually contains abundant granules of one of major two types, α- or β-granules (Despommier and Müller, 1976). Presumably because of this heterogenity all stichocytes did not stain in the same manner by PAS and AZAN, and exhibited a cross-striated appearance in one third of the lower stichosome (Figs. 1, 4, 5, and 8). Our current working hypothesis derived from a direct comparison between adjacent ultrathin sections for electron microscopy and PAS stained sections is that β-stichocytes yield a higher density of PAS positive staining than α-stichocytes (Yoshikawa *et al.*, 1988). Since both α- and β-granules were PAS positive, the observed difference in staining density may be due to the amount of glycogen in the cytoplasm.

Of particular interest was the prominent blue staining by AZAN in the stichosome, which exhibited a granular appearance in a high power (Fig. 8). Since this blue staining was encountered in one third of the lower stichosome and the distribution pattern (cross striation) closely resembled to that of α-stichocytes, the most probable structure responsible for this prominent blue staining is α-granules with a sharp inclusion. The greatest advantage of AZAN staining lies on the fact that blue staining in α-granules is prominent and the fate of them in parasite's life cycle can be monitored with excellent easiness. Granules in α-stichocytes have been reported to be antigenic during the initial course of *T. spiralis* infection (Despommier and Müller, 1976; Silberstein and Despommier, 1984; Uno *et al.*, 1988), and to be secreted from stichocytes to the lumen of the esophagus by the 30th hour of infection (Despommier, 1974). We have already confirmed that conspicuous blue staining was absent or undetectable in the adult worms recovered from the host intestine after oral inoculation (to be published elsewhere). The biological function of stichocyte granules responsible for this conspicuous blue staining and the immunological meaning of their disappearance should be investigated from the point of host-parasite relationships. The other major granules in the stichosome, β-granules, stained red by AZAN, suggesting to be acidphilic. Minor granules cannot be characterized because of limitation in microscope's resolution.

The Banded Structure

In a previous article we documented the presence of the banded structure detected by HE staining, situated at the fore-end of a typical stichosome and characterized by an alternative occurrence of bright cells and eosinophilic cells (Takahashi *et al.*, 1987). In this study, these bright cells exhibited strong PAS positive staining (Fig. 1) and stained very light blue by AZAN (Fig. 5). This staining profile strongly suggests that bright cells are equipped with abundant glycogen. The function of the banded structure, especially the biological meaning of huge accumulation of glycogen, is a matter for future investigation.

EOS/MOS

The lumen of the esophagus (E in Figs. 3 and 7) is occasionally filled with an amorphous

material, referred to as esophagus-occupying substance (EOS) (Furuki *et al.*, 1987). The ampullar portion of the midgut accommodates a similar substance (M in Figs. 2 and 6), termed midgut occupying substance (MOS) by Takahashi *et al.* (1987a). Both substances are composed of a homogeneous material with medium electron density (Furuki *et al.*, 1987; Takahashi *et al.*, 1987a) and are antigenic during the course of an initial infection in rats (Uno *et al.*, 1988), which prompted us to trace the fate of EOS and MOS after encystation in order to explore the possibility that these may account in part for the origin of excretory/secretory antigen. EOS and MOS were PAS positive (Figs. 3 and 2, respectively) suggesting to be of glycoprotein nature and stained red by AZAN (Figs. 7 and 6, respectively) suggesting to be acidic. The place of synthesis of EOS and MOS are thus far unknown.

The Genital Primordium

In general, the genital primordium stained orange or yellow by AZAN (GP in Figs. 5 and 6). The female larvae presented minute spots that were PAS positive and stained very light blue by AZAN (data not shown), while the male lacked such spots (Figs. 5 and 6). This phenomenon has been used as a mean for sex differentiation in tissue sections of a given muscle larva (Takahashi *et al.*, 1987c). According to this criteria the larva in Fig. 1 is a female and the one in Fig. 5 is a male.

Hemolymph

The pseudocoelom, the body cavity of nematodes, is filled with a fluid, namely hemolymph. Hemolymph was most prominent in the anterior and posterior end of the genital primordium; however, it was often lost from tissue sections because of its liquid nature. When successfully preserved, hemolymph stained red by AZAN and was weak positive by PAS (data not shown). This PAS positive staining shows the presence of carbohydrate contents. Hemolymph is reported to be antigenic (Uno *et al.*, 1988)

Glycogen Aggregates

The muscle larva of *T. spiralis* mostly contains rich glycogen (Beckett and Boothroyd, 1962). The glycogen reserve in the larva comprises approximately 15 percent of its dry weight (Ferguson and Castro, 1973). Under electron microscopy it appears as massive aggregates in a wide variety of cells including the stichocytes, the non-contractile portion of the muscle, the cord, the epithelial cells of the mid- and hindgut, and the germinal cells of the female genital primordium (Kim *et al.*, 1987; Yoshikawa *et al.*, 1988). During our histochemical analysis, glycogen aggregates yielded strong PAS positive staining, stained very light blue (sometimes barely visible) by AZAN and distributed in a spotty pattern at places listed above.

