[Jpn. J. Parasitol., Vol. 38, No. 5, 285—289, October, 1989]

Hematoxylin and Eosin Staining Profile of Adult Worms of *Trichinella spiralis*

YUZO TAKAHASHI, TAKAKO UNO, NAOTO MIZUNO, HIDEKAZU SUZUKI, CHIYOKICHI TOKUDA AND TSUNEJI ARAKI

(Accepted for publication; August 22, 1989)

Abstract

Longitudinal sections of adult worms of *Trichinella spiralis* were prepared by "squashing and fixation" technique (Jpn J. Parasitol., 36, 361—366, 1987) and an hematoxylin and eosin staining profile was described. The hypodermal glands lined up longitudinally with regular intervals. The stichosome was composed of uniform stichocytes equipped with a round nucleus and a prominent nucleolus. The stichocyte granules were strongly eosinophilic. The posterior two thirds of the male adult was occupied by the reproductive organs, including the testis and the seminal vesicle. Both were long tubular structures folded in two near the ampullar portion of the midgut and running parallel to each other with numerous sperms inside the tube. Nuclei of the sperms were round and stained violet by hematoxylin. Its cytoplasm was structureless at the light microscopic level. The posterior half of the testis was occupied by immature male germ cells. The vagina was a fine tubular structure running longitudinally between the body wall and the stichosome. It began from the uterus and ended at the vaginal opening. The uterus was a huge organ occupying two thirds of the body length. A variety of stages of embryogenesis were observed in the uterus. There was a crowd of sperm in the seminal receptacle. Generally the ovary was lacking prominent structure. The more mature ova were situated on the more anterior side. There was a clear line that separates the neighboring ova.

Key words: *Trichinella spiralis*, adult worm, histology, hematoxylin and eosin staining

Introduction

Anatomical knowledge of any parasite is important not only for the basic understanding of the parasite such as its life cycle, physiology and taxonomy, but also for the progress of clinico-pathological studies. The recent progress of immunology-oriented studies on nematode *Trichinella spiralis* prompted its morphological description to be expanded beyond *in to-to* observation under a light microscopic (Villella, 1970). Light and electron microscopical investigation has been carried out on muscle larvae of *T. spiralis* to some extent (Bruce *et al.*, 1965; Takahashi *et al.*, 1987, 1988a, b; Despommier, 1983; Wright *et al.*, 1985). Adult worms of *T. spiralis*, however, have received little attention in spite of their potential importance in establishing parasitism in the host, and consequently morphology of adult worms is still insufficiently understood.

We have already established the "squashing and fixation" technique to obtain a longitudinal section through the entire length of muscle larvae of *T. spiralis*, which allowed crystal clear orientation of each organ of interest (Takahashi *et al.*, 1987). Adapting the technique in this paper, an hematoxylin and eosin (H & E) profile of the adult worm is described.

Department of Parasitology, Nara Medical University, Kashihara, Nara, 634, Japan
Correspondence: Yuzo Takahashi, MD. Associate Professor, Department of Parasitology, Nara Medical University, Kashihara Nara 634 Japan
高橋優三　宇野貴子　水野直人　鈴木秀和　徳田千代吉　荒木恒治（奈良県立医科大学寄生虫学教室）

Materials and Methods

Adult worms were recovered from the intestines of mice 6 days after oral inoculation with muscle larvae, then suspended in the half strength Karnovsky fixative at 4°C and squashed between two glass slides with "appropriate" pressure, 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 longitudinally and stained with H & E according to the standard method.

Results and Discussion

For orientation purposes the location of each anatomical structure is shown in Fig. 8 which is a phase contrast micrograph of a male and a female. The parasite is composed of the body wall (cuticle, hypodermis, muscle cells and cords) and internal organs including the stichosome (ST), the intestinal gland (IG), the esophagus (E in Fig. 6), the midgut, the hindgut (G in Fig. 13) and the reproductive system. Adult worms have additional structure known as hypodermal glands (Bruce, 1970); in a section cut tangenitially through the body wall, the glands (HY in Fig. 7) run longitudinally with regular intervals. Drastic morphological alterations occur on the cords, the stichosome and the reproductive system during the development from larva to adult, therefore we restrict this paper to H & E histology of these structures.

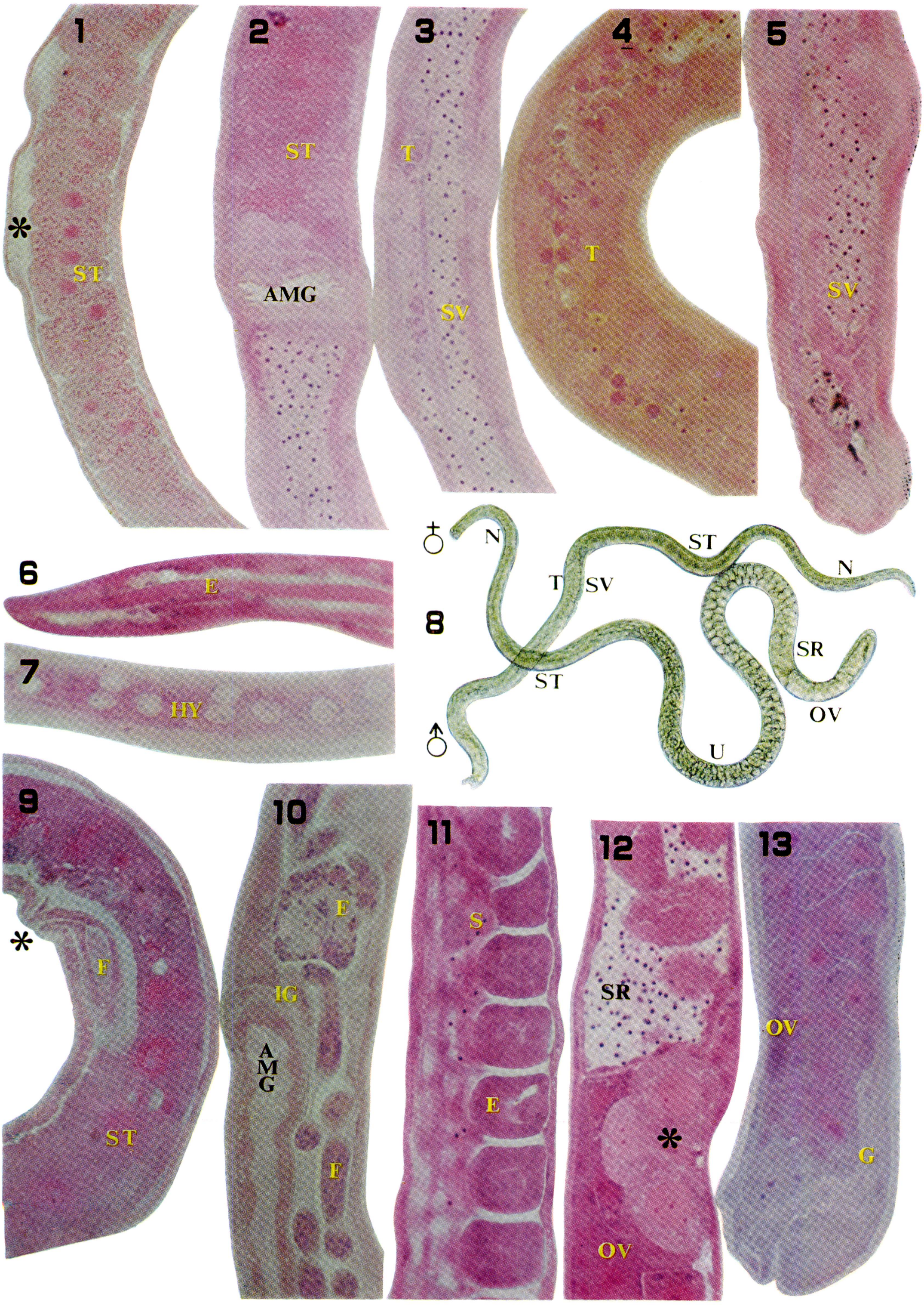
The stichosome

The stichosome of adult worms (ST in Figs. 1 and 9) has a round nucleus with a prominent nucleolus just like that of muscle larvae. A space between the body wall and the stichosome, known as the pseudocelom, is widened and the stichosome is more segmented than that of muscle larvae (asterisk in Fig. 1). Although the stichosome of muscle larvae is characterised by a striated appearance due to alternate occurrences of α and β stichocytes (Takahashi *et al.*, 1987),

Fig. 1. A longitudinal section through the stichosome of a male adult. Note widened pseudocelom (asterisk) and the segmented stichosome.
Fig. 2. A longitudinal section through the stichosome, the ampullar portion of the midgut and the male reproductive organ filled with mature sperms.
Fig. 3. The testis and the seminal vesicle run in parallel.
Fig. 4. Immature male germ cells are located in the distal portion of the testis.
Fig. 5. A longitudinal section of the distal end of an adult male worm.
Fig. 6. A longitudinal section of the frontal end of an adult worm. The esophagus is running longitudinally in the center.
Fig. 7. A tangenitial section through the hypodermal glands.
Fig. 8. A phase contrast micrograph of the adult male and female.
Fig. 9. A longitudinal section through the stichosome, the vaginal opening (asterisk) and the uterus with a fetal larva.
Fig. 10. A longitudinal section through the ampullar portion of the midgut and the uterus.
Fig. 11. A longitudinal section through the uterus with embryos and sperms.
Fig. 12. A longitudinal section through the seminal receptacle with sperms and the ovary.
Fig. 13. A longitudinal section of the distal end of a female adult.
Original magnification of each micrograph is ×250.