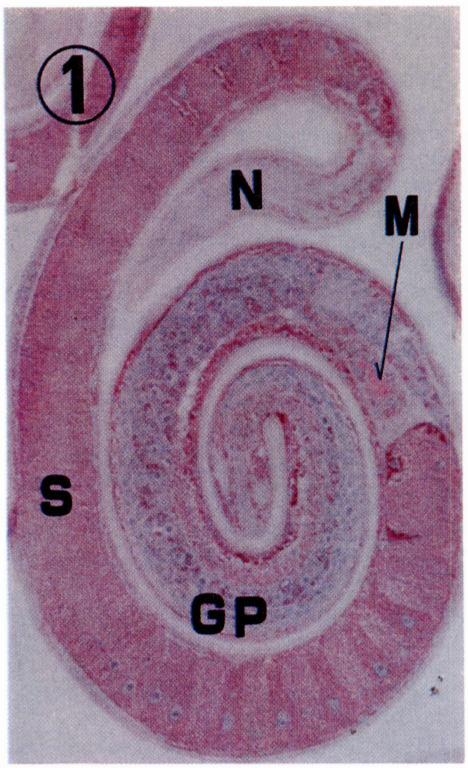
The Cuticle

The cuticle is an external covering of nematodes which is composed of collagen (Ouazana, 1982), however, its precise chemical nature is still obscure. The cuticle is the most obvious point of contact between a host and parasites