abbreviations used in figures

ST	: stichosome	U	: uterus
AMG	: ampullar portion of the midgut	E	: embryo
T	: testis	IG	: intestinal gland
SV	: seminal vesicle	S	: sperm
N	: neck	SR	: seminal receptacle
ES	: esophagus	OV	: ovary
HY	: hypodermal gland	G	: gut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that of adult worms is devoid of such striation (Figs. 1, 2 and 9). The stichocyte granules are secreted within 30 hours after infection and regained as the worms grow (Chipman 1957). The stichocyte granules of the adults are more eosinophilic than that of larvae. In some adult worms, granules form many clusters which line up along the esophagus with periodical intervals (Fig. 9). Our preliminary electron microscopical observation revealed that each cluster is located in the vicinity of the canalicular tree. Van Someren (1939) suggested the presence of two types of stichocytes in the adult worms. But our results by means of H & E histology did not confirm the presence of two types of stichocytes.

Male reproductive system

The posterior two thirds of the male adult is occupied by the reproductive organs, including the testis (T in Figs. 3 and 4) and the seminal vesicle (SV in Figs. 3 and 5). Both are long tubular structures folded in two near the ampullar portion of the midgut (AMG) and running parallel to each other with numerous sperms inside the tube (Figs. 2, 3 and 5). Nuclei of the sperms are round and stain violet by hematoxylin. Cytoplasm is structureless at the light microscopic level. The posterior half of the testis is occupied by immature male germ cells (Fig. 4).

Female reproductive system

The vagina is a fine tubular structure running longitudinally between the body wall and the stichosome. It begins from the uterus and ends at the vaginal opening (asterisk in Fig. 9) which is located to the level corresponding to the midpoint of the stichosome. In the vagina several fetal larvae (F in Fig. 9) of whose morphology are identical to that of newborn larvae, can be observed. The uterus is a huge organ occupying two thirds of the body length (Figs. 10 and 11). A variety of stages of embryogenesis can be observed in the uterus. The more anterior the embryos are located in the uterus, the more mature they are (E in Fig. 10). The more posterior the embryos are located, the more eosinophilic and the more round they are (Fig. 11). Sperms are seen in both the vagina and the uterus (S in Fig. 11). Particularly there is a crowd of sperms in the seminal receptacle (SR in Fig. 12) as reported by Villella (1970). Generally the ovary is lacking prominent structure, however, at the anterior end where the most matured ova are supposed to be located, a couple of large, bright and round cells are lined (asterisk in Fig. 12). The nucleus of the cells is larger than those of the other ova and contains two or three fine dots. The dots are stained violet but apparently are smaller than the nucleolus in the other ova. Whether they have been fertilized or not is a matter of future investigation. The more mature ova are situated on the more anterior side. There is a clear line that separates the neighboring ova (Fig. 13).

In summary, we have first described H & E staining profile of *T. spiralis* adult worms. The micrographs in the figures are representative which depict all of the profiles of this worm. Therefore this contribution is expected to serve as a colour atlas of this parasite for future histological investigations.

References

1) Bruce, R. G. (1966): The fine structure of the intestine and hindgut of the larva of *Trichinella spiralis*. Parasitol., 56, 359—365.
2) Bruce, R. G. (1970): *Trichinella spiralis*: Fine structure of body wall with special reference to formation and moulting of cuticle. Exp. Parasitol., 28, 499—511.
3) Chipman, P. B. (1957): The antigenic role of the excretions and secretions of adult *Trichinella spiralis* in the production of immunity in mice. J. Parasitol., 43, 593—598.
4) Despommier, D. D. (1983): Biology, In *Trichinella* and Trichinosis Plenum Press, Campbell WC ed., New York and London, 75—151.
5) van Someren, V. D. (1939): On the presence of a buccal stylet in adult *Trichinella,* and the mode of feeding of the adults. J. Helminthol., 17, 83—92.
6) Takahashi, Y., Yoshikawa, Y., Furuki, J., Yamada, S. and Araki, T. (1987): Morphological study of *Trichinella spiralis*: an overall picture of a muscle larva as revealed by longitudinal sectioning. Jpn. J. Parasitol., 36, 361—366.
7) Takahashi, Y., Uno, T., Yagi, J., Yamada, S. and Araki, T. (1988a): Morphology of the alimentary tract of *Trichinella spiralis* muscle larva with emphasis on the esophagus. Parasitol. Res., 75, 42—49.

8) Takahashi, Y., Uno, T., Furuki, J., Yamada, S. and Araki, T. (1988b): The morphology of *Trichinella spiralis*: ultrastructural study of the mid- and hindgut of the muscle larvae. Parasitol. Res., 75, 19—27.
9) Villella, J. B. (1970): Life cycle and Morphology, In Trichinosis in man and animals, Gould SE, ed. C.C. Thomas, Springfield Illinoi, 19—60.
10) Wright, K. A., Lee, D. L. and Shivers, R. R. (1985): A freeze-fracture study of the digestive tract of the parasitic nematode *Trichinella*. Tissue. Cell., 17, 189—198.