Abbreviations used in figures

S: stichosome
GP: genital primordium
N: neck
M: midgut
E: esophagus

Fig. 1 General view of *T. spiralis muscle* larva stained with PAS.
Fig. 2 High power of a longitudinal section through the ampullar portion of the midgut, stained with PAS.
Fig. 3 High power of a transverse section through the esophagus showing its contents (EOS) is PAS positive.
Fig. 4 High power of a longitudinal section through the stichosome, stained with PAS.
Fig. 5 General view of the muscle larva stained with AZAN.
Fig. 6 High power of a longitudinal section through the ampullar portion of the midgut, stained with AZAN.
Fig. 7 High power of a transverse section through the esophagus showing its contents (EOS) stains red.
Fig. 8 High power of a longitudinal section through the stichosome, stained with AZAN.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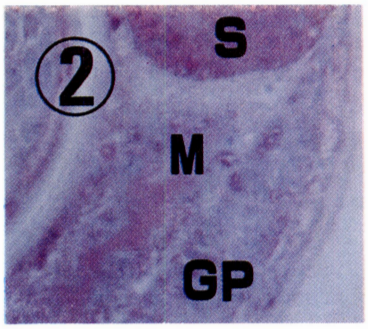
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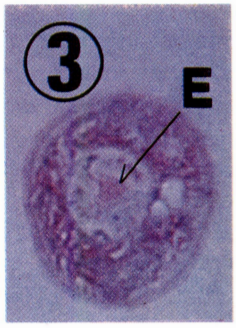
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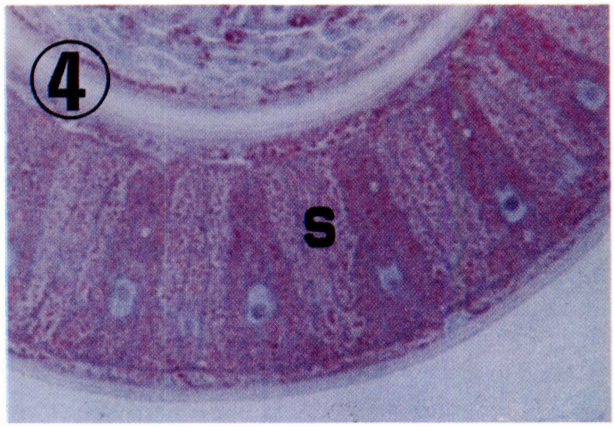
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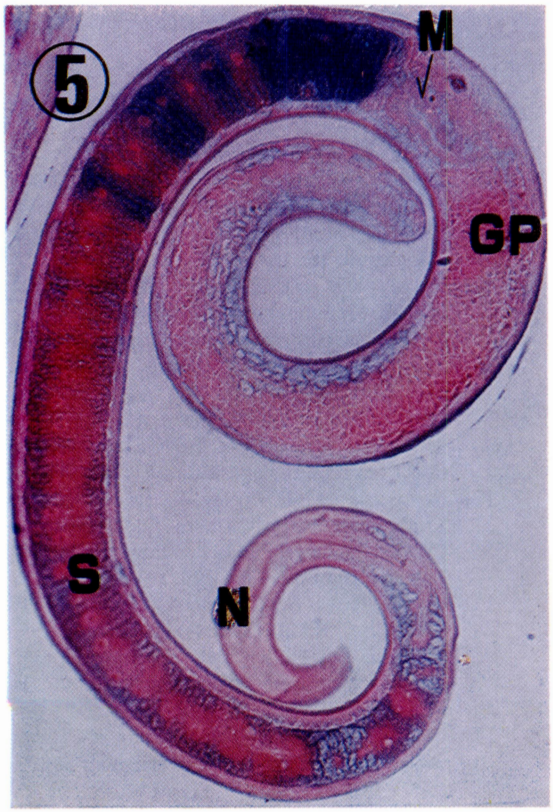
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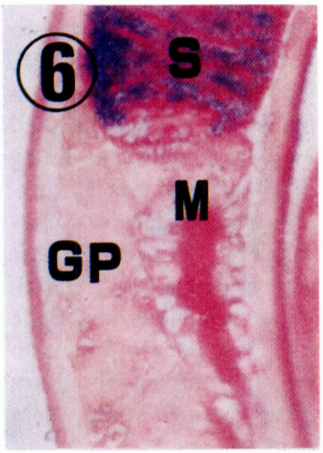
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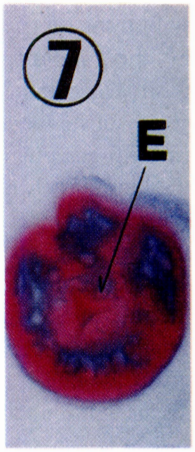
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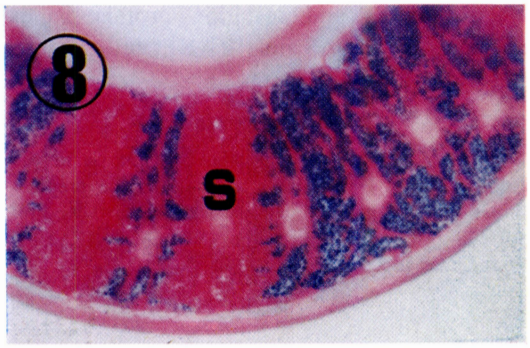
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and its antigenicity is attracting a great deal of attention in terms of protection inducing activity (Novoselska, *et al.*, 1978) and stage specificity (Jungery and Ogilvie, 1982; Mackenzie *et al.*, 1978; Maizels *et al.*, 1982; Ortega-Pierres *et al.*, 1984; Parkhouse *et al.*, 1983; Philipp *et al.*, 1981; Pritchard *et al.*, 1984). Since it was PAS negative (Figs. 1 through 4) polysaccharide is unlikely major components of the cuticle. It may be due to its fibrous components that cuticle's inner-layers stained red by AZAN (Figs. 5 through 8). Sometimes the cuticle surface stained blue by AZAN (Fig. 7).

In summary, we demonstrated that PAS and AZAN staining methods provide a more accurate and informative description of the morphology of *T. spiralis* muscle larva disclosing chemical nature of each component, which cannot be achieved by conventional HE staining. The histochemical information described herein enhances our understanding of muscle larvae, which have attracted a great deal of attention with the reason being that they play a major role in the pathogenesis and clinical symptoms of trichinosis. Such example is given by the similarity in the distribution of PAS-positive substances to that described for antigenic substances (Kim *et al.*, 1987), which led us to focus our interest in glycoprotein during the immunochemical characterization of antigenic substances. Another example is AZAN staining. This staining is so conspicuous that AZAN staining may be used as a tool to monitor the fate of stichocyte α-granules in the life cycle of *T. spiralis*. The staining information thus obtained is being applied in our laboratory to the immunochemistry-oriented study where antigenic substances are separated by means of electrophoresis and resulting protein bands are subjected to PAS and AZAN stainings.

